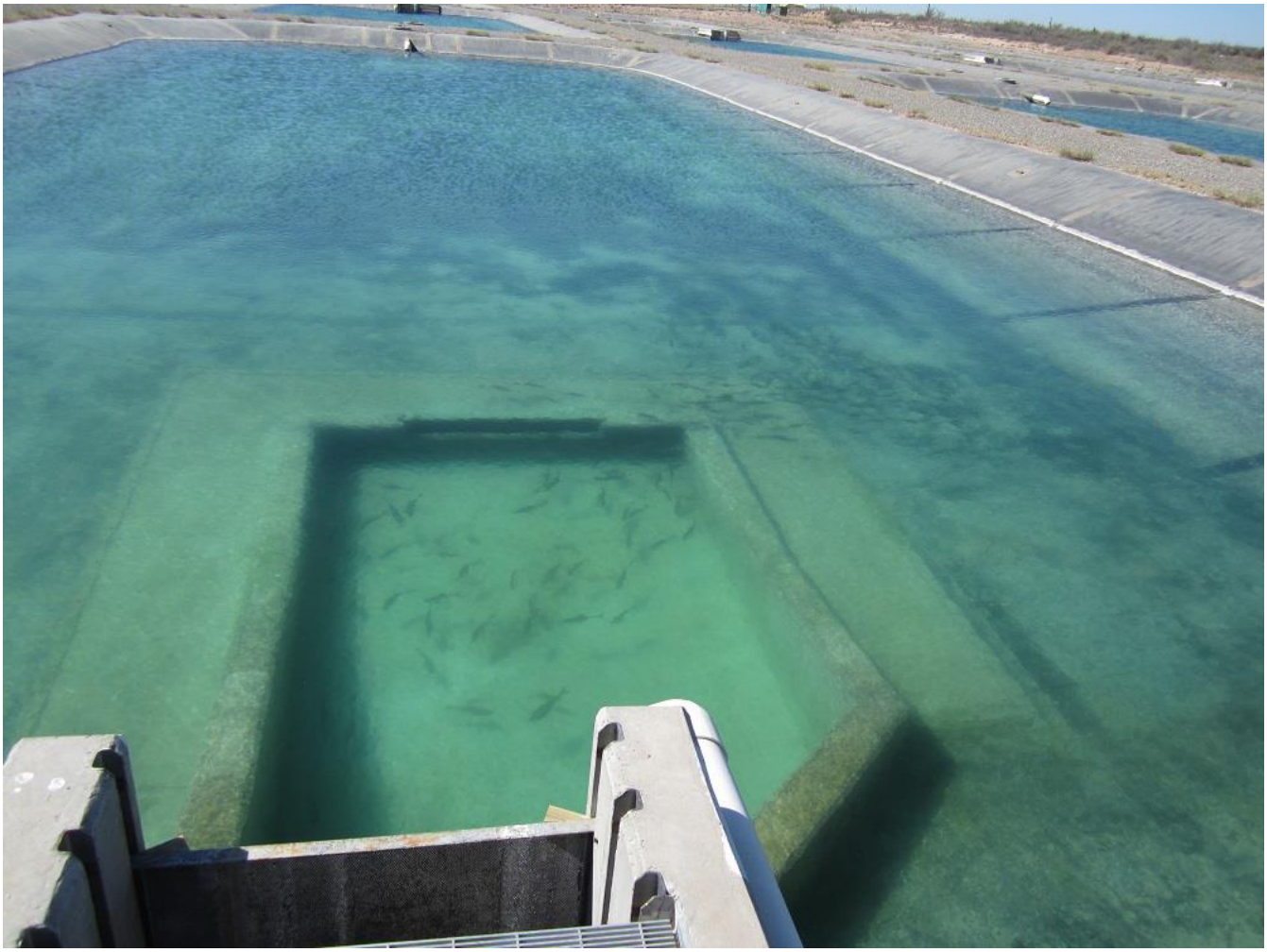
Lower Colorado River Multi-Species Conservation Program

Balancing Resource Use and Conservation

Genetic Monitoring and Biological Control of Recruitment in Bonytail Rearing Ponds



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Lower Colorado River Multi-Species Conservation Program

Genetic Monitoring and Biological Control of Recruitment in Bonytail Rearing Ponds

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ACRONYMS AND ABBREVIATIONS

Achii Hanyo NFRF	Achii Hanyo Native Fish Rearing Facility
A_R	allelic richness
BW	body weight
CPM	Colorado pikeminnow
d.f.	degrees of freedom
F_{IS}	average inbreeding coefficients
g	gram(s)
ha	hectare(s)
H_E	expected heterozygosity
H_O	observed heterozygosity
HW	Hardy-Weinberg
mm	millimeter(s)
N_A	number of alleles per locus
$N_{e(SA)}$	effective size calculated from sibship assignments
PIT	passive integrated transponder tag
SD	standard deviation
SGR	specific growth rate
SNARRC	Southwestern Native Aquatic Resources and Recovery Center
TL	total length
USFWS	U.S. Fish and Wildlife Service
Uvalde NFH	Uvalde National Fish Hatchery
YC	year class

Symbols

Δ	change
°C	degrees Celsius
>	greater than
≥	greater than or equal to
<	less than
≤	less than or equal to
µL	microliter(s)
%	percent

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Attachment

1 Box Plots Showing Allele Frequencies (x axis) in Each of the Experimental Ponds (y axis) by Microsatellite Locus (15 Loci)

EXECUTIVE SUMMARY

To address the management implications of natural recruitment (i.e., young-of-year entering a population within a given year) in grow-out ponds, this study has three objectives, the first two being: (1) explore the use of Colorado pikeminnow (*Ptychocheilus lucius*), a piscivorous fish, as a means of biological control to reduce natural recruitment in production grow-out ponds at the Southwestern Native Aquatic Resources and Recovery Center (SNARRC) and (2) optimize growth and stocking rates specifically for pond culture of bonytail (section 1). The results from section 1 of this study (Biological Control of Natural Recruitment) showed a strong negative effect of Colorado pikeminnow on the abundance of bonytail (*Gila elegans*) recruitment and total pond biomass. The number of bonytail recruits (i.e., individuals within the recruitment population) harvested from each pond was significantly reduced in ponds with Colorado pikeminnow (range = 3–63) compared to ponds without pikeminnow (2,854–6,969), as was total biomass harvested from each pond. However, there was no difference in the final number of recruits or differences in total biomass between the two levels of the Colorado Pikeminnow treatment, suggesting densities as low as 50 pikeminnow per 0.4 hectare can effectively control unwanted recruitment of bonytail.

The third objective was to use microsatellite markers to assess the genetic diversity of recruitment spawned naturally in grow-out ponds and conduct parentage assignments to determine how many parents contribute (section 2). The results of section 2, combined with the portion of section 1, suggest that although recruitment can be genetically diverse and similar to the parental population in terms of total number of alleles and allelic richness, as seen in the experimental control samples, the demographics and genetic diversity of the parental population are key. Section 1 suggested that females were larger than males, and with current management actions of selecting (grading) and stocking large individuals (size $\geq$ 300 millimeters), gender ratios may be skewed toward males after grading. If a captive population that has been graded several times produces recruitment, it is likely that few parental females were available to maintain genetic diversity. Unless the parental population is large, has not been graded, and is the product of many genetically diverse paired spawns, recruitment should not be used for production or recovery purposes. That is, unless the recruitment population is evaluated for genetic diversity and compared to data of diverse populations such as the broodstock at the SNARRC or other production populations that have been evaluated for genetic diversity. Production of genetically diverse bonytail from natural recruitment is an option when few individuals (< 5,000) are needed and if the stable conditions similar to those used in the experimental portion of this study are replicated. It is not an option to produce large numbers similar to current the SNARRC bonytail production commitments (> 40,000) due to space and other restrictions. Results may change as environmental and demographic conditions change, and these results cannot be applied to other systems.

INTRODUCTION

The Southwestern Native Aquatic Resources and Recovery Center (SNARRC), Dexter, New Mexico, maintains the only genetically managed captive stock of the endangered bonytail (*Gila elegans*) and endemic species of the Colorado River basin. A genetic management plan was developed in 2004, and SNARRC personnel have diligently followed the tasks and management actions outlined in the plan (U.S. Fish and Wildlife Service [USFWS] 2004b). Since the founding of the original broodstock, which was developed using 10 paired spawns (Hamman 1982), captive populations are monitored to ensure each successive generation of broodstock maintains genetic diversity comparable to the preceding stock. Subsequent broodstocks were created between 2000 and 2004 (83 paired spawns), and again between 2012 and 2014 (50 paired spawns). Spawning and rearing strategies for the species are geared toward maintaining the existing genetic diversity of the species, which is essentially extirpated from the wild, but also reducing effects on wild populations through processes such as the Ryman-Laikre effect (Ryman and Laikre 1991). This effect occurs when wild populations are augmented with production fish that have been produced from few parents (low effective size), in turn reducing the effective size of the recipient population (Christie et al. 2012).

The SNARRC bonytail broodstock is spawned for production purposes on an annual basis. The larvae are reared in ponds at the SNARRC until reaching target stocking size or are shipped to several regional facilities for grow-out. The targeted stocking size (> 300 millimeters [mm]) requires a multi-year grow-out period, and precocious individuals often spawn during the second summer of rearing. The inadvertent spawning may not only violate the broodstock management protocols designed to ensure genetic diversity in production fish, but it may also negatively impact growth due to feed rates that cannot be tailored due to the indeterminate number of fish in each pond. In addition, unintended recruitment (i.e., young-of-year entering a population within a given year) can lead to overcrowding. High fish densities foster poor water quality conditions, such as oxygen depletion, which in turn can lead to increased susceptibility to disease and potential loss of production fish. To address the management implications of natural recruitment in grow-out ponds, this study has three objectives: (1) explore the use of Colorado pikeminnow (*Ptychocheilus lucius*), a piscivorous fish, as a means of biological control to reduce natural recruitment in production grow-out ponds at the SNARRC; (2) optimized growth and stocking rates specifically for pond culture of bonytail (section 1); and (3) use microsatellite markers to assess the genetic diversity of the recruitment populations spawned naturally in grow-out ponds and conduct parentage assignments to determine how many parents contribute (section 2).

Section 1 – Biological Control of Natural Recruitment

METHODS

Experimental Fish

All individuals of both bonytail and Colorado pikeminnow in the study were spawned and maintained on site prior to the experiment. Two different year classes (YC) of adult bonytail were utilized between years due to limitations with the availability of experimental fish (i.e., fish not used for recovery needs). Age-4 (2009 YC) adults were used in 2013, and Age-2 fish (2012 YC) were used in 2014 (figure 1). Both age classes were of reproductive maturity (Hamman 1982, 1985). Prior to the experiment, each adult was individually marked with a passive integrated transponder (PIT) tag (Hamman and Ulibarri 2005) in order to track individuals during the experiment.

The SNARRC spawns Colorado pikeminnow to augment the wild San Juan River population to aid recovery efforts: "Recovery Goals Criteria – Reestablish populations with hatchery-produced fish" (USFWS 2014). Colorado pikeminnow historically co-occurred with bonytail throughout the Colorado Basin (Quartarone 1995; Minckley et al. 2003; Gobalet et al. 2005), and preyed on bonytail in their natural environment (Bestgen et al. 2008). The availability of onsite, disease-free fish and the evolutionary history of predator-prey interactions made Colorado pikeminnow a logical biological control candidate for this study. Thus, we used juvenile Age-1 Colorado pikeminnow, spawned May 30, 2012, and May 30, 2013, respectively (Hamman and Ulibarri 2005, 2008; Knight and Ulibarri 2013) to act as control agents on bonytail recruitment while preventing predation on adults.

Experimental Design

Pond preparation and fish stocking was conducted according to standard SNARRC protocols (Hamman and Ulibarri 2005, 2008; Knight and Ulibarri 2013). The experiment was set up in a randomized complete block design in nine 0.4-hectare (ha) lined ponds with each of two treatments and a control being conducted in triplicate (three ponds per treatment and control) in 2013 and independently repeated in 2014 (figure 2). Each of the nine ponds was initially stocked with 200 bonytail (100 males and 100 females); thereafter, 6 ponds received an additional stocking of either 50 Colorado pikeminnow per pond (treatment one) or 100 Colorado pikeminnow per pond (treatment two).
The remaining three ponds served as controls and received zero Colorado pikeminnow. The stocked density of 200 bonytail per 0.4 ha is the same as

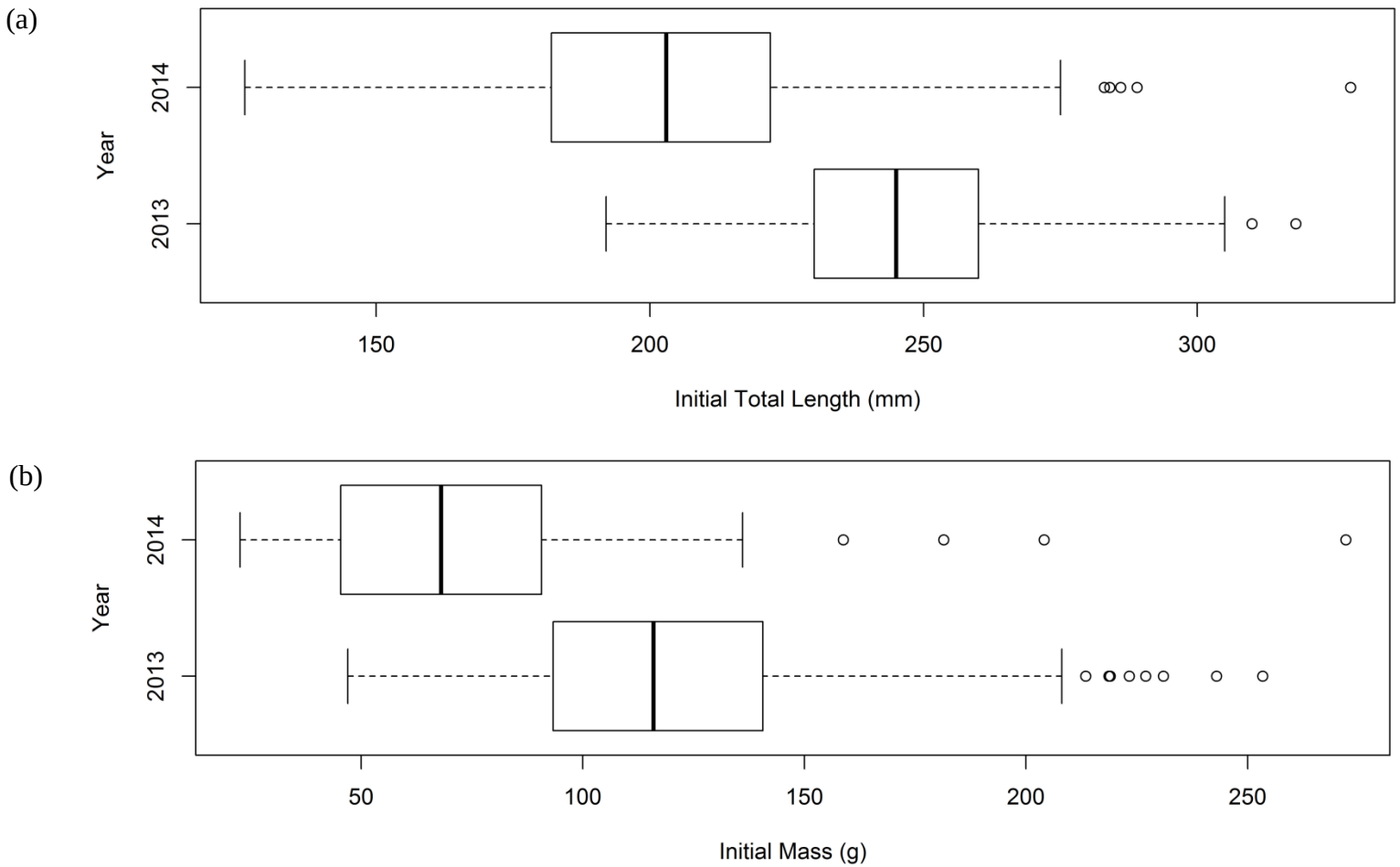

Figure 1.—Box plot showing bonytail initial total lengths (a) and initial mass (b) for each year of the study (2013 and 2014).
Individuals used in 2013 were the 2009 year class (Age-4), and those used in 2014 were the 2012 year class (Age-2). A total of 1,800 individuals were stocked each year (200 in each of 9 ponds).

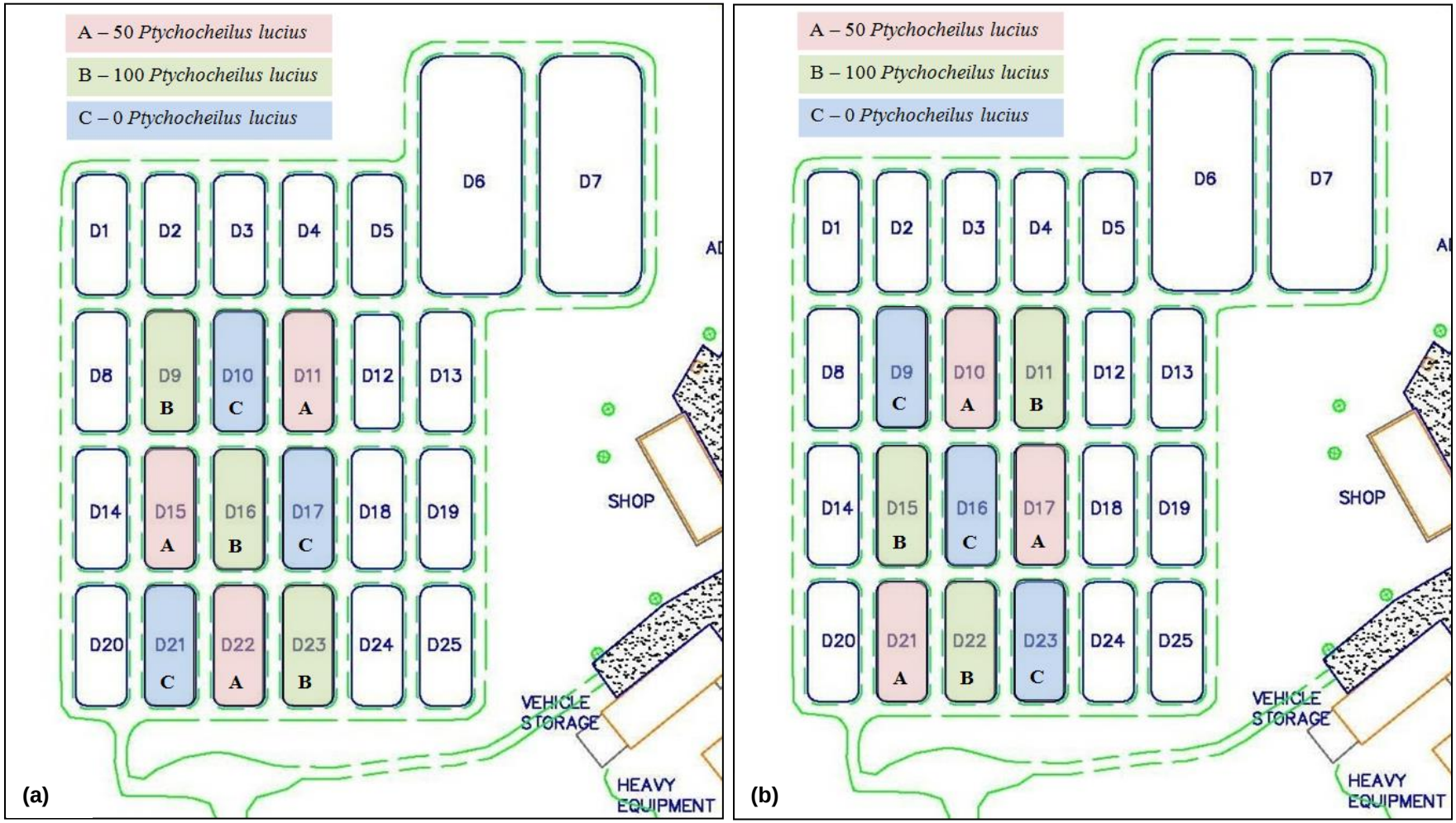

Figure 2.—Experimental pond treatment layout for 2013 (a) and 2014 (b).

the density used in maintenance of bonytail broodstock and production ponds. Ponds used in both years were the same, except the treatment type was rotated and not replicated in the same pond between years. New individuals of both bonytail and Colorado pikeminnow were used in 2014. Prior to stocking, both individual length and weight recorded for a random subset of fish stocked into each pond (2013 N = 20; 2014 N = 40); in addition, PIT tags were recorded for each individual. Sex data were also recorded for all individuals in 2014.

Basic water quality parameters (dissolved oxygen and temperature) were measured daily using a hand-held HACH HQ40d meter (Hach Company, Loveland, Colorado). Fish were fed in accordance with standard feed protocols used for culture practices at the SNARRC (Hamman and Ulibarri 2005, 2008; Knight and Ulibarri 2013). Any mortality observed during the experiment was recorded, and individuals were removed. After 5 months (May to September in 2013 and June to October in 2014), all individuals were harvested, scanned for PIT tags, inventoried, and sorted by species and age class. Recruits were individually counted for treatment ponds, whereas the total number of recruits in control ponds were estimated from five sample weights and extrapolated on total batch weight due to the large number of individuals harvested in each control group. Final batch weight for bonytail (adults and recruitment) and Colorado pikeminnow was recorded for each pond. To estimate growth during the study period, individual length and weight data were collected on the same individuals randomly selected prior to stocking (2013 N = 20; 2014 N = 40).

Data Analysis

Biological Control of Bonytail Recruitment

General linear models were used to assess the effects of treatment (i.e., number of Colorado pikeminnow) on recruitment (defined as the number of Age-0 individuals harvested after 5 months), mean mass of individual recruits (grams [g]), mean Colorado pikeminnow mass (g), percent survivorship for both bonytail and Colorado pikeminnow, and total biomass (g). Due to the large difference in the number of recruits harvested, data were log transformed for final analyses. In order to obtain enough statistical power for some of the analyses, both years were analyzed in the same model; however, in order to control for annual variation, the year was entered as a fixed effect in each respective model. Interaction effects were tested with significant interactions retained in respective models. Statistical significance was set at $P < 0.05$, and effect sizes were estimated with partial eta^2 (η^2; the proportion of variance accounted for by each effect). If the results indicated a significant difference among treatments, a Tukey's HSD (Tukey 1949) test was used to identify significance among treatment levels.

Growth Rate

Adult bonytail used in 2013 were significantly larger in both total length (TL) (mean = 246.9 mm TL, standard deviation [SD] = 24.8) and mass (mean = 122.1 g, SD = 41.8) compared to 2014 (mean = 203.0 mm TL, SD = 29.3; mean = 77.4 g, SD = 32.4) (independent-samples t-test; TL, $t_{391.8} = 17.9$; mass, $t_{272.8} = 12.3$). Due to these differences, each year was analyzed separately.

In order to assess effects of treatment type on adult bonytail growth, we quantified metrics related to changes in TL, mass, and growth rates:

Change in total length (ΔTL) = final TL - initial TL

Change in body mass (Δg) = final mass (g) - initial mass (g)

Percent body weight gain (%BW) = 100 x {(final weight - initial weight)/initial weight}

Specific growth rate (SGR) = 100 x {($\log_e$ [final weight] - $\log_e$ [initial weight])/ total experiment days}

All variables, except for SGR, were square root transformed to approximate normality of residuals. Outliers, assumed to be data recording errors, were removed from the data set prior to final analyses. General linear models were used to assess the effects of sex (2014 only) and treatment on the four growth metrics. Initial size or mass was entered as a covariate in each respective model, and interaction effects were tested, but only significant interactions were retained in the final models. For this section of the study (section 1), we used the R statistical language (R Development Core Team 2015) to analyze each dataset and produce all figures.

RESULTS

Biological Control of Bonytail Recruitment

Bonytail recruitment harvested at the end of each treatment had a similar effect each year ($F_{1, 14} = 1.263$, $P = 0.280$, $\eta^2 = 0.083$), with fewer fish ($F_{2, 14} = 125.601$, $P < 0.001$, $\eta^2 = 0.95$) harvested from the two treatments (50 and 100 Colorado pikeminnow) compared to the controls (table 1). Tukey's HSD post-hoc analysis indicated a significant difference between both treatment levels and the control (50–0 $P < 0.001$; 100–0 $P < 0.001$), but showed no significant difference between treatments (50–100 $P = 0.481$). General linear model results assessing the effect of Colorado pikeminnow on total biomass in each pond suggest a significant reduction in biomass ($F_{1,12} = 19.48$, $P < 0.001$, $\eta^2 = 0.929$); however, there was a significant interaction between treatment and year, which was due to the difference in biomass between the control and treatment groups between the two years (figure 3).

Table 1.—Results from the 2013 and 2014 experiment showing treatment (number of Colorado pikeminnow, total recruitment (number of Age-0 bonytail) per pond (replicate), mean mass per individual for bonytail and Colorado pikeminnow, percent survival for bonytail and Colorado pikeminnow, and total pond biomass

Study year	Treatment	Replicate	Bonytail			Colorado pikeminnow		Total biomass (g)
			Total recruitment	Mean individual mass (g)	Adult survival (percent)	Mean individual mass (g)	Survival (percent)	
2013	0 (control)	1	3,931	2.9	99.0	–	–	44,839
		2	4,472	2.7	99.0	–	–	49,059
		3	2,854	3.9	98.0	–	–	46,458
	50	1	14	10.4	98.0	43.3	90	37,748
		2	24	4.1	99.0	45.4	92	40,257
		3	35	13.6	99.0	40.2	70	39,275
	100	1	62	12.1	96.5	35.8	90	39,417
		2	11	6.9	98.0	32.3	97	39,034
		3	3	5.8	99.0	32.0	95	37,934
2014	0 (control)	1	4,239	5.0	99.0	–	–	56,990
		2	4,441	5.1	98.5	–	–	62,898
		3	6,968	3.4	98.5	–	–	61,843
	50	1	27	10.9	99.0	44.3	84	44,874
		2	63	20.2	99.0	53.5	100	46,729
		3	15	12.1	99.0	49.6	86	46,814
	100	1	22	10.3	99.5	42.3	88	47,092
		2	23	9.9	100	42.1	95	45,866
		3	18	10.1	99.5	49.9	90	45,086

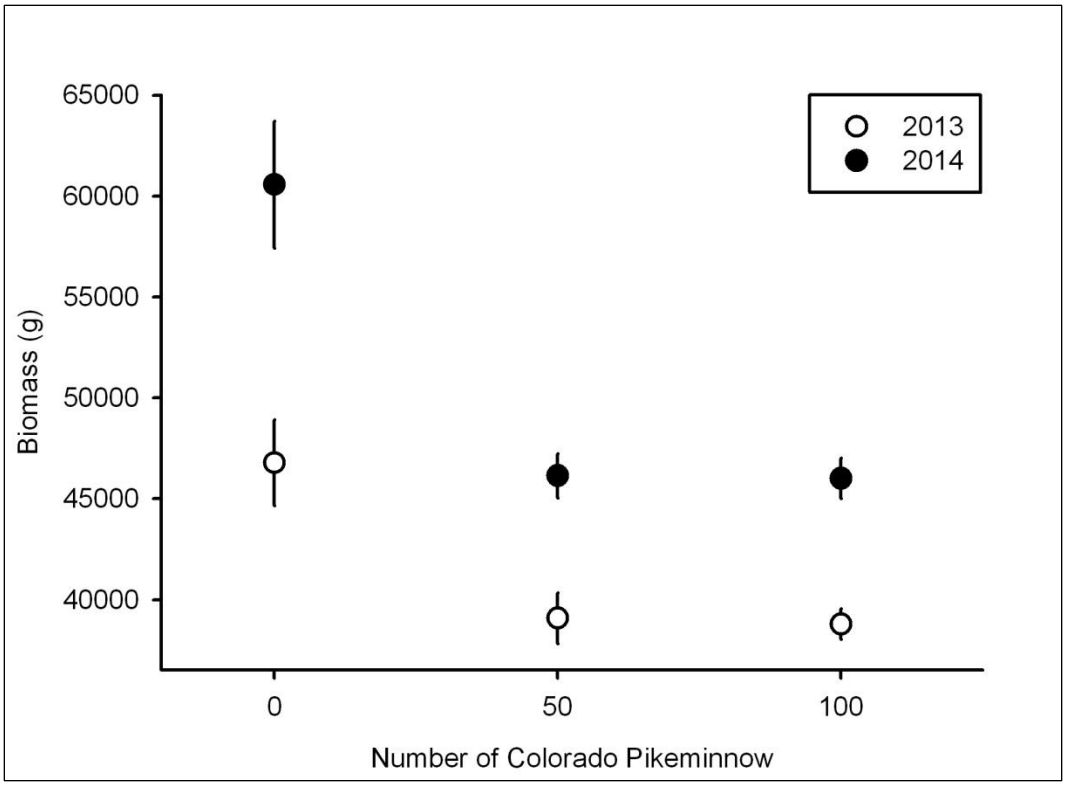

Figure 3.—Total pond biomass (± 1 SD) for each treatment.
2013 results are displayed in white and 2014 in black.

The recruitment mean mass per individual (g) varied significantly among treatments ($F_{2, 45} = 10.4$, $P = 0.002$), with individuals from the two treatments being larger than those from the control (see table 1); a similar result was observed between years ($F_{1, 14} = 3.4$, $P = 0.087$). The results from the post-hoc analyses showed significant differences between treatments and the control (50–100 $P = 0.001$; 100–0 $P = 0.026$) but no difference between treatments (100–50 $P = 0.319$). Additional post-hoc results from the general linear model demonstrated a significant difference ($P = 0.017$) in Colorado pikeminnow mean mass (g), with individuals from the treatment of 50 Colorado pikeminnow being larger than those from the treatment of 100 Colorado pikeminnow (see table 1).

Adult bonytail survivorship was high for both years (see table 1), with 98.4 percent (%) surviving the first year and 99.1% surviving in 2014. General linear model results did not detect an effect of the treatment on survivorship of adults ($F_{2,14} = 0.076$, $P = 0.927$, $\eta^2 = 0.011$), with both years exhibiting similar results ($F_{1,14} = 4.271$, $P = 0.058$, $\eta^2 = 0.234$). Colorado pikeminnow survivorship was less than what was observed for adult bonytail, with a survival of 89% of individuals in 2013 and 90.5% in 2014. Additional post-hoc results from the general linear model testing the difference in Colorado pikeminnow survivorship between treatments did not detect a difference ($P = 0.320$).

Growth Rate

In regard to model predictions of adult bonytail growth rates (table 2; figure 4), in 2013, initial TL, but not treatment, had significant effects ($P = 0.045$) on ΔTL, indicating that overall, individuals that were shorter initially were shorter at harvest; however, the effect size was relatively small. The remaining 2013 models (Δg, SGR, %BW) had a significant interaction effect (treatment*initial mass), suggesting treatment effects varied over initial starting sizes. Although initial size had the strongest effect on all treatment variables, the treatment effect seemed to be only apparent in the smaller individuals. However, results suggest initial mass had the strongest effect on all treatment variables, with a larger effect size for initial mass than treatment.

Sex data were not collected for the treatment groups in 2013; however, due to the data collection requirements in the genetic portion of the study (section 2), sex data were collected for the control group. Analyses of these data indicated a strong size difference between sexes for adult bonytail, with females being larger than males (see figure 6). Consequently, sex data were collected in 2014 to account for variation attributed to sexual dimorphism in body size. When sex data were added (2014), there was a significant effect of treatment and sex for ΔTL but not initial TL. Post-hoc analyses for ΔTL found significant differences between both treatment groups from the control; however, there was no difference between the two treatment groups (50–100). Initial mass for 2014 was mistakenly measured in pounds rather than grams, therefore initial weight values in figure 5 were clumped following the conversion to grams. Thus, data points were altered in the figure to increase the visibility of data points. There was a significant effect of treatment, initial size, and sex for SGR, with initial mass having the largest effect size. Both Δg and %BW had a significant interaction, suggesting the effects of initial mass varied by sex and treatment, respectively. However, the effect sizes of these interactions were relatively small. A significant interaction between initial size and sex was found for Δg, whereas, similar to 2013 results, a significant interaction between treatment and initial mass was found for %BW. Plotted data for Δg for 2014 (figure 5) suggests a treatment effect could be present, with the 100 Colorado pikeminnow treatment showing a greater change in mass relative to the control group; however, this effect was likely only observed in the initially smaller individuals, and this effect size was substantially smaller than other terms in the model.

Water Quality

Treatment effect on water quality measures was highly variable for both years of the study (figure 7). There was a gradual increase in water temperature during both study periods, with little to no variation in water temperature between replicates. Percent saturation of dissolved oxygen in the ponds varied between

Table 2.—General linear model results from analyses of adult bonytail growth rates after 5 months in study ponds

(Results show year, growth metrics [response], Colorado pikeminnow treatment variable [effect], degrees of freedom [d.f], F-statistic [F], *P*-value [*P*], and effect size [η^2]. ΔTL = change in total length, Δg = change in mass, SGR = specific growth rate, %BW = percent body weight gain)

Year	Response	Effect	d.f.	F	*P*	η^2
2013	ΔTL	Treatment	2,167	0.913	0.403	0.011
		Initial TL	1,167	4.088	0.045	0.024
	Δg	Treatment	2,165	4.446	0.013	0.024
		Initial mass	1,165	35.149	< 0.001	0.232
		Treatment*initial mass	2,165	3.650	0.028	0.042
	SGR	Treatment	2,165	3.974	0.021	0.027
		Initial mass	1,165	194.353	< 0.001	0.747
		Treatment*initial mass	2,165	3.152	0.045	0.037
	%BW	Treatment	2,165	5.469	0.005	0.040
		Initial mass	1,165	0.614	0.435	0.046
		Treatment*initial mass	2,165	4.263	0.016	0.049
2014	ΔTL	Treatment	2,345	25.489	< 0.001	0.129
		Initial TL	1,345	0.354	0.552	0.001
		Sex	1,345	422.123	< 0.001	0.551
	Δ g	Treatment	2,344	45.450	< 0.001	0.209
		Initial mass	1,344	146.911	< 0.001	0.324
		Sex	1,344	1.304	0.254	0.317
		Initial mass*sex	2,344	13.721	< 0.001	0.038
	SGR	Treatment	2,345	44.873	< 0.001	0.206
		Initial mass	1,345	599.469	< 0.001	0.635
		Sex	1,345	133.776	< 0.001	0.279
	%BW	Treatment	2,344	11.334	< 0.001	0.197
		Initial mass	1,344	14.263	< 0.001	0.207
		Sex	1,344	129.347	< 0.001	0.274
		Treatment*initial mass	2,344	4.484	0.012	0.025

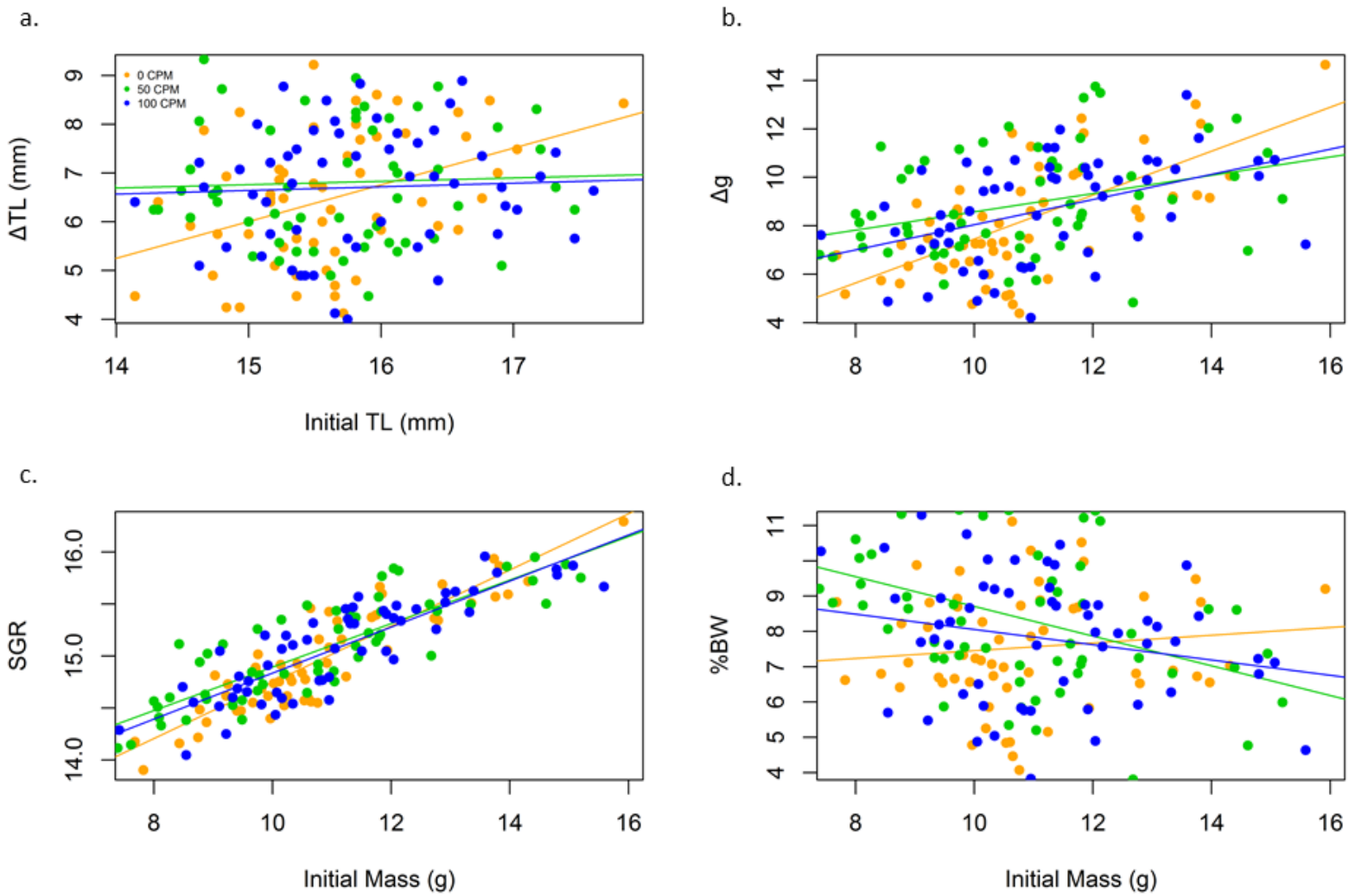

Figure 4.—Relationships between initial size (Δg and initial TL) and growth rate metrics (ΔTL, ΔMass, SGR, and %BW) for 2013.
The control group is plotted in yellow (0 CPM), green shows the 50 Colorado pikeminnow treatment (50 CPM), and blue shows the 100 Colorado pikeminnow treatment (100 CPM).

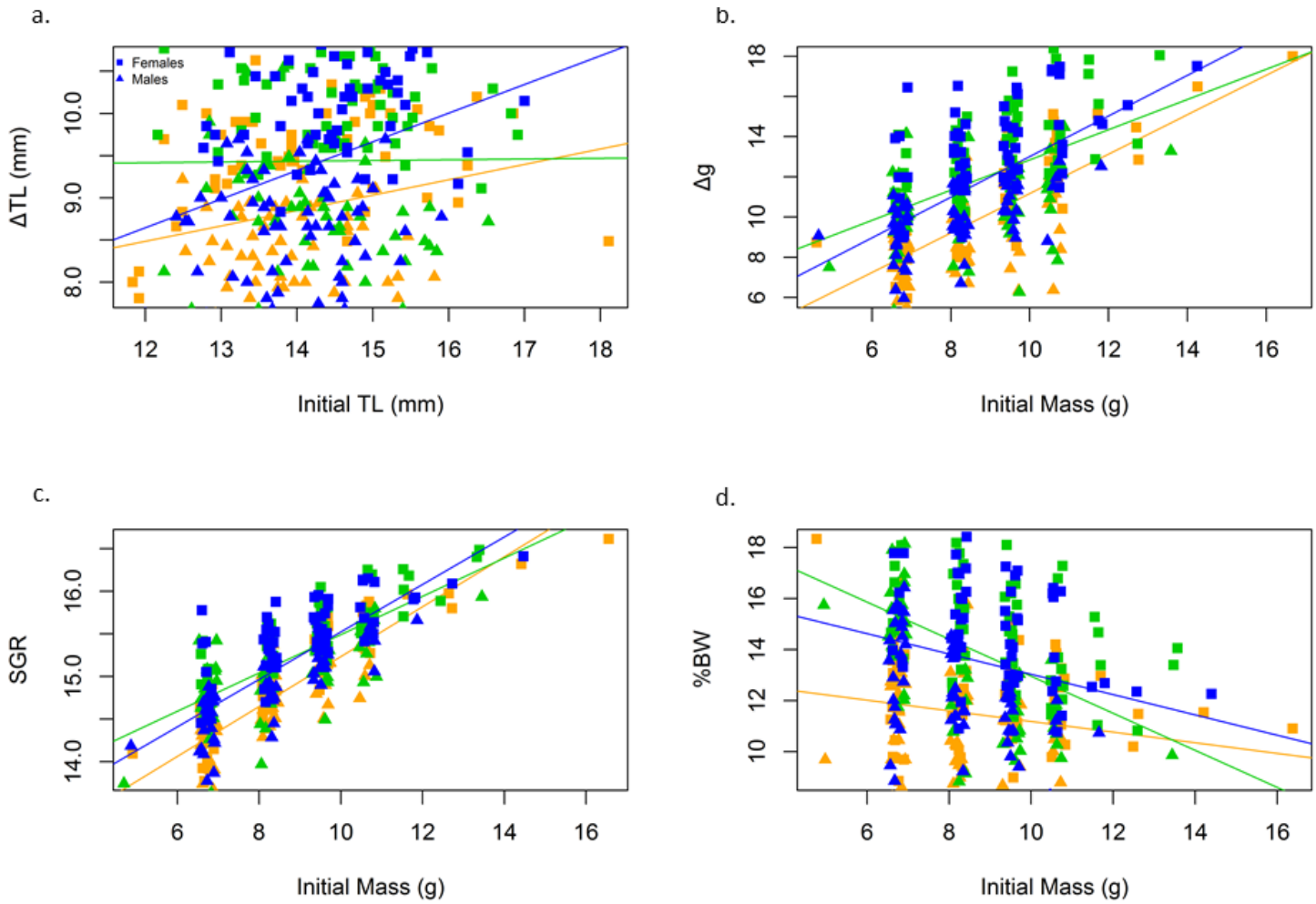

Figure 5.—Relationships between initial size (Δg and initial TL) and growth rate metrics (ΔTL, ΔMass, SGR, and %BW) for 2014.
The control group is plotted in yellow (0 CPM), green shows the 50 Colorado pikeminnow treatment (50 CPM), and blue shows the 100 Colorado pikeminnow treatment (100 CPM). Sex data are shown in the 2014 plots, with females shown in squares and males shown in triangles.

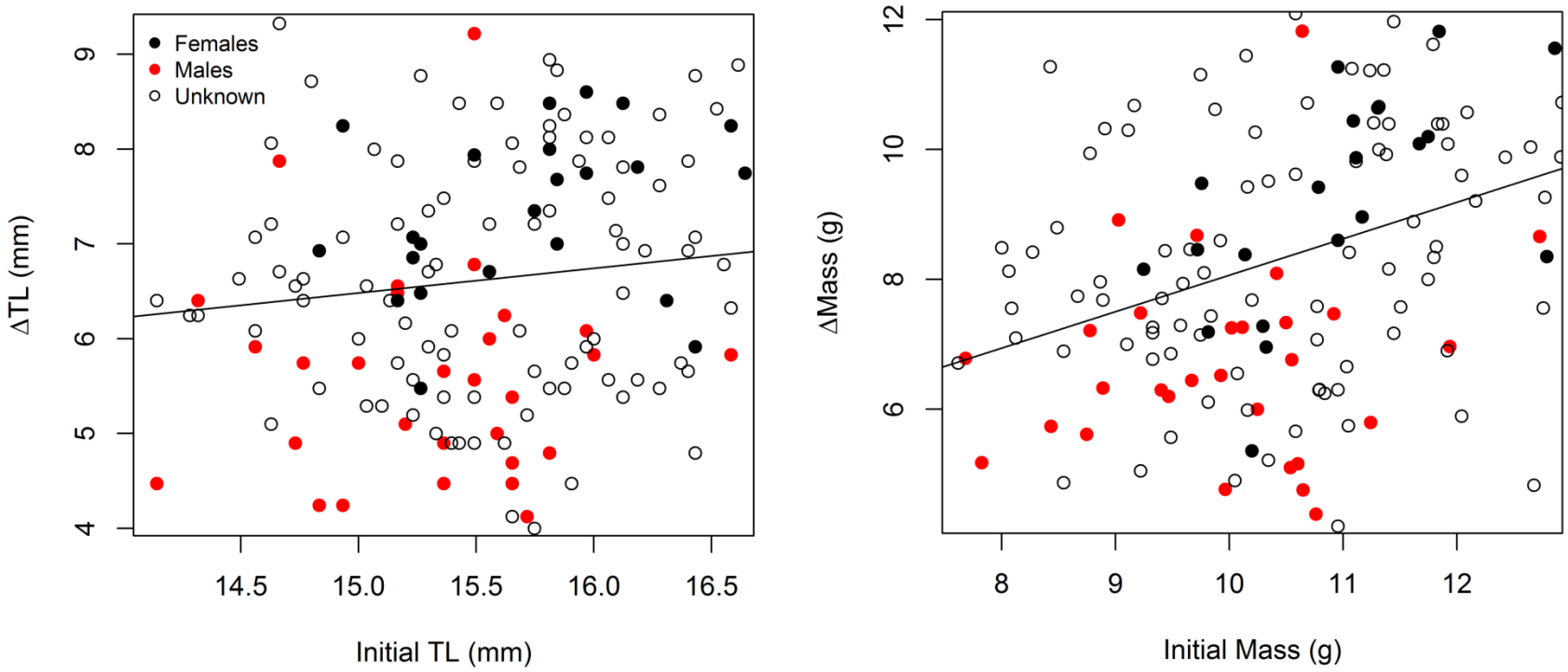

Figure 6.—Sex data for the control group plotted on the 2013 total length and mass data plots from figure 4.
Females are displayed in solid black circles, males in solid red circles, and unknown sexes in open circles.

Figure 7.—Water quality data results showing 2013 data.
The red line shows mean temperature data (degrees Celsius) with standard error bars for all ponds. The squares below the temperature data show individuals (A–H) that operated the water quality meter. Mean percent dissolved oxygen with standard error bars for each treatment is reported below operator data. Control ponds are reported in yellow, the 50 Colorado Pikeminnow (50 CPM) treatment is reported in green, and the 100 Colorado Pikeminnow treatment (100 CPM) is reported in blue.

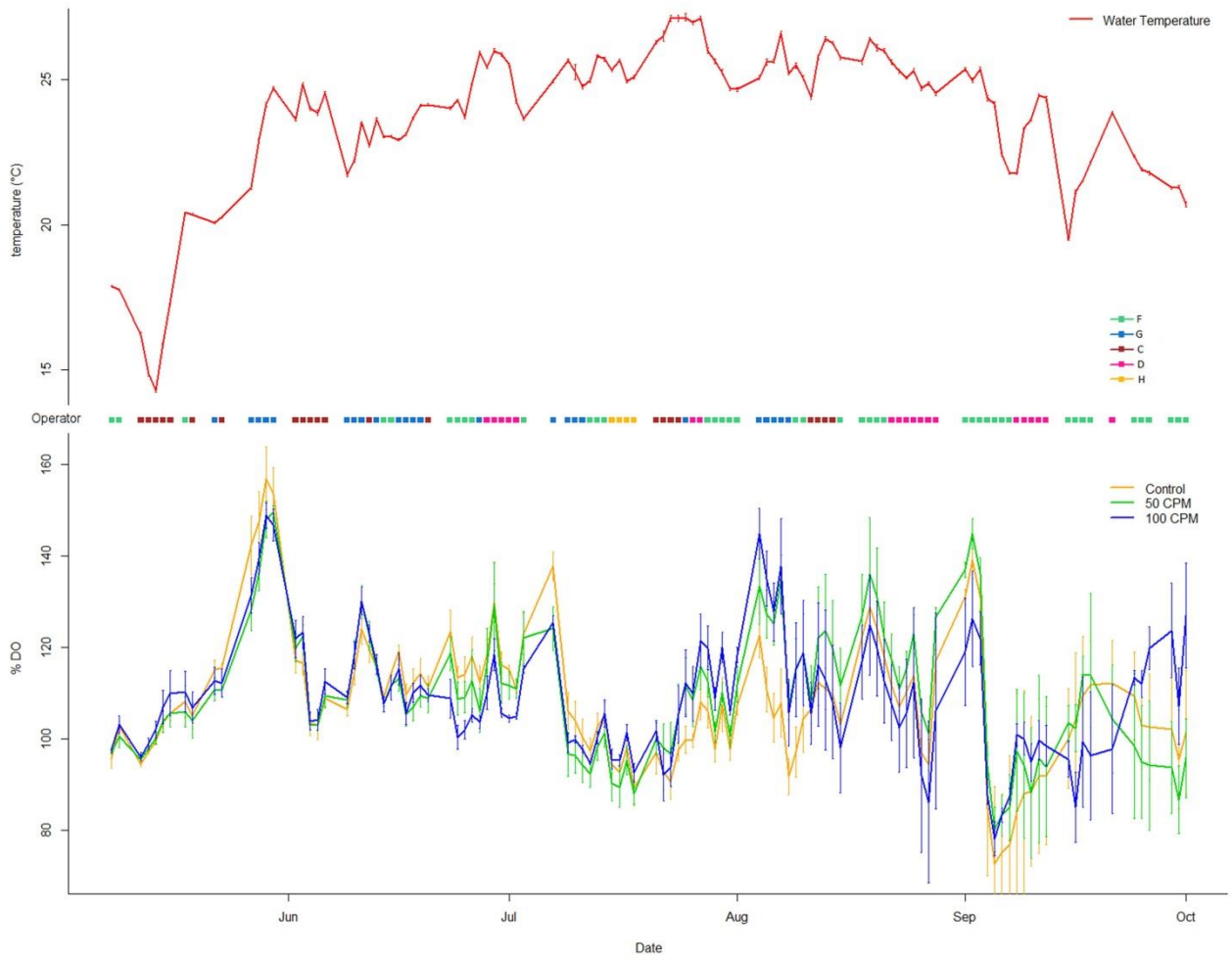

Figure 8.—Water quality data results showing 2014 data.
The plot shows the mean percent dissolved oxygen for each treatment group with standard error bars. The control group is shown in yellow, the 50 Colorado pikeminnow (50 CPM) treatment is in green, and the 100 Colorado pikeminnow (100 CPM) treatment is in blue. Mean percent dissolved oxygen with standard error bars for each treatment is reported below operator data. Control ponds are reported in yellow, the 50 Colorado Pikeminnow (50 CPM) treatment is reported in green, and the 100 Colorado Pikeminnow treatment (100 CPM) is reported in blue.

and among treatment groups. As time progressed, ponds gradually deviated from the starting values when ponds were filled. During September 2014, 5.4 inches of rain were recorded on station, which could have affected water chemistry, leading to the decrease in percent saturation values observed toward the end of the experiment. Other deviations and variation of percent saturation is difficult to explain.

DISCUSSION

Biological Control of Bonytail Recruitment

Bonytail recruitment in wild populations is extremely low if not non-existent (Marsh 2004; USFWS 2004a); however, in hatchery ponds, they are precocious, with bonytail reproducing as early as Age-2 (Hamman 1982, 1985). Contradictory to potential positive outcomes associated with natural recruitment in a hatchery system (i.e., increased production), recruitment can have negative impacts on various factors related to overcrowding (Wedemeyer 2001; Tatara et al. 2009; Kavanagh and Olson 2014). Integrating fish as biological controls can effectively control biota, including non-native species (Chaichana and Jongphadungkiet 2012; Lemmens et al. 2015) and pathogen-transmitting vectors (i.e., mosquitos) (Frenkel and Goren 2000) in hatchery settings.

The results from this study showed a strong negative effect of Colorado pikeminnow on the abundance of bonytail recruitment. The number of bonytail recruits harvested from each pond was significantly reduced in ponds with Colorado pikeminnow (range = 3–63) compared to ponds without Colorado pikeminnow (2,854–6,969); however, there was no difference in the final number of recruits between the two levels of the Colorado pikeminnow *s* treatment, suggesting densities as low as 50 Colorado pikeminnow per 0.4 ha can effectively control unwanted recruitment of bonytail. Although a significant interaction for total biomass was found between treatment and year, results (see table 1; figure 3) show Colorado pikeminnow had an impact on total biomass by effectively controlling unwanted recruitment. In the wild, Colorado pikeminnow is highly piscivorous after reaching 200 mm TL (Vanicek and Kramer 1969), which likely contributed to the high predation levels observed here. Other investigations may be warranted to assess the effectiveness of other predators in limiting unwanted bonytail recruitment.

The total biomass of recruitment in control treatments differed between the two years, which could be attributed to increased numbers and larger recruits harvested in 2014. In 2013, the mean number of bonytail recruitment harvested from the control group was 3,752, with a mean mass of 3.19 g, whereas in 2014, there were 5,216 recruits that averaged 4.51 g. The 2013 adult bonytail (2009 YC) had been graded once prior to the experiment to meet stocking quotas during

that year, and the larger fish meeting the 300 mm TL target length were stocked into Reach 3 of the lower Colorado River. Thus, adults used in the 2013 experiment were likely the slower-growing fish from the 2009 cohort, whereas the 2014 adults (2012 YC) were not graded prior to the experiment and were likely more representative of normal broodstock populations at the SNARRC. Therefore, we cannot rule out the possibility of a genetic component to the slower growth observed in 2013. Further work may be needed to assess the effects of adult size and growth rates on recruitment abundance and mass.

Fish reared in high densities can have higher mortality rates and increased risk of disease compared to fish reared in lower densities (Wedemeyer 2001; Tatara et al. 2009; Kavanagh and Olson 2014); however, our results showed no effect of Colorado pikeminnow on survivorship of adult bonytail, with high survival rates observed in all treatments (see table 1). Survival rates of adult bonytail used in this study were similar for broodstock and production fish of this age range and density (Hamman and Ulibarri 2005). Conversely, Colorado pikeminnow had higher mortality rates than adult bonytail in both years; however, these rates were within the normal range observed for this size class of Colorado pikeminnow production fish (Hamman and Ulibarri 2006, 2008). It was initially proposed (study plan) that inadvertent recruitment can lead to overcrowding in ponds with high densities that may foster poorer water quality, cause oxygen depletions, and increase the susceptibility of fish to disease. Daily water quality values collected during both years varied immensely within years and between treatments, suggesting there is no detectible difference in water quality measures between treatments and controls; however, it is important to consider differences in environmental variables between facilities with bonytail when maintaining either production or broodstock fish.

High-density environments can greatly reduce the growth of fish (Ward and Slaney 1988; Ward et al. 1989; Wedemeyer 2001; Kavahagh and Olson 2014). The presence of Colorado pikeminnow had a significant effect on average recruitment mass: individuals harvested from ponds with Colorado pikeminnow were larger than those harvested without. There was no difference in mean recruitment mass (g) between Colorado pikeminnow treatments, indicating the effect on bonytail recruitment mass was the same for both treatments. Thus, recruits in ponds treated with the biological controls likely increased their growth rates due to the lower densities of potential competitors (i.e., other recruits). In addition, we observed differences in the mean mass of Colorado pikeminnow between the two treatment groups. The treatment groups containing 50 Colorado pikeminnow were significantly heavier than those in the 100 treatment group, suggesting increased competition for Colorado pikeminnow in ponds with higher predator densities. The results from this study indicate bonytail recruitment can be effectively controlled with as few as 50 Colorado pikeminnow, with no difference in total recruitment and increased mass in both bonytail recruits and Colorado pikeminnow.

Growth Rate

Post-stocking survival of bonytail is negligible (Pacey and Marsh 2008; Bestgen et al. 2008; Portz 2009), with studies showing extremely high post-stocking mortality on bonytail ≤ 225 mm TL. Low survivorship is likely attributed to predation from non-native species (Bestgen et al. 2008; Pacey and Marsh 2008). Management practices for the Lower Colorado Multi-Species Conservation Program require a target stocking size of 300 mm TL to reduce predation (Bureau of Reclamation 2006; Portz 2009; Kappenman et al. 2012); however, grow-out to this size can take several years. Therefore, understanding and implementing aquaculture practices that maximize growth at hatchery facilities will likely be important to the management and recovery of bonytail.

Several studies have assessed propagation techniques (i.e. ,cage culture, diet improvement, poly-culture, and thermal regimes) to facilitate growth of bonytail (Sowka and Brunknow 1999; Henne et al. 2006, 2007; Kappenman et al. 2012). Managing fish in high-density environments can greatly reduce growth and the condition of fish (Ward and Slaney 1988; Ward et al. 1989; Wedemeyer 2001; Kavahagh and Olson 2014). Using biological controls can greatly reduce high densities while controlling unwanted biota. Results from this study show Colorado pikeminnow can be effective at reducing total biomass in bonytail production ponds by decreasing bonytail recruitment. Although significant interactions were found in several models tested and effects varied between years, there appears to be a generally positive effect of Colorado pikeminnow on adult bonytail growth (ΔTL, Δg, % BW, and SGR). However, these positive effects were most evident in individuals that started at smaller sizes compared to larger individuals. Using Colorado pikeminnow to control unwanted recruitment could increase the growth rates of at least smaller adult bonytail, which could aid in management goals.

A difference in results between the two years was likely due to differences in sample sizes (2013 N = 20; 2014 N = 40) and the addition of sex as a predictor variable in 2014. Sex data collected on the control group in 2013 (see section 2) revealed a difference in both length and weight between females and males (see figure 6). Consequently, sex data were collected for all individuals used in 2014. Sex had significant effects in all 2014 models that did not have a significant interaction, and sex also had stronger effects than treatment in all models. A significant effect was detected for %BW in 2013, but this effect varied by starting mass of fish. Smaller sample sizes collected in 2013 (N = 20) and the absence of sex as a predictor variable likely limited our ability to detect effects of Colorado pikeminnow in 2013. Initial size had the strongest effect in nearly all models, indicating the starting sizes of fish had strong effects on their growth over time: fish that started small tended to finish small and vice versa. The significant

effects of initial size (TL or g) and sex on growth of fish observed here may warrant further investigation regarding bias in stocking of sexes into the Lower Colorado River Basin and the effect of selecting for faster growing individuals.

Water quality measures were within an acceptable range for culture practices at the SNARRC (Knight and Ulibarri 2013) and for maximizing bonytail growth. Water temperature was similar between years, with little variation among ponds. At the start of the experiment, temperatures were relatively low, but by early to mid-May, temperatures rose to 18–20 degrees Celsius (°C), reaching the preferred range of bonytail for spawning (Marsh 2004; Kappenman et al. 2012). Indeed, ideal spawning temperatures corresponded to dates when larval fish were first observed in the experimental ponds. Water temperature continued to rise from mid-May to early June and by mid-June stabilized between 22–27 °C for the rest of the experiment. Temperature experiments of bonytail has shown the ideal temperature range for increased growth and condition to be between 22–26 °C (Kappenman et al. 2012). Experimental fish in both years were subjected to temperatures that maximized their growth for the majority of the experiment. Dissolved oxygen also varied somewhat unpredictably among ponds in both years, but this variation was seemingly unrelated to Colorado pikeminnow treatments.

Section 2 – Genetic Assessment of Natural Recruitment

METHODS

Experimental Fish

See section 1, "Experimental Fish."

Tissue Collection

A small portion of the caudal fin was clipped from all adults prior to stocking the experimental ponds (controls). During harvest, all recruits were also fin clipped. All fin clips were stored in 95% ethanol until DNA extraction. In addition, fin clips were collected from young-of-the-year recruitment from the Achii Hanyo Native Fish Rearing Facility (Achii Hanyo NFRF), SNARRC, and Uvalde National Fish Hatchery (Uvalde NFH) (table 3).

Molecular Methods

Multilocus genotypes were generated using 15 microsatellite loci (table 4). Genomic DNA was extracted from fin clips using Qiagen DNeasy® 96 Blood and Tissue Kits following the manufacturer's instructions, after which samples were stored at -80 °C. Multiplex (3–4 microsatellite primer pairs) polymerase chain reaction amplifications (10 microliters) consisted of 4.0 µL Qiagen Multiplex Mastermix®; 0.375 µL each, forward and reverse primers; 2.0 µL ddH20; and 1 µL DNA. Forward primers were labeled with one of four fluorescent dyes (6-FAM, PET, NED, VIC) (Applied Biosystems, Inc.). Amplification for all samples consisted of a touchdown protocol performed in an ABI 9700 GeneScanTM thermal-cycler. The thermal profile included a denaturing step of 95 °C for 15 minutes (to activate the HotstarTaq polymerase), followed by 33 cycles of 94 °C for 45 seconds, an initial annealing temperature of 56 °C for 45 seconds, and an extension temperature of 72 °C for 60 seconds. The annealing temperature decreased by 0.2 °C each cycle. The final extension cycle was 30 minutes at 70 °C. Polymerase chain reaction products were processed on an ABI 3130xl genetic analyzer using a GeneScanTM 500 LIZ® size standard. Composite genotypes for individual fish were compiled with GeneMapperTM 4.0 software (Applied Biosystems, Inc.).

Table 3.—Collection information and genetic descriptive statistics of natural recruits (offspring) and parental bonytail using 15 microsatellite loci (DX = SNARRC [Dexter], N_A = number of alleles, A_R = allelic richness, H_O = observed heterozygosity, H_E = expected heterozygosity, and F_{IS} = inbreeding coefficient)

Facility	Pond	Sample number	DX sample name	Parent/recruit	Year class	N_A	A_R	H_O	H_E	F_{IS}
Achii Hanyo NFRF	Raceway	100	Gele09AH	Recruit	2009	9.80	8.91	0.803	0.779	-0.029
	1	100	Gele10AH	Recruit	2010	10.87	10.17	0.833	0.806	-0.034
	2	100	Gele11AH	Recruit	2011	10.40	9.87	0.812	0.800	-0.014
	1	100	Gele1AH10	Recruit	2009	10.93	10.20	0.808	0.810	0.004
	2	100	Gele2AH10	Recruit	2010	9.93	9.18	0.797	0.790	-0.008
	3	100	Gele3AH10	Recruit	2010	10.93	10.17	0.831	0.814	-0.021
	C	100	GeleCAH10	Recruit	2010	9.93	9.30	0.794	0.784	-0.013
Uvalde NFH	13	50	Gele13UV10	Recruit	2010	8.27	8.10	0.747	0.732	-0.026
	14	50	Gele14UV10	Recruit	2010	7.93	7.82	0.762	0.731	-0.047
	23	50	Gele23UV10	Recruit	2010	8.17	7.99	0.783	0.758	-0.039
	24	50	Gele24UV10	Recruit	2010	8.61	8.40	0.765	0.721	-0.064
SNARRC	10D	100	GeleDX09recruit	Parent females	2009	10.60	9.93	0.828	0.813	-0.017
		100	GeleDX09recruit	Parent males	2009	10.53	9.97	0.812	0.799	-0.019
		100	GeleDX13recruit	Recruit	2013	10.27	9.53	0.811	0.798	-0.017
	17D	100	GeleDX09recruit	Parent females	2009	10.13	9.71	0.799	0.812	0.015
		100	GeleDX09recruit	Parent males	2009	10.60	9.78	0.815	0.785	-0.043
		100	GeleDX13recruit	Recruit	2013	10.27	9.70	0.819	0.799	-0.021

Table 3.—Collection information and genetic descriptive statistics of natural recruits (offspring) and parental bonytail using 15 microsatellite loci (DX = SNARRC [Dexter], NA = number of alleles, AR = allelic richness, HO = observed heterozygosity, HE = expected heterozygosity, and FIS = inbreeding coefficient)

Facility	Pond	Sample number	DX sample name	Parent/recruit	Year class	N_A	A_R	H_O	H_E	F_{IS}
SNARRC (continued)	21D	100	GeleDX09recruit	Parent females	2009	10.53	9.92	0.824	0.815	-0.012
		100	GeleDX09recruit	Parent males	2009	10.67	9.71	0.793	0.781	-0.019
		99	GeleDX13recruit	Recruit	2013	10.60	9.85	0.811	0.808	-0.004
	9D	100	GeleDX12recruit	Parent females	2012	10.20	9.61	0.829	0.814	-0.015
		100	GeleDX12recruit	Parent males	2012	10.13	9.56	0.882	0.791	0.011
		100	GeleDX14recruit	Recruit	2014	10.13	9.35	0.808	0.809	0.005
	16D	100	GeleDX12recruit	Parent females	2012	10.27	9.69	0.814	0.807	-0.007
		100	GeleDX12recruit	Parent males	2012	10.13	9.41	0.806	0.796	-0.014
		100	GeleDX14recruit	Recruit	2014	9.80	9.35	0.782	0.810	0.038
	23D	100	GeleDX12recruit	Parent females	2012	10.07	9.46	0.797	0.806	0.016
		100	GeleDX12recruit	Parent males	2012	10.53	9.77	0.812	0.811	-0.001
		100	GeleDX14recruit	Recruit	2014	9.73	9.19	0.806	0.809	0.008
	3D	100	GeleDX12c	Recruit	2012	9.93	9.34	0.781	0.788	0.007
	4B	100	GeleDX12c	Recruit	2012	10.40	9.52	0.790	0.792	0.001
	3B	90	GeleDX12c	Recruit	2012	10.40	9.63	0.793	0.790	-0.003
Total		**2,913**								

Table 4.—Information of the 15 microsatellite loci used to genotype adult and recruitment bonytail

Primer name	Sequence information	Reference	Reference species
*Gbi*G39F	GAGCGGGTGGATTTTTACTATTAT	Meredith and May (2002)	Tui chub (*Gila bicolor obesa*)
*Gbi*G39R	ATTCATTATCCGGGGTCTCAT		
*Ca*12F	GTGAAGCATGGCATAGCACA	Dimsoski et al. (2000)	Central stoneroller (*Campostoma anomalum*)
*Ca*12R	CAGGAAAGTGCCAGCATACAC		
*Gel*227F	TTGCACATGAACTTACATAGAGG	Keeler-Foster et al. (2004)	Bonytail/humpack chub
*Gel*227R	ACCGTAGATAAAAACAATACAACG		
*Gel*225F	CCTGTGGATCAAAAAGTAGATG	Keeler-Foster et al. (2004)	Bonytail/humpack chub
*Gel*225R	CGATTCCCACACAGTAAGAAC		
*Gel*234F	GCCAATTAACGGTTTTGC	Keeler-Foster et al. (2004)	Bonytail/humpack chub
*Gel*234R	CCATTAATTCTGACCACATCTG		
*Gel*300F	GTTACAGAGGCCATAGTCCG	Keeler-Foster et al. (2004)	Bonytail/humpack chub
*Gel*300R	AGTTCAAGAAGACAAAATATGTGTG		
*Cyp*G26F	TTGGTTAGACACGATTGATGT	Baerwald and May (2004)	Cyprinid
*Cyp*G26R	TCCAAGTCTAAAGTATACAACAGTC		
*Cyp*G5F	AGTTGATGCCTGTTTTGTTTGTAT	Baerwald and May (2004)	Cyprinid
*Cyp*G5R	AGTTGTGTCAGGGTCTGTAGAA		
*Cyp*G22F	AGTCAGTCAGTCCACAAGGGTATCT	Baerwald and May (2004)	Cyprinid
*Cyp*G22R	TTGGTGGGAATTATTAACAGAGTTC		

Table 4.—Information of the 15 microsatellite loci used to genotype adult and recruitment bonytail

Primer name	Sequence information	Reference	Reference species
*Gel*228F	TGTGAGATGGTTGTGCAAAG	Keeler-Foster et al. 2004	Bonytail/humpack chub
*Gel*228R	TTTTAATTGGCCACGACACA		
*Gel*223F	CATAACTGATTTTTTTAATTAAGCTTG	Keeler-Foster et al. (2004)	Bonytail/humpack chub
*Gel*223R	GTTACTGTAGTGGTTGAGGGAAC		
*Cyp*G3F	AGTAGGTTTCCCAGCATCATTGT	Baerwald and May (2004)	Cyprinid
*Cyp*G3R	GACTGGACGCCTCTACTTTCATA		
*Gbi*G13F	TCAAAAGATAAACAATAGAAACC	Meredith and May (2002)	Tui chub
*Gbi*G13R	CTGTCAGAAATTGTCAAGTGTAAC		
*Gel*287F	CTACAACACGGGACAGGTATC		Bonytail/humpack chub
*Gel*287R	TGAAGACTGTCAGATGTGCC		
*Ca*8F	ACGCAGACATATTTTAGATG	Dimsoski et al. (2000)	Central stoneroller
*Ca*8R	AATAATACAACTCGCTCTCA		

Quality Control/Quality Assurance

Accuracy in genotyping was ensured by a quality control/quality assurance process consisting of two actions: (1) rescoring of all genotypes by a second researcher and (2) independently re-extracting, amplifying, and scoring 10% of the samples. Genotypes produced by the different researchers were compared, and all inconsistencies were resolved; thus, no samples were removed prior to analyses.

Data Analysis

Departures from the Hardy-Weinberg (HW) equilibrium and global tests of linkage equilibrium among all pairs of loci and populations were tested using GENEPOP v4.0 (Raymond and Rousset 1995; Rousset 2008). The test for HW equilibrium used the heterozygote deficiency method (Raymond and Rousset 1995), a global test that examines either the population(s) or locus but not both simultaneously. The test of linkage disequilibrium examines the relationship between genotypes at each pair of loci (i.e., composite linkage disequilibrium) (Weir 1996). Expected heterozygosity (H_E), observed heterozygosity (H_O) on a per locus basis, the number of alleles per locus (N_A), average inbreeding coefficients (F_{IS}), and allele frequencies were estimated using GenAlEx v6 (Peakall and Smouse 2006, 2012). Allelic richness (A_R) was estimated using FSTAT v2.9.3.1 (Goudet 1995) using the methods described by Petit et al. (1998) and a rarefaction and repeated random subsampling method (Leberg 2002). Multiple tests were accounted for by using a Bonferroni correction (Rice 1989).

Colony v2.0.5.9 (Jones and Wang 2009) was used for assignment of parents to estimate the number of offspring per individual, the number of mates per individual, and the inbreeding effective size using the sibship assignment method ($N_{e(\mathrm{SA})}$). Parameter settings used included: male and female polygamy, with inbreeding, medium length of run, full likelihood, medium precision, update allele frequency, and no prior. Marker settings included: allele frequency unknown. Four independent runs with different seeds were completed for each sample.

RESULTS

A total of 2,913 individuals were sampled and genotyped (see table 3) using 15 microsatellite loci. Of the 15 loci used, all samples were in HW equilibrium. Two additional loci (*Gbig*87 and *Gel*257) were included in initial genotyping but were removed due to HW disequilibrium. Linkage equilibrium varied across samples with two loci (*Gel*226 and *Gel*287) consistently in linkage disequilibrium across parents and recruitment, so *Gel*226 was removed. Linkage disequilibrium

in the parental samples was common but was resolved within the recruitment populations and thus not related to markers. This is likely due to non-random mating in the parents with random mating in the experimental ponds being allowed and restoring linkage equilibrium.

Allelic richness (A_R) was lowest (ANOVA/Tukey's HSD, $P < 0.0001$) in the recruitment from the Uvalde NFH ($\mu = 8.25$) compared to non-experimental recruitment at the Achii Hanyo NFRF ($\mu = 10.40$) or the SNARRC ($\mu = 10.24$) (see table 3). In the experimental samples, there was not a significant difference in the mean number of alleles per locus (equal sample sizes) when comparing replicates (year one) or parents (2009 YC) versus recruitment (2013 YC). In year two, there was not a difference in the mean number of alleles between replicates; however, there was a significant difference ($P = 0.03$) between parents (2012 YC, $\mu = 10.22$) and recruits (2014 YC, $\mu = 9.89$). The total number of alleles in the experimental samples ranged from 174 (23DO) to 192 (21DM and 10DM). The total number of alleles between males versus females within the 2009 YC or 2012 YC or parents (2009) versus recruitment (2013) did not differ. There was a difference in parents (2012, $\mu = 182.8$) versus recruitment (2014, $\mu = 178.5$, $P < 0.05$), which was driven by the 23D replicate. The comparison was not significant ($P = 0.44$) after removal of these samples (23D). Total number of alleles in the recruitment at the Achii Hanyo NFRF was lower ($\mu = 157$) than the 2013 and 2014 recruitment at the SNARRC ($\mu = 157$, $P < 0.001$) but not lower than the 2012 non-experimental recruitment at the SNARRC ($\mu = 154.7$). Overall, allele frequencies ranged from 0.005 to 0.690, with an overall mean of approximately 0.09 for the experimental samples (attachment 1) and the Achii Hanyo NFRF samples; allele frequencies ranged from 0.01 to 0.810 for the Uvalde NFH samples.

In the experimental samples, the total number of spawners by gender (table 5) ranged from 38 (16D females and 23D males) to 56 (10D males). The number of spawners did not differ between replicates for either the 2009 YC or 2012 YC and did not differ between genders. There were more spawners in the 2009 YC ($\mu = 52.17$) versus 2012 ($\mu = 45.5$, $P = 0.032$). The number of offspring per individual ranged from 1.79 (10D males, 2009 YC) to 2.52 (16D females and 23D males, 2012 YC) with the 2012 YC having more offspring per individual than the 2009 YC ($\mu = 2.16$ versus $\mu = 1.88$ respectively, $P = 0.036$); all other comparisons were non-significant (replicates and gender). The number of mates per individual ranged from 1.57 (10D males, 2009 YC) to 2.18 (23D males, 2012 YC); thus, there was a correlation between the number of offspring per individual and the number of mates per individual ($r = 0.958$, $P < 0.00001$), and is significant for both genders, but stronger for females ($r = 0.980$, $P = 0.0006$) than males ($r = 0.942$, $P = 0.005$). Effective size ranged from 80 (16D, 2012 YC) to 132 (17D, 2009 YC) and did not differ between the 2009 YC and 2012 YC.

Table 5.—The total number of spawners, number of offspring, number of mates, and effective size with 95% confidence interval by experimental control pond (replicate) and or gender at the SNARRC

(Each control pond contained 100 adult bonytail males and 100 adult females. The number of recruitment sampled in each pond was 100.)

Parent year class	Pond	Gender	Total number of spawners	Mean number of offspring/individuals (maximum)	Mean number of mates/individuals (maximum)	Effective size $N_{e(SA)}$ (95% confidence interval)
2009	10D	Male	56	1.79 (5)	1.57 (4)	
	10D	Female	50	1.92 (7)	1.72 (5)	
	10D					111 (83–150)
	17D	Male	54	1.83 (5)	1.61 (4)	
	17D	Female	49	1.92 (5)	1.80 (4)	
	17D					132 (99–175)
	21D	Male	52	1.90 (6)	1.71 (5)	
	21D	Female	52	1.90 (6)	1.71 (5)	
	21D					110 (84–148)
2012	9D	Male	52	2.00 (9)	1.73 (6)	
	9D	Female	49	2.00 (6)	1.80 (5)	
	9D					103 (77–141)
	16D	Male	49	2.04 (7)	2.04 (7)	
	16D	Female	38	2.52 (9)	2.05 (6)	
	16D					80 (57–112)
	23D	Male	38	2.52 (7)	2.18 (5)	
	23D	Female	47	1.89 (5)	1.66 (4)	
	23D					92 (67–128)

DISCUSSION

Bonytail is an endangered species that only persists due to continued augmentation with captively propagated individuals; thus, maintaining genetic diversity in both the captive broodstocks and production fish is a high priority (USFWS 2004b). The natural reproductive ecology of the bonytail is not well known. Vanicek (1969) reported that Colorado chub, including roundtail (*G. robusta*) and bonytail – Vanicek did not specify species/subspecies in reproductive findings) in the Green River were found to be ripe at Age-5–7. Hamman (1985) reported captive bonytail were reproductive at Age-2. In an effort to better understand natural recruitment and reproduction in captive populations and how genetic diversity varies in recruitment, the genetic component of this study used microsatellites to examine genetic diversity of recruitment at three facilities (Achii Hanyo NFRF, Uvalde NFH, and SNARRC) and used parentage assignment methods at one facility (SNARRC). The results of this study revealed: (1) genetic diversity in natural recruitment, as measured by total number of alleles and allelic richness, varies with some samples having lower diversity (Achii Hanyo NFRF, Uvalde NFH, SNARRC) and other samples being similar to the parental population (SNARRC experimental samples); (2) low frequency alleles can be maintained in recruitment; (3) bonytail is polygamous, and each gender spawns with multiple mates; (4) recruits were not the product of a few spawning parents or pairs, with a minimum of 38%/gender/pond spawning in the experimental samples.

The results of this section combined with section 1 suggest that although recruitment can be similar to the parental population in terms of genetic diversity as seen in the experimental samples, the demographics and genetic diversity of the parental population are key. The management and history of the parental stocks of the non-experimental captive populations is largely unknown; however, section 1 suggested that females were larger than males, and with current management action of selecting by size (grading) and stocking large individuals (size $\geq$ 300 mm), gender ratios may be skewed toward males after grading. If a captive population that has been graded several times produces recruitment, it is likely that few parental females were available to maintain genetic diversity. An additional study is being conducted at the SNARRC to further examine the relationship of size and gender in production populations of bonytail. If the parental population is not skewed in terms of gender ratios and is moderately large demographically, as was the case of the experimental control ponds, then genetic diversity can be maintained and genetic drift (change in allele frequency over time) minimized. The loss of an allele most often occurred when present in a single potential parent (e.g., female but not male) but did occur rarely when two potential parents (one male and one female) had the allele.

The moderately larger percentage of spawning parents found in this study is an underestimate of the number of spawners, number of offspring/individuals, and

number offspring/individuals due to sampling; that is, not all recruits (see table 1) were genotyped. Given the low number of offspring/individuals and mates/individuals, it would be expected that more individuals contributed, but their recruitment was not sampled. Studies that examine genetic diversity of captive parental populations and natural recruitment are rare, with spawning often taking place in culture tanks and not in naturalized conditions (Hoskin et al. 2015).

In conclusion, unless the parental population is large, has not been graded, and is the product of at least 20 successful paired spawns (USFWS 2004b), recruitment should not be used for production or recovery purposes. That is, unless the population is evaluated for genetic diversity and compared to data of diverse populations such as the broodstock at the SNARRC or other production populations that have been evaluated for genetic diversity. Production of genetically diverse bonytail from natural recruitment using the stable conditions similar to those used in the experimental portion of this study is an option when few individuals (< 5,000) are needed. It is not an option to produce large numbers similar to current SNARRC bonytail production commitments (> 40,000) due to space and other restrictions. Results may change as environmental and demographic conditions change, and these results cannot be applied to other systems.

ACKNOWLEDGEMENTS

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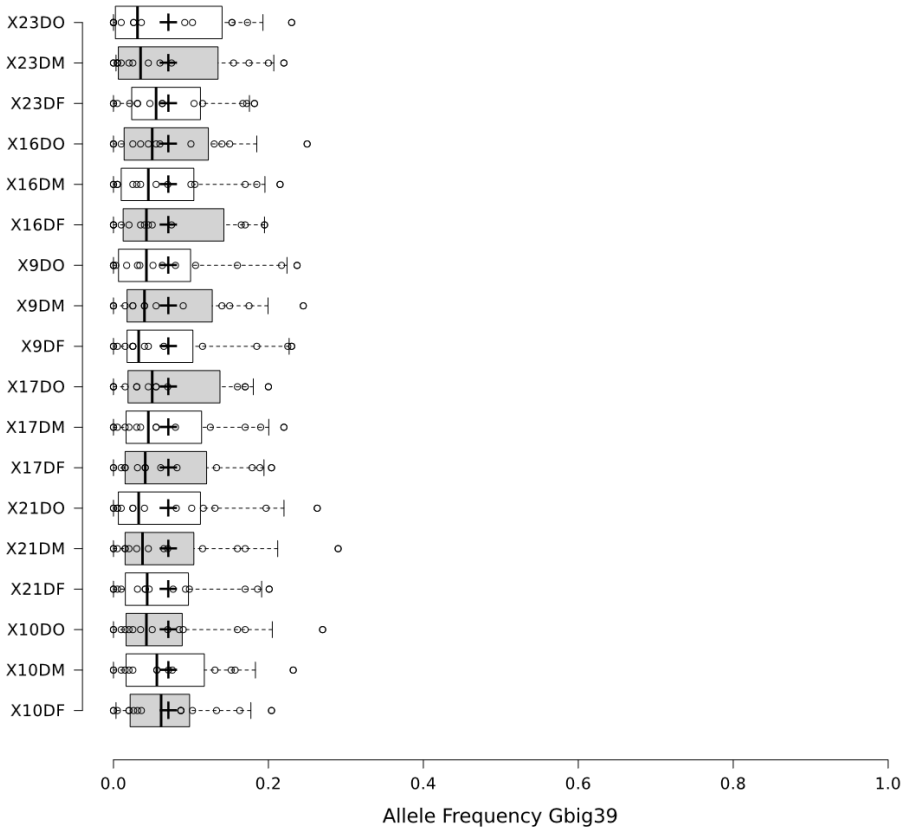
ATTACHMENT 1

Box Plots Showing Allele Frequencies (x axis) in Each of the Experimental Ponds (y axis) by Microsatellite Locus (15 Loci)

Center lines show the medians, box limits indicate the 25th and 75th percentiles as determined by R software, whiskers extend to 5th and 95th percentiles, sample values are represented by dots, and crosses represent sample means.

(Sample name format: X-Pond Number and Series-Parental Gender [F = Female, M = Male] or Recruitment [O]).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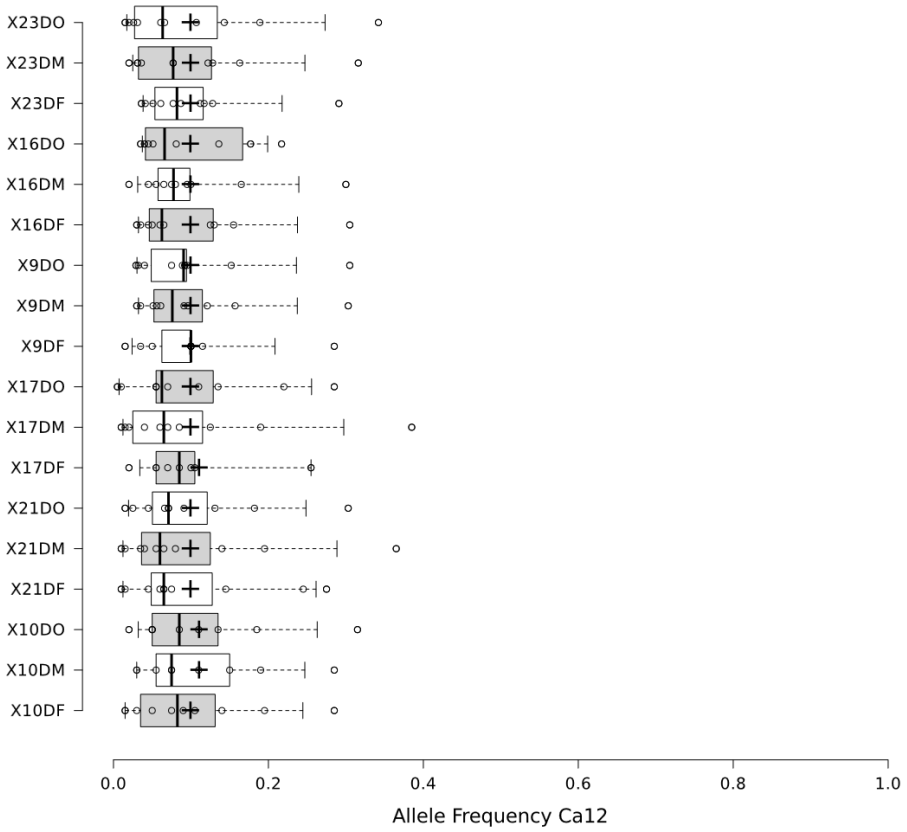
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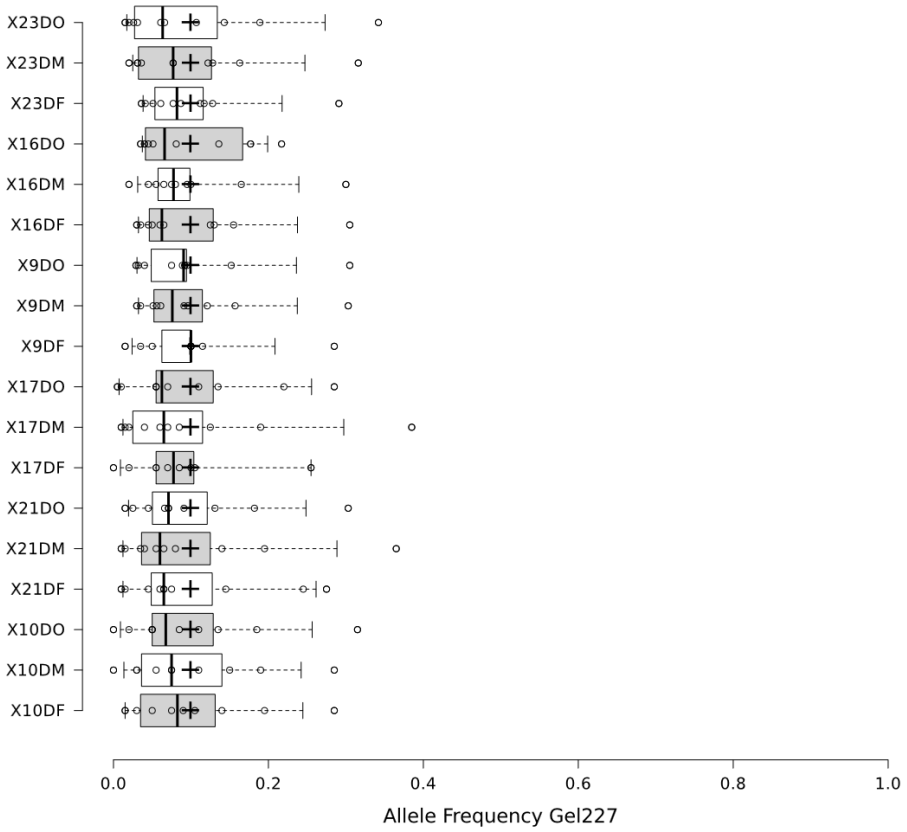
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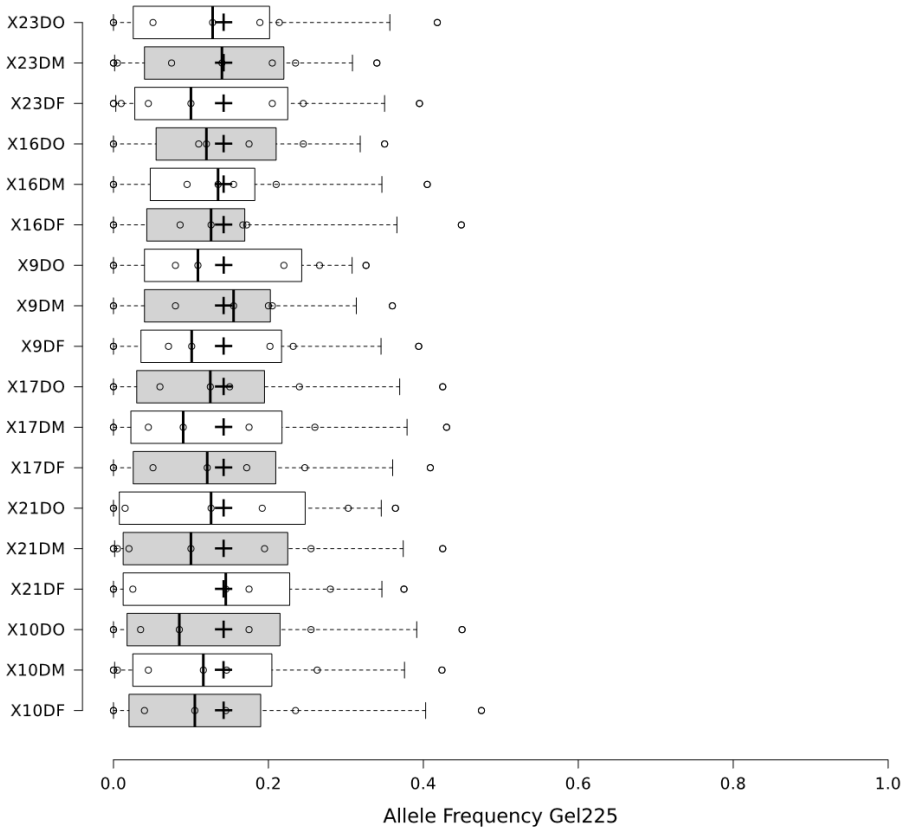
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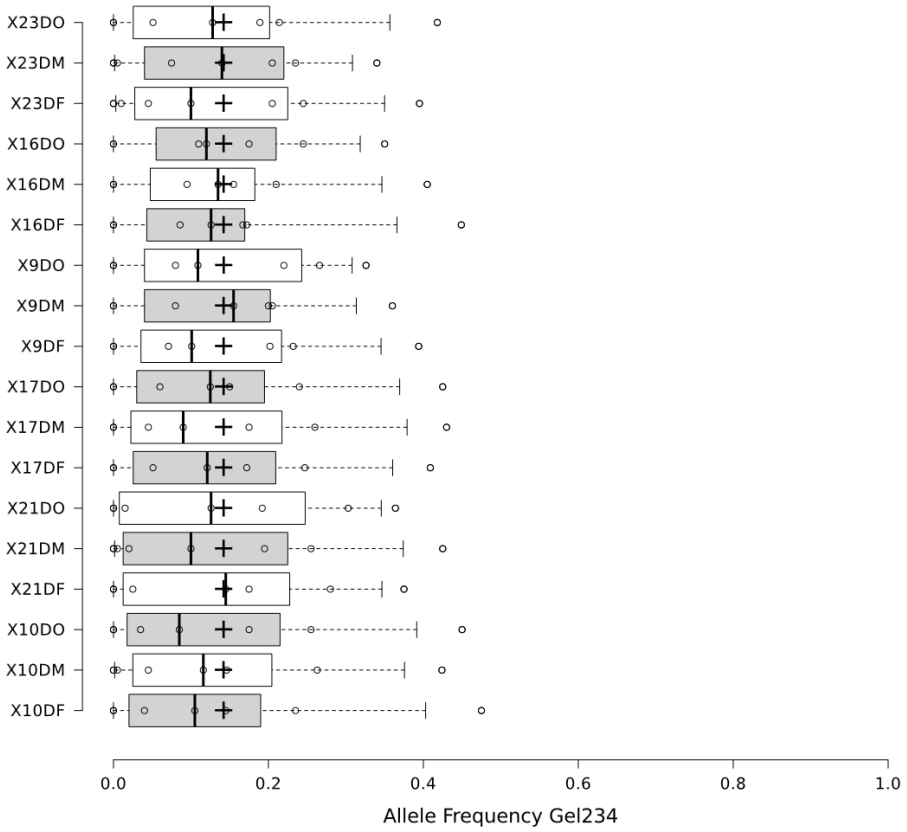
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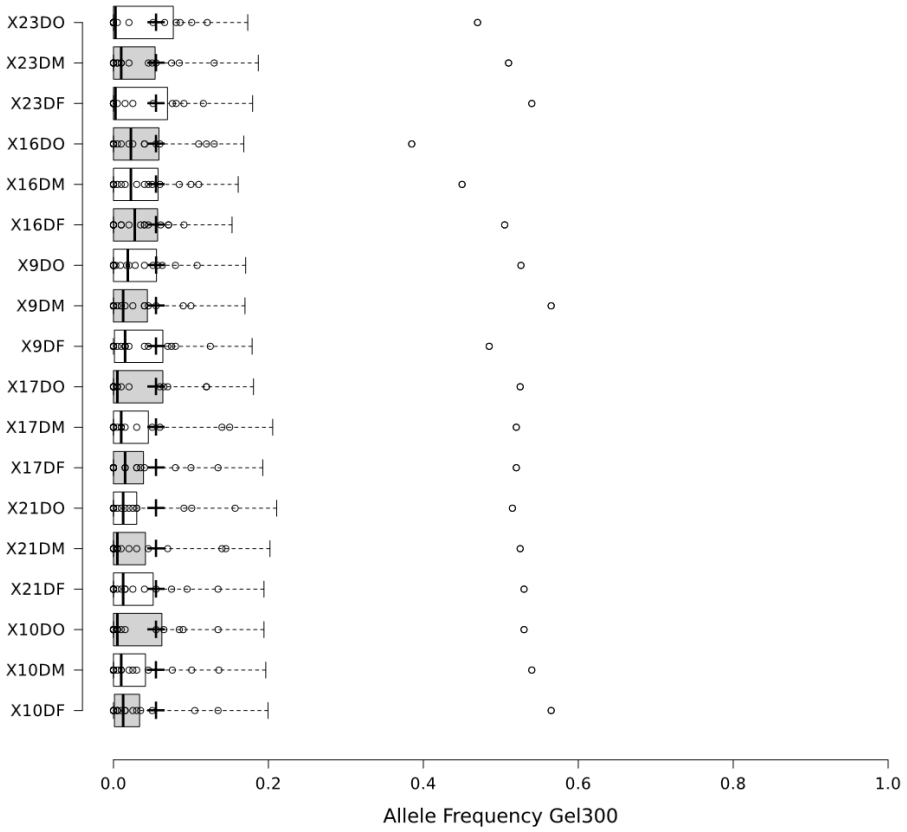
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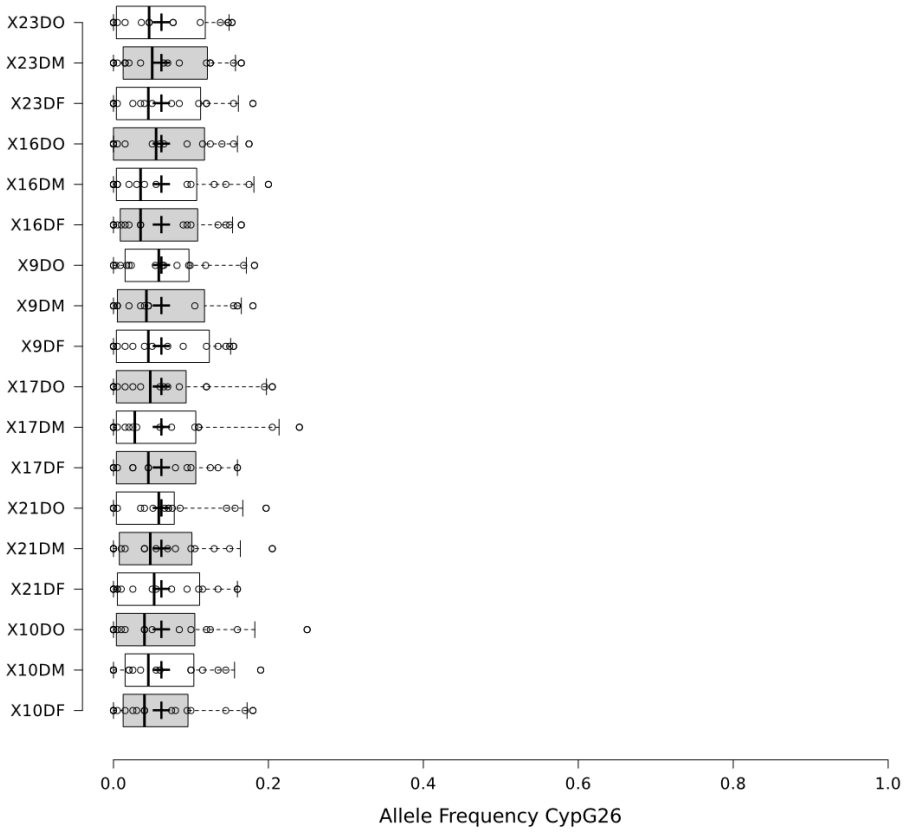
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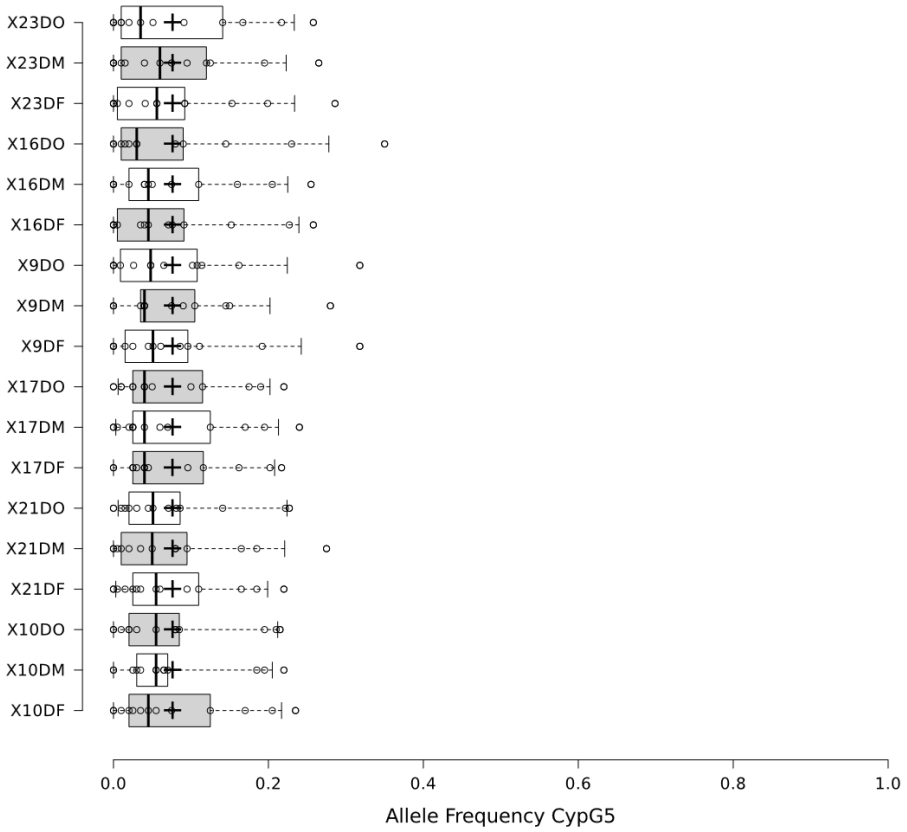
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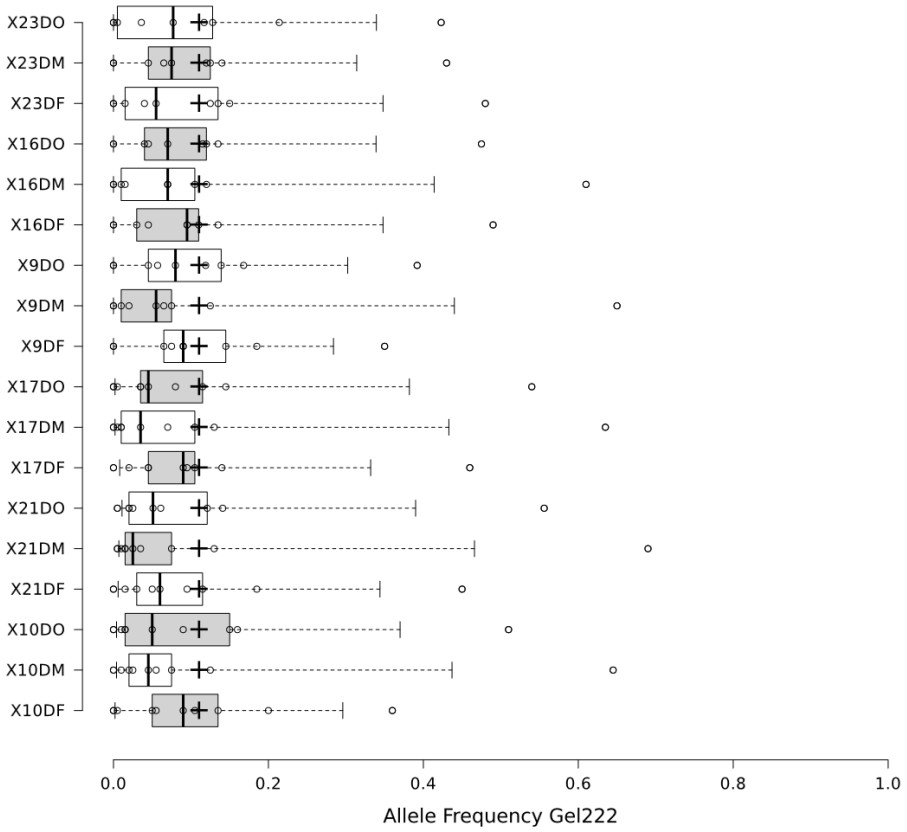
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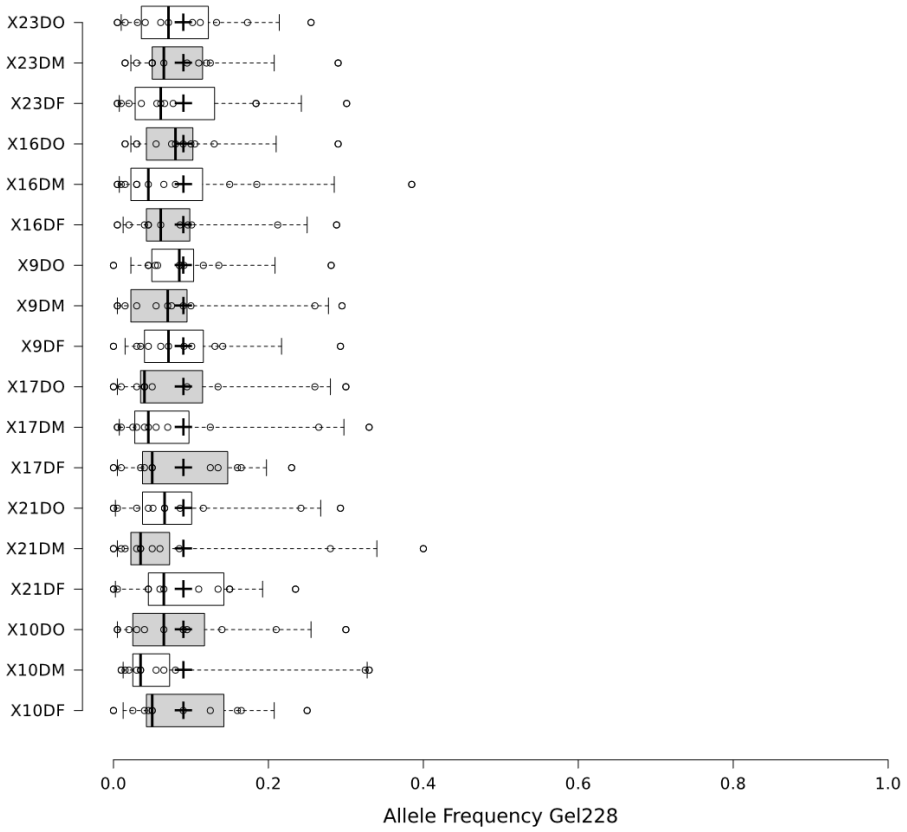
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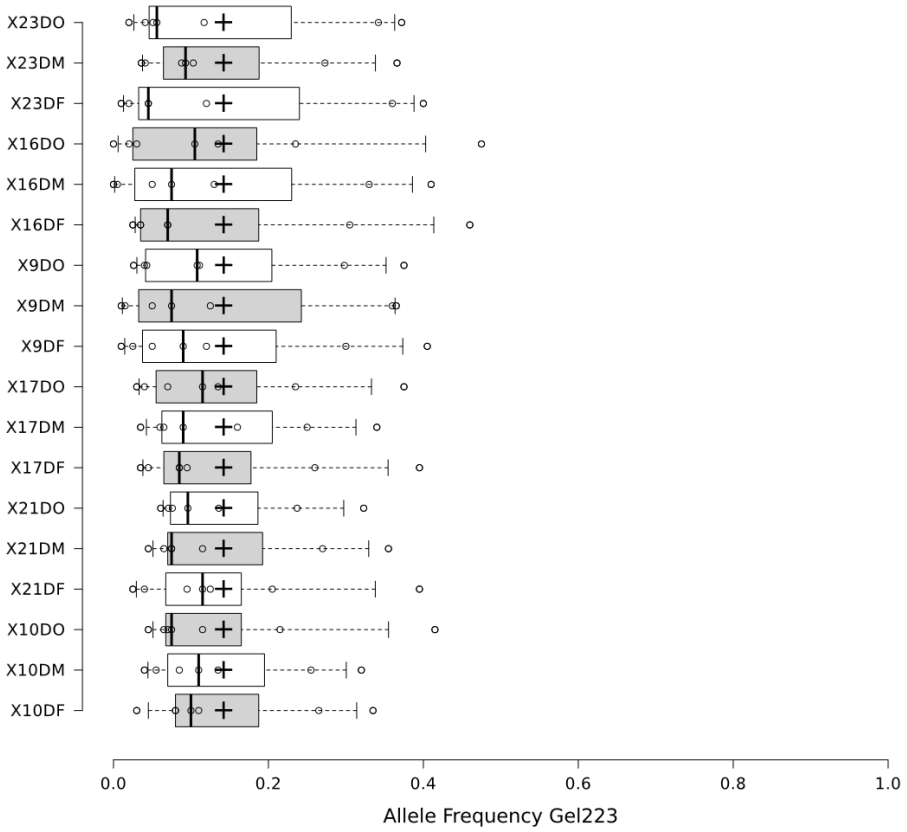
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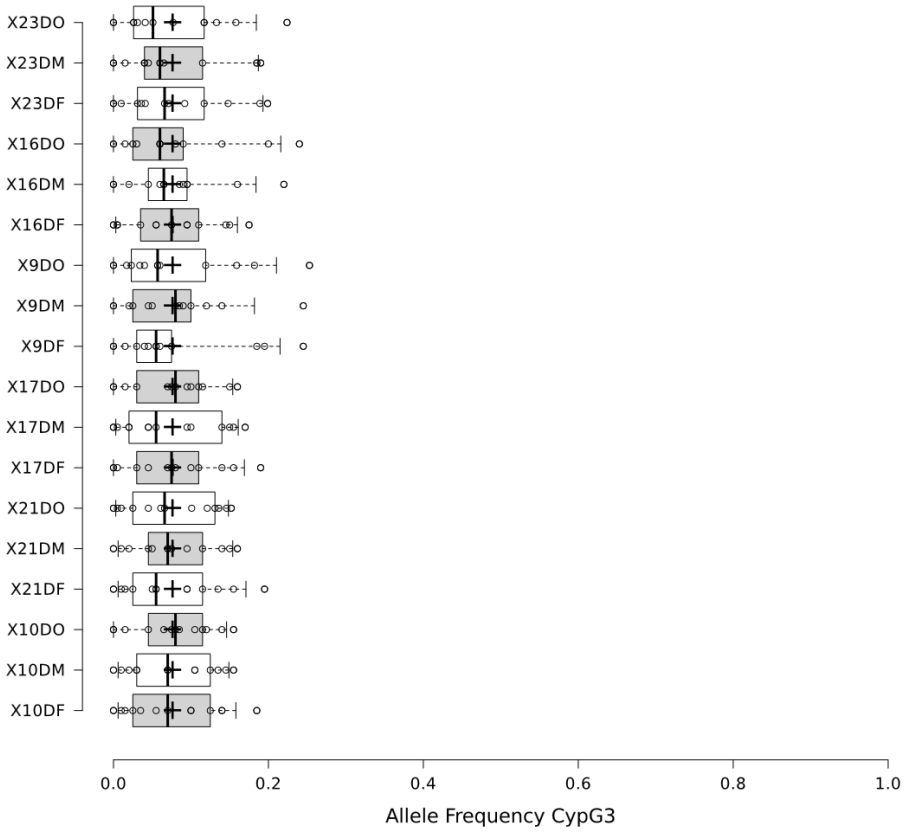
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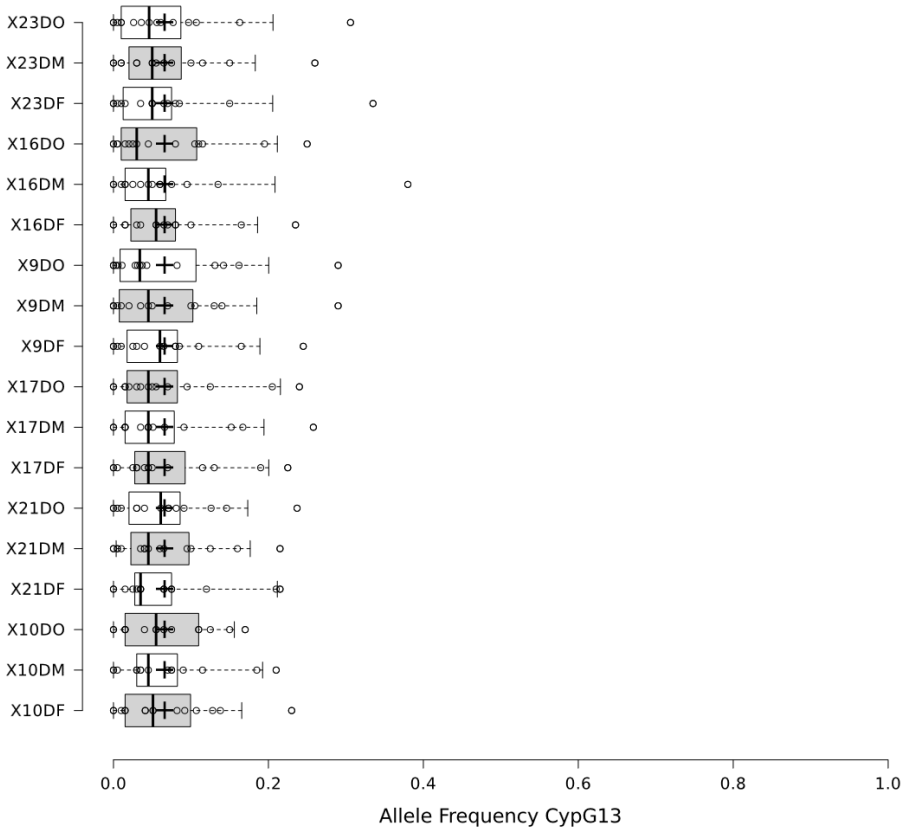
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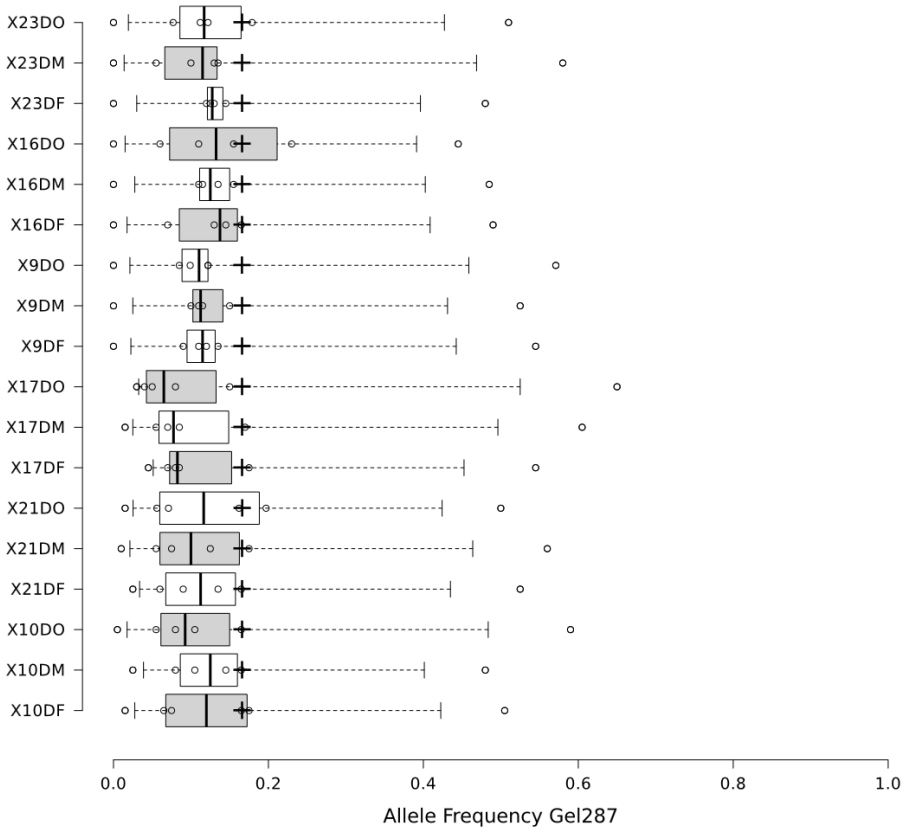
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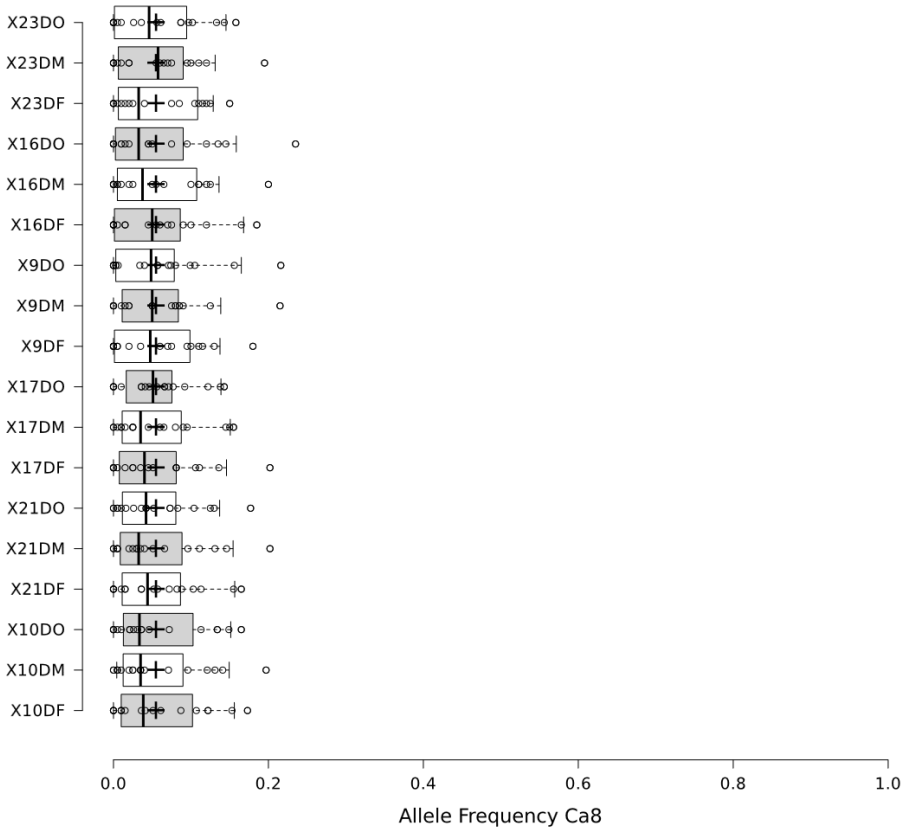
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