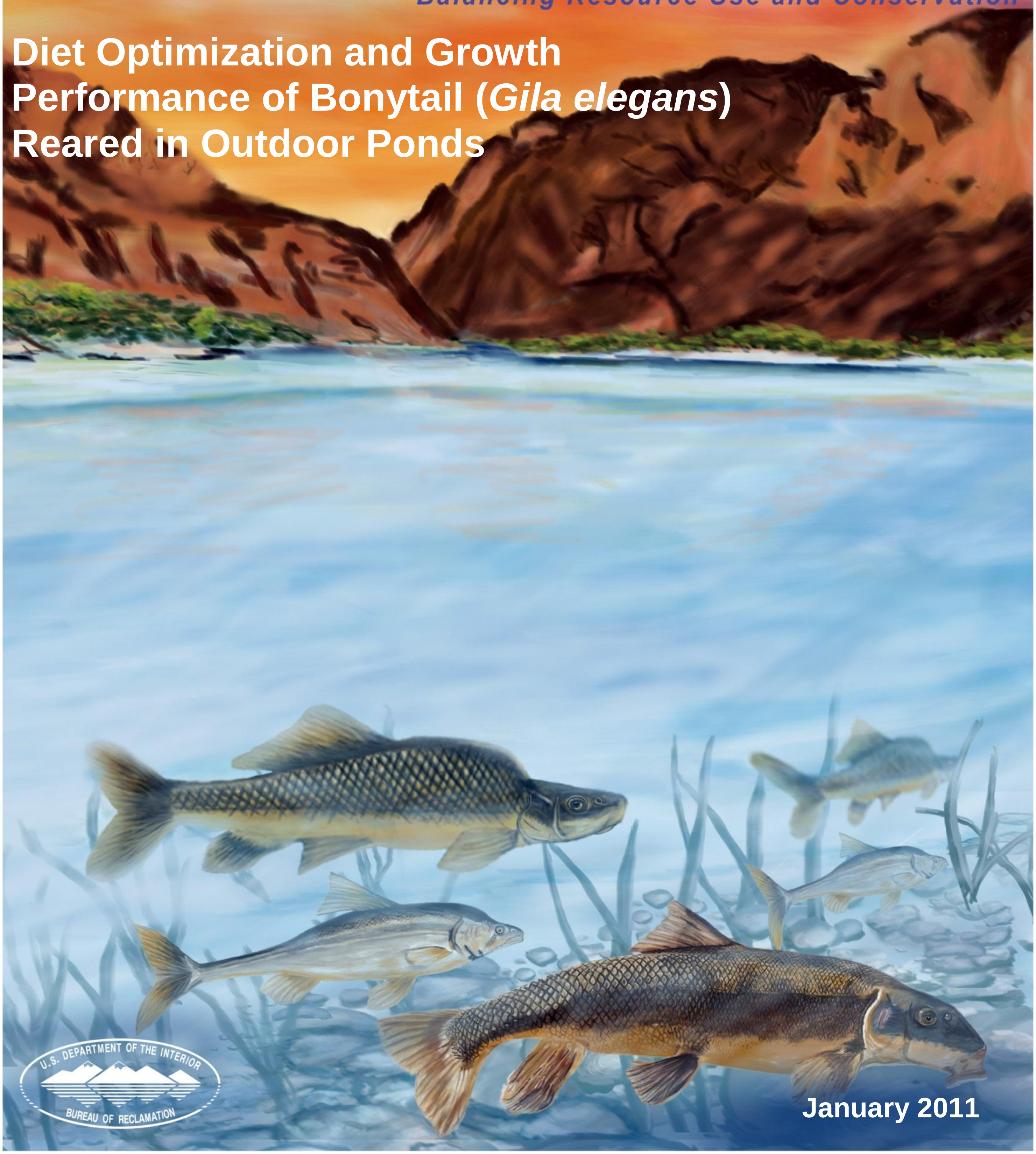
Lower Colorado River Multi-Species Conservation Program

Balancing Resource Use and Conservation

Diet Optimization and Growth Performance of Bonytail (*Gila elegans*) Reared in Outdoor Ponds



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January 2011

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Multi-Species Conservation Program

Diet Optimization and Growth Performance of Bonytail (*Gila elegans*) Reared in Outdoor Ponds

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Executive Summary

No extant populations of the endangered bonytail *Gila elegans* are known to exist in the Colorado River basin. Hatchery rearing of bonytail to 300 mm for stocking is a critical component of the recovery efforts for this species. Four experimental diets, consisting of fish meal or chicken meal with either 13 or 19% lipid, were tested against the current razorback sucker (RBS) diet to determine if alternative protein source and/or lipid levels will improve growth of bonytail reared in outdoor ponds. Currently, the RBS diet is the most commonly used feed for bonytail at rearing facilities. All five diets performed equally well on the following variables measured at the end of the study: % body weight gain, total length increase, specific growth rate (wet weight), feed conversion ratio, condition factor, and survival. Neither protein source nor lipid level produced significantly different results in juvenile bonytail performance over the 131-d study. While chicken meal produced equal growth in bonytail compared to fish meal, other physiological variables such as swim performance, stress vulnerability, disease resistance and reproductive performance should be tested before recommendations can be made regarding the replacement of fish meal in bonytail feed. An additional important factor in testing the performance of a feed is fish density. To further support research in the development of the best diet for bonytail we recommend conducting a density study to test the efficiency of the RBS diet against other manufactured diets. Until further research can be conducted on alternative fish feeds, our management recommendation is to continue use of the RBS diet for rearing bonytail in outdoor ponds.

Introduction

Bonytail is a unique cyprinid critically endangered primarily due to the transition of the Colorado River from a rigorous lotic environment to a series of large manmade impoundments (Minckley 1995; Starnes 1995). No extant populations of bonytail are known to occur in the Colorado River or its tributaries. Predation on stocked fish and recruitment failure are two of the most critical challenges in the lower Colorado River basin. Until the ecological habitat is conducive to bonytail recruitment and survival, production and stocking of hatchery-cultured bonytail is the only available method for recovery efforts. Dexter National Fish Hatchery and Technology Center (Dexter NFHTC) has reared all life stages of bonytail since 1990 for distribution into the upper and lower Colorado River basins and most recently to partners participating in fish augmentation activities of the Lower Colorado River Multi-Species Conservation Program (LCR MSCP). Annually, Dexter cultures 4000-6000 juvenile bonytail for stocking into Reach 2 and Reach 3 of the Lower Colorado River. In conjunction with this stocking commitment Dexter also provides larval bonytail for grow-out to program partners in the Upper and Lower Colorado River basin. Bonytail are reared in hatcheries to approximately 300 mm to reduce the threat of predation by piscivorous non-native fishes upon release into native waters. To reach that target size the average grow-out time is ≥ 2 years. Production of adequate numbers at the target size of 300 mm on a yearly basis is a critical component of the bonytail recovery program.

Diet trials with juvenile bonytail conducted by the Bozeman Fish Technology Center (BFTC) tested six diets of fish meal combined with varied levels of corn gluten meal, wheat gluten meal, and krill meal (see Appendix A, BFTC Project Update - Interim Progress Report). Although slight differences among the diets in the parameters measured were observed, results cannot be explained at this time as data analyses have not been finalized from that study. Additional diet trials were conducted by USGS New Mexico Cooperative Fish and Wildlife Research Unit (USGS) with larval bonytail (see Appendix B, USGS 2009 Interim Report). The larval diet study compared a feed formulated for razorback suckers (Bozeman Razorback 0301, Nelson and Sons, Murray, Utah) to six experimental diets of fish meal comprised of two protein levels and three lipid levels. No general trend was observed between the diets although greater growth was noted in the razorback sucker (RBS) diet.

Currently, most bonytail production facilities are feeding the RBS 0301 diet. Although the fish appear to grow well on this diet, little is known about the nutritional needs of bonytail through the various life stages. In addition, growing concerns over the availability of fish meal and fish oil as a primary protein source for fish feed as well as possible contaminants in fish meal have been the basis for research in developing alternative feed products. Plant products such as soybeans have been tested with varying degrees of success (Gatlin III et al. 2007); however, published studies in the use of poultry meal as an alternative protein source have reported comparable growth performance results in fish fed poultry versus fish meal (Giri et al. 2010, Pine et al. 2008, Thompson et al. 2007).

Our objectives for this research project were to develop four diets with varied ingredient composition including chicken meal and compare performance of juvenile bonytail fed the experimental diets to bonytail fed the RBS diet. Evaluating nutritional needs of bonytail and developing a quality feed designed to maximize growth and reduce rearing time in hatcheries will assist resource managers in meeting production goals.

Methods

Experimental fish.—Age-1 bonytail (2009 year class, mean total length and weight of 80.2 mm and 3.2 g) were obtained from the U.S. Fish and Wildlife Service, Dexter NFHTC, Dexter, New Mexico. All fish had been reared in outdoor ponds since swim-up and maintained on the RBS diet. Twenty-seven days prior to the study, fish were transferred from outdoor ponds to an indoor culture facility and graded using a stainless steel floating fish grader to obtain uniform size. As a prophylactic treatment following handling, fish were treated with oxytetracycline at 15 mg/L and salt at 0.5% for eight hours for two consecutive days.

Diets.—Bonytail were fed four experimental diets and one commercial diet (Table 1). The commercial diet was a RBS formulated pellet feed. The RBS diet is the most commonly used feed at bonytail rearing facilities and was included as a reference diet. The four experimental diets were formulated with fish meal or chicken meal and included two lipid levels in a 2 X 2 factorial treatment design (Table 1). The experimental diets were manufactured as pellet feed 2.0, 3.0, and 4.0 mm in diameter at the U.S. Fish and Wildlife Service's Bozeman Fish Technology Center, Bozeman, Montana. All ingredients were ground to less than 250 µm using an air-swept pulverizer (Jacobsen, model 18H, Minneapolis, Minnesota) and mixed in a horizontal paddle mixer (Marion, Iowa) for 5 minutes prior to extrusion. Experimental diets were manufactured using a twin-screw cooking extruder (DNDL-44, Buhler AG, Uzwil, Switzerland) with an 18 second exposure to 127°C in the extruder barrel and were preconditioned with steam (Extru-tech, Sabetha, Kansas) prior to entering the extruder. Pellets were then dried in a pulse bed drier (Buhler AG, Uzwil, Switzerland) for 25 minutes at 102°C with a 10 minute cooling period, resulting in final moisture levels less than 10%. All feeds were coated with the fish and poultry oils under vacuum (AJ Phlaur, Ontario, California) and packaged in plastic lined paper bags for shipment to Dexter NFHTC.

Experimental design.—The feed study was conducted in 0.04-ha rubber-lined ponds filled with water from three wells (pH 6.9, hardness 3325 mg/L $CaCO_3$, alkalinity 162 mg/L $CaCO_3$). Pond water levels were maintained with an average flow of 19-30 liters per minute. Prior to filling, the ponds were swept and washed to remove organic matter. On April 12, 2010 bonytail were randomly assigned to 20 ponds and stocked at 12,500 fish/ha (500 fish/pond) and ponds were randomly assigned one of five diets with four replicates per diet (Figure 1). A completely randomized design was used to allow testing for any potential edge effect on outer ponds based on the percent of the pond exposed to roads (ponds D2, D3, D4, D5, D21, D22, D23, D24 = 15%; ponds D8 and D14 = 35%; ponds D1 and D20 = 50% exposure). Initial lengths and weights were taken on a subsample of 30 fish from each pond prior to stocking. A subsample of 30 fish were euthanized and submitted to A&L Great Lakes Laboratories (Fort Wayne, Indiana) for mineral and proximate analysis.

Dissolved oxygen and water temperature were recorded in each pond 5 d per week before 0800 h using a handheld HACH HQ40d meter (Hach Company, Loveland, Colorado). In addition, daily water temperature profiles were monitored in 10 ponds (two ponds in each diet) with HOBO® Pro v2 data loggers (Onset Computer Corporation, Bourne, Massachusetts) programmed to record water temperatures every four hours. Observed mortalities were recorded and removed from ponds. Visual observations of pond conditions were noted, but ponds were not treated for phytoplankton and algal blooms during the feed study.

Feeding technique, frequency and amount was similar to standard feeding methods used for species cultured in ponds at Dexter NFHTC and was based on water temperature and fish life stage. Fish were fed their respective diets for 131 d (April 19, 2010 to August 27, 2010). For the first 39 d, fish were fed 2.0 mm pellets by hand three times daily (0900, 1230 and 1500 h) at

15% body weight (BW)/d (5.75 kg/ha/d) each Monday, Wednesday and Friday. At d 40, feed amounts were adjusted for the remainder of the study for each pond to 5% BW/d according to average weight of fish and feeding was reduced to two times daily (1000 and 1430 h) on Monday, Tuesday, Thursday and Friday. From d 89 through the end of the study a 50/50 mix of 2.0 and 3.0 mm pellets for each of the diets was fed to ensure feed availability for all size fish. A subsample of 30 fish was collected from each pond by seining on May 25 (day 37) and June 28 (day 71). Lengths and weights were recorded and feeding amounts adjusted accordingly. The sampled fish were removed from the feed study to preclude bias on growth or survival from handling stress. Fish were not sampled in July due to deteriorating pond conditions and high ambient temperatures that posed a potential risk to the fish if handled.

Ponds were slowly drained over a 24 h period at the end of the study. The fish were concentrated into concrete catch basins where they were netted and placed in a hauling tank and transported to indoor raceways. Individual lengths and weights were taken on a subsample of 60 fish from each pond and bulk weights and final counts were recorded for all fish from each pond. The following calculations were performed for each diet at each sampling interval:

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};

Feed conversion ratio (FCR) = g dry feed/g weight gain

Condition factor (K, g/cm^3) = 100 X [fish weight/(total length)^3].

Necropsies were performed on 20 fish from each diet (n = 100 total) for comparison of percent mesenteric fat and overall condition of fish using a fish health assessment developed by Goede and Barton (1990). Five fish randomly sampled from each pond were euthanized and sent to A&L Great Lakes Laboratories for mineral and proximate analysis.

Data analysis.—The effects of diet were initially tested using a multiple factor analysis of variance (ANOVA) including pond edge effect. When edge effect did not reduce the unexplained variation in the analyses it was removed. Subsequently, SGR and FCR data was tested using a two-way ANOVA to compare response variables and to examine each response variable over time. Data for % BW, survival, and proximate analysis were tested using a one-way ANOVA. Statistical detectability was determined at $P \leq 0.05$ for all tests. Unless otherwise stated data are reported for sampling periods by the collection day (April 12 to May 25 = day 37, May 26 to June 28 = day 71, and June 29 to August 27 = day 131). Values are reported as treatment means ± standard errors of the mean.

Results and Discussion

Dietary lipid levels and protein source did not have a significant effect on growth of bonytail juveniles reared in outdoor ponds (Figure 2). Fish were observed actively feeding on all diets when presented, suggesting there was no difference in the palatability of the diets. Weight and total length at day 131 ranged from a mean of 73.99 ± 1.46 g and 202.14 ± 1.42 mm, respectively, in the RBS diet to 78.55 ± 1.31 g and 207.43 ± 1.22 mm in the low lipid chicken meal (LLC) diet (Table 2). The maximum % BW at the end of the study was observed in the LLC diet (2457.6%) while the minimum was in the RBS diet (2193.4%), but a statistical evaluation of weight gain revealed no detectable differences ($P \geq 0.05$) among the five diets (Table 3). Analyses of the data also revealed no edge effect among ponds based on percent exposure to roads around the perimeter of the study area.

Daily increase in length measured at the end of the study averaged 0.95 mm/d and was similar among all diets ($P = 0.583$). The greatest mean increase in length within a sampling period was observed at day 71 (1.07 ± 0.02 mm/d) and was detectably different ($F_{2,45} = 21.488$, $P = 0.000$) than day 37 (0.94 ± 0.01) and day 131 (0.90 ± 0.2), but all diets were similar within each sampling period (Table 2). Condition factors were consistent among all diets and averaged 0.90 (Table 3). Hampton and Ulibarri (2008) reported lower average growth rates (0.62 to 0.72 mm/d) but comparable condition factors (0.87 to 1.02) for age-1 bonytail stocked at the same density as the current study and fed the RBS diet. However, data from the two studies cannot be accurately compared as the 2008 study encompassed a longer period of time (April 4 to November 13) and was designed to test smaller fish growth potential when cultured with larger fish. The fish stocked were not of uniform size and were sampled only once at the end of the study. Results from our study suggest that under optimum rearing conditions, including fish density and manipulation of water temperature, bonytail can potentially achieve the targeted 300 mm size before the current two year time frame.

Overall SGR at the end of the study was similar among all diets, ranging from 2.37 to 2.45% per day ($P = 0.542$). Differences in SGR were detected between sampling periods ($F_{2,45} = 1030.81$, $P = 0.000$), but not among diets within each period ($P = 0.687$). The highest SGR observed (3.30 to 3.50% per day) was at day 37 and the lowest (1.37 to 1.50% per day) was day 131 (Table 2). Water temperatures during the observed highest SGR ranged from 16.3 to 23°C, with an average of 19.5°C (Table 4). These are slightly lower temperatures than the optimum growing temperatures (22 to 24°C) reported by BFTC for young-of-year bonytail (Appendix A). However, temperature is only one of several factors that influence growth rates of fish. Other factors include density, age of fish, and rearing conditions. The study at Dexter NFHTC was conducted with age-1 fish in outdoor ponds while the study at BFTC used age-0 fish tested in an indoor culture facility.

Feed conversion ratios in this study were relatively low among all diets within all sampling periods, indicating efficient feed utilization by bonytail of fish meal and chicken meal at both lipid levels (Table 2). Mean FCR was not detectably different ($P = 0.989$) among the five diets at the end of the study (range 0.92 to 0.96). Similar to the SGR, differences were detected ($F_{2,45} = 117.05$, $P = 0.000$) between sampling periods (FCR higher at day 131 compared to day 37 and 71), but not among diets within each period ($P = 0.384$). Despite a less efficient FCR in August, which may reflect an increase in energy requirements for maintenance with rising water temperatures, dietary energy requirements were equally met by all five diets for continued bonytail growth. In contrast, Henne et al. (2006) reported FCRs ranging from 2.5 to 6.2 in juvenile bonytail reared in an indoor facility at an average water temperature of 21.6°C for 120 d and fed four commercial diets formulated for other fish species with varied protein and lipid levels.

One interesting note, bonytail had the most efficient FCR at day 71, but the greatest SGR at day 37. This may be explained by two changes that occurred between the first and second sampling periods: 1) the amount of feed was lowered from 15% to 5% BW/d, and 2) the feed time was changed from feeding three times daily three days a week to two times daily four days a week. Bonytail were presented with adequate feed to allow maximum percent growth during the first period but the FCR may have been higher due to excessive feeding. Initially feeding 15% BW/d is standard protocol at Dexter NFHTC to ensure feed is available for all the fish when stocking ponds with juveniles at the densities used in this study. The change in the number of feedings per day and the number of days per week is dictated by water temperature. As the water approaches optimum temperature feed is offered an additional day.

Proximate composition (% dry basis) of bonytail sampled prior to the study was 71.8% moisture, 58.2% crude protein, 30.4% crude lipid, and 13.0% ash. Results of the proximate analysis conducted on bonytail tissue at the end of the study are presented in Table 5. Within the variables measured, no differences were observed among the five diets ($P \geq 0.05$).

Survival was not significantly different among the diets and ranged from 94.7 to 99.5% (Table 3). Pond D24 (LLC diet) experienced the lowest survival at 85.3%, which was attributed to deteriorating water quality conditions. The pond received a high load of allochthonous organic material causing algal blooms when water temperatures began increasing in late July and early August. Dissolved oxygen reached levels of 185-190% saturation (13.9 mg/L) in the afternoon and gas bubble disease was diagnosed in the moribund fish. Elevated dissolved oxygen levels were not observed in the other 19 ponds.

Necropsies revealed no difference in bonytail mesenteric fat levels or overall health condition among the five diets. Overall rating of internal organs (spleen, liver, and kidney) was normal and most external abnormalities (fin abrasions, petechial hemorrhage, loss of scales, etc.) were due to handling and confinement stress during collection from the ponds. All fish had complete coverage of viscera by fat (Figure 3). Fat deposits provide potential energy reserves for fish adjusting to new environments and for gonad development (Goede and Barton 1990, Yaron and Sivan 2006), but at what point excessive fat may affect liver function and other critical aspects of survival such as immune responses and predator avoidance is unknown. Further research is needed to determine what level of fat deposit is most beneficial for bonytail that are to be stocked into native waters.

In conclusion, all five diets performed equally well within the variables measured in this study. Neither protein source nor lipid level produced significantly different growth or survival in bonytail reared in outdoor ponds. Results from this study suggest chicken meal is a suitable replacement for fish meal when measuring growth performance in juvenile bonytail. However, fish growth may be affected by multiple factors including density. Observations during the culture of bonytail suggest this species performs differently depending on the density of fish in the rearing unit. In addition, determining the response of bonytail in other physiological variables may be as equally important as growth to the recovery of this species. Sink et al. (2010) reported slightly lower fecundity, hatch success, and fry survival in channel catfish *Ictalurus punctatus* fed a diet of poultry byproduct meal (PBM) compared to catfish fed a fish meal/PBM mix. While chicken meal produced equal growth in bonytail compared to fish meal, other variables such as swim performance, stress vulnerability, disease resistance and reproductive performance should be tested to determine the suitability of a diet based on chicken meal for culture of native fishes. Our priority recommendation is to conduct a density study comparing the RBS diet to the experimental diets in this study or other comparable manufactured diets. Feeding the RBS diet should be continued until further research can be conducted on alternative sources of protein and lipids in fish feed.

ACKNOWLEDGEMENTS

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BUDGET

Project Charges for 2008

Bozeman FTC and USGS/NMSU	
O&M Labor Costs	$ 9,180.50
Materials and Supplies	5,926.50
Utilities & Equipment Maintenance	814.00
Contract transfer to USDA-ARS	38,500.00
Subtotal-Expended as of March 2010	**$ 54,421.00**
21% Admin Overhead	11,428.00
Total Project Funds for 2008	**$ 65,849.00**

Project Charges for 2009

Bozeman FTC, Dexter NFHTC and USGS/NMSU	
O&M Labor Costs	$ 12,299.50
Materials and Supplies	5,679.38
Utilities & Equipment Maintenance	10,195.66
Contract transfer to USDA-ARS	38,500.00
Subtotal-Expended as of March 2010	**$ 66,674.54**
Carryover to 2010	**5,846.46**
21% Admin Overhead	15,229.00
Total Project Funds for 2009	**$ 87,750.00**

Project Charges for 2010

Bozeman FTC and Dexter NFHTC	
O&M Labor Costs	$ 17,671.00
Materials and Supplies	7,894.29
Utilities & Equipment Maintenance	387.08
Contract transfer to USDA-ARS	35,000.00
Subtotal-Expended as of March 2010	**$ 60,952.37**
21% Admin Overhead	12,800.00
Total Project Funds for 2010	**$ 73,752.37**
Total received in 2010	**$ 67,906.00**
Carry over from 2009	**5,846.46**
	$ 73,752.46

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Table 1.— Ingredients and proximate composition of five diets fed to bonytail reared in outdoor ponds. In the fish meal and chicken meal diets, HL = high lipid and LL = low lipid.

Ingredient	Fish Meal Diets HL	Fish Meal Diets LL	Chicken Diets HL	Chicken Diets LL	Bozeman Razorback 0301 Diet
Fish meal	36.51	36.51	--	--	42.91
Corn protein Concentrate	24.32	24.32	20.99	20.99	--
Chicken 42	--	--	18.91	18.91	--
Soy Protein Concentrate	--	--	13.35	13.35	--
Krill meal	4.98	4.98	4.98	4.98	9.98
Corn Gluten	--	--	--	--	9.23
Wheat gluten	--	--	4.00	4.00	4.74
Wheat flour	14.80	20.80	11.30	17.93	18.31
Fish oil	11.25	5.25	5.00	5.00	8.23
Poultry oil	--	--	9.28	3.18	--
Lecithin	3.00	3.00	3.00	3.00	2.99
Dical	2.74	2.74	6.11	6.11	2.74
Vitamin premix	1.50	1.50	1.50	1.50	.59
Trace mineral Premix	0.10	0.10	0.10	0.10	.09
Vitamin C	0.20	0.30	0.30	0.30	.14
Choline CL	0.60	0.60	0.60	0.60	--
Potassium Chloride	--	--	0.56	0.56	--
Sodium Chloride	--	--	0.28	0.28	--
Magnesium oxide	--	--	0.05	0.05	--
Taurine	--	--	0.50	0.50	--
Lysine	--	--	1.29	1.29	--
Methionine	--	--	0.22	0.22	--
Threonine	--	--	0.15	0.15	--
PROXIMATE COMPOSITION:					
Protein, Crude (%)	48.0	48.6	48.0	48.6	48.0
Fat (%)	19.0	13.1	19.0	13.0	13.0
Phosphorus (%)	1.58	1.58	1.58	1.58	1.6

Table 2.— Mean (± SE) within each sampling period (day) for length, weight, specific growth rate (SGR), and feed conversion ratio (FCR) of bonytail fed five diets (Table 1). No significant difference was observed in the measured variables among the diets ($P \geq 0.05$).

		Diet				
Variable	Day	LL Chicken	HL Chicken	LL Fish	HL Fish	RBS
Length (mm)	0	79.58 ± 0.51	80.27 ± 0.56	80.21 ± 0.46	80.30 ± 0.52	80.61 ± 0.56
	37	115.18 ± 0.80	114.86 ± 0.74	114.99 ± 0.74	114.47 ± 1.08	114.70 ± 0.81
	71	154.83 ± 1.09	151.08 ± 1.03	150.02 ± 1.07	150.03 ± 1.05	150.14 ± 1.16
	131	207.43 ± 1.22	206.31 ± 1.23	204.50 ± 1.24	205.09 ± 1.37	202.11 ± 1.42
Weight (g)	0	3.08 ± 0.06	3.24 ± 0.07	3.14 ± 0.06	3.18 ± 0.07	3.22 ± 0.07
	37	14.38 ± 0.30	13.80 ± 0.28	13.76 ± 0.29	13.76 ± 0.33	13.81 ± 0.31
	71	33.99 ± 0.70	30.74 ± 0.59	30.81 ± 0.67	30.85 ± 0.63	30.74 ± 0.68
	131	78.55 ± 1.32	77.06 ± 1.27	75.10 ± 1.26	76.99 ± 1.50	73.99 ± 1.46
SGR (%)	37	3.50 ± 0.08	3.30 ± 0.09	3.40 ± 0.12	3.33 ± 0.09	3.31 ± 0.07
	71	2.53 ± 0.06	2.36 ± 0.11	2.32 ± 0.16	2.37 ± 0.12	2.35 ± 0.05
	131	1.37 ± 0.07	1.50 ± 0.06	1.46 ± 0.02	1.49 ± 0.06	1.43 ± 0.09
FCR	37	0.71 ± 0.03	0.76 ± 0.03	0.75 ± 0.04	0.76 ± 0.03	0.76 ± 0.03
	71	0.62 ± 0.02	0.69 ± 0.05	0.72 ± 0.07	0.69 ± 0.05	0.69 ± 0.02
	131	1.19 ± 0.07	1.08 ± 0.06	1.08 ± 0.07	1.04 ± 0.04	1.10 ± 0.03

Table 3.— Mean (± SE) weight gain, condition factor (*K*), and survival for bonytail fed five diets (Table 1) for 131 days. No significant difference was observed in the measured variables among the diets ($P \geq 0.05$). The % survival in the LL Chicken diet was affected by mortalities in pond 24D due to water quality issues.

	Diet				
Variable	LL Chicken	HL Chicken	LL Fish	HL Fish	RBS
Weight gain (% BW)	2458 ± 145	2285 ± 95	2301 ± 109	2334 ± 220	2193 ± 138
K (g/cm^3)	0.91 ± 0.008	0.89 ± 0.005	0.90 ± 0.008	0.90 ± 0.01	0.90 ± 0.006
Survival (%)	94.7 ± 3.2	99.5 ± 0.1	98.7 ± 1	99.5 ± 0.2	97.7 ± 0.8

Table 4.— Weekly mean (± SE) morning (AM) dissolved oxygen and morning (AM) and afternoon (PM) water temperatures in feed study ponds. Dissolved oxygen values are means of 20 ponds. Water temperatures are means of 10 ponds.

	Dissolved Oxygen (mg/L)	Temperature (°C)	
Week	AM	AM	PM
1	9.0 ± 0.06	17.3 ± 0.2	19.5 ± 0.1
2	9.0 ± 0.05	16.9 ± 0.1	19.3 ± 0.1
3	10.4 ± 0.07	17.6 ± 0.2	20.2 ± 0.2
4	8.6 ± 0.05	17.8 ± 0.1	20.2 ± 0.1
5	9.4 ± 0.07	20.6 ± 0.1	23.5 ± 0.1
6	8.4 ± 0.06	22.2 ± 0.1	25.1 ± 0.1
7	9.1 ± 0.07	23.2 ± 0.1	26.2 ± 0.2
8	8.0 ± 0.07	24.7 ± 0.1	27.2 ± 0.1
9	7.6 ± 0.08	23.9 ± 0.1	26.9 ± 0.1
10	7.5 ± 0.05	25.3 ± 0.1	28.2 ± 0.1
11	8.3 ± 0.06	24.0 ± 0.1	25.7 ± 0.2
12	7.9 ± 0.08	25.6 ± 0.1	28.0 ± 0.1
13	8.2 ± 0.09	26.7 ± 0.1	29.3 ± 0.1
14	7.1 ± 0.08	26.0 ± 0.1	28.6 ± 0.1
15	8.2 ± 0.08	25.3 ± 0.1	27.5 ± 0.1
16	7.1 ± 0.07	25.9 ± 0.1	28.6 ± 0.1
17	6.4 ± 0.07	25.7 ± 0.1	28.0 ± 0.1
18	7.8 ± 0.13	25.6 ± 0.1	27.8 ± 0.1
19	7.5 ± 0.08	23.6 ± 0.3	25.5 ± 0.2

Table 5.— Proximate tissue composition (whole body, % dry basis) from bonytail fed five diets. Prestudy samples collected on the day fish were stocked into the ponds. Post-study samples collected on final day of study. No significant difference was observed in the measured variables among the diets ($P \geq 0.05$)

	Prestudy	Post-Study				
		Diet				
Variable		LL Chicken	HL Chicken	LL Fish	HL Fish	RBS
Moisture	71.84	63.53	62.75	62.14	61.36	62.24
Nitrogen	9.31	8.02	8.06	7.25	7.92	8.10
Crude Protein	58.20	50.10	50.35	45.28	49.47	50.59
Crude Fat	30.35	41.75	43.07	42.40	44.16	45.64
Ash	12.97	10.11	9.69	9.14	9.15	9.57
Calcium (Ca)	4.04	2.46	3.24	2.59	2.44	2.61
Potassium (K)	1.32	0.93	0.85	0.83	0.87	0.86
Magnesium (Mg)	0.14	0.11	0.12	0.11	0.11	0.11
Sodium (Na)	0.28	0.23	0.23	0.21	0.23	0.22
Phosphorus (P)	2.58	1.48	1.85	1.57	1.48	1.60
Sulfur (S)	0.73	0.60	0.56	0.53	0.59	0.56
Aluminum (Al)	1.00	24.75	32.75	25.00	22.50	32.50
Boron (B)	1.00	1.00	1.00	1.00	1.00	1.00
Copper (Cu)	4.00	6.25	7.00	7.00	6.75	5.75
Iron (Fe)	47.00	40.75	44.50	37.75	39.00	36.25
Manganese (Mn)	2.00	1.75	3.50	2.50	2.25	1.50
Zinc (Zn)	242.00	80.25	80.75	74.00	75.75	97.75

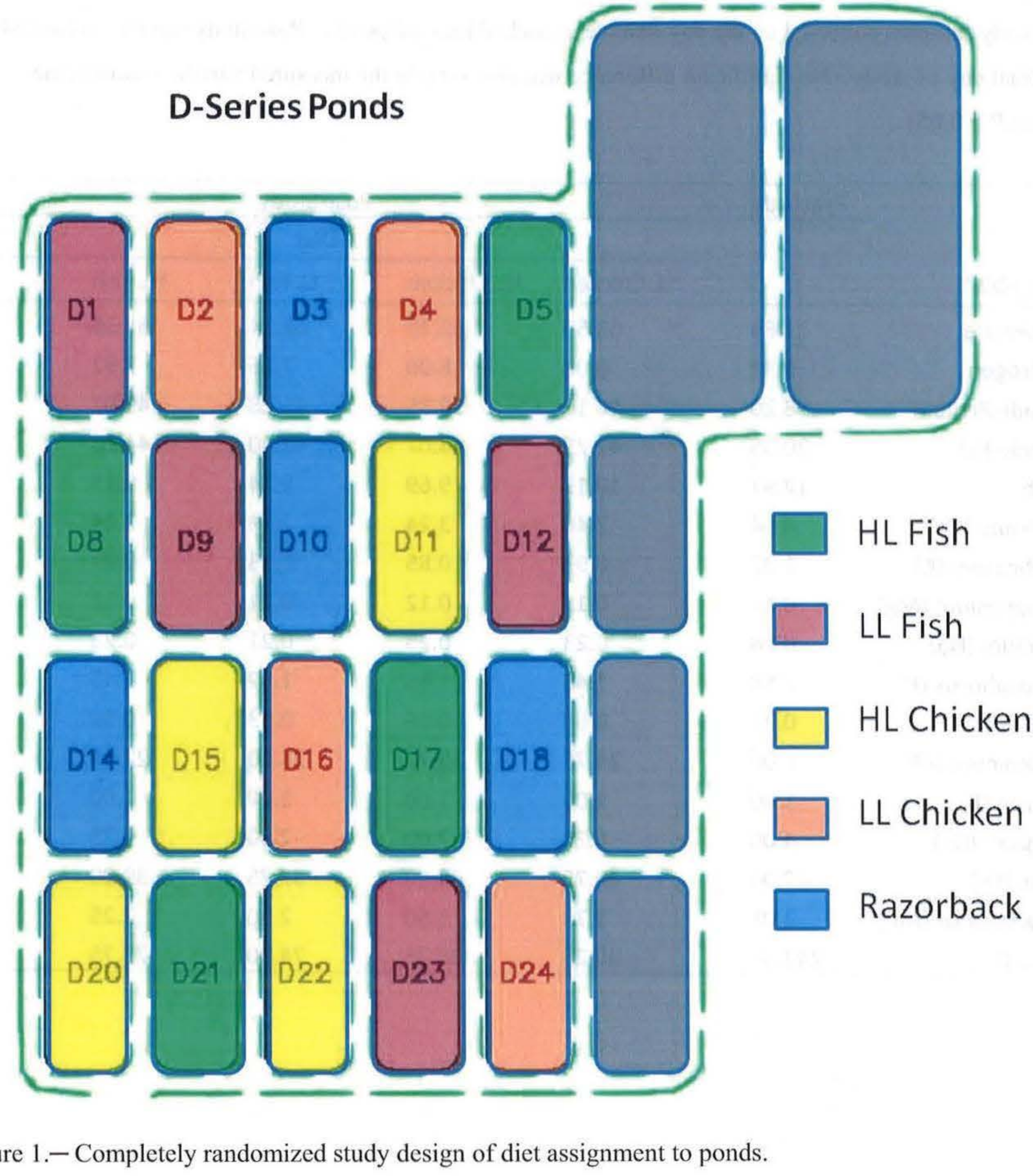

Figure 1.— Completely randomized study design of diet assignment to ponds.

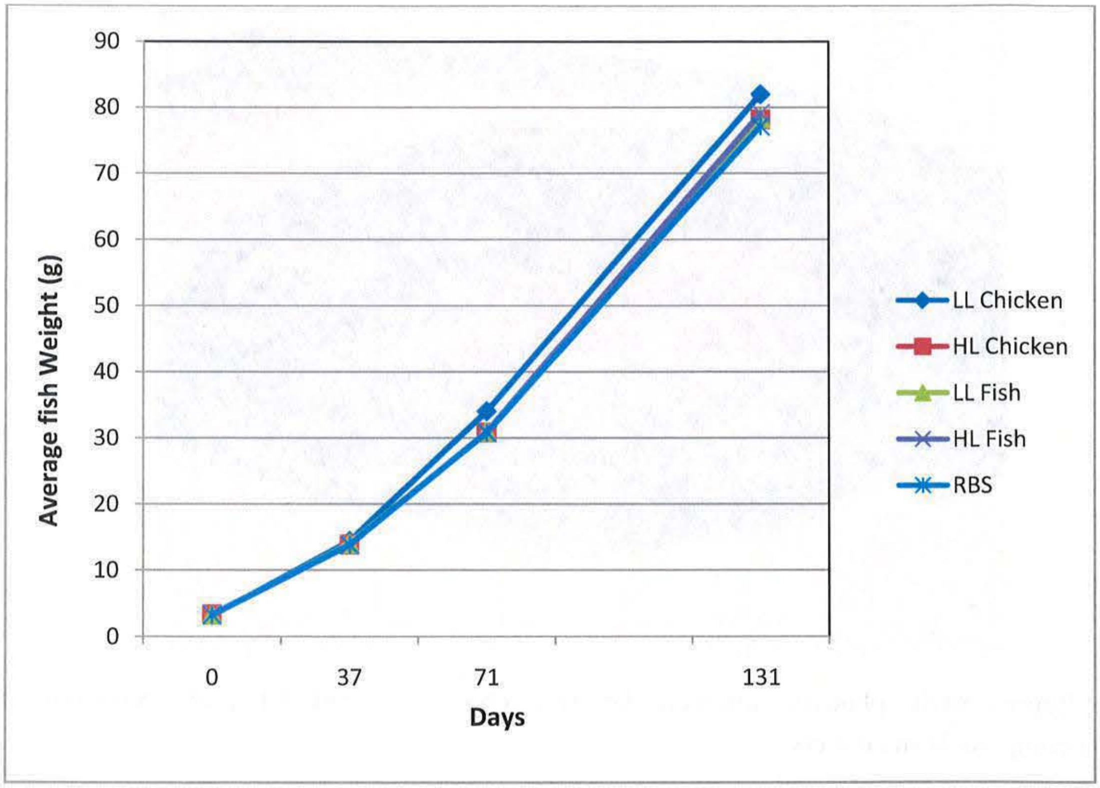

Figure 2.— Mean weight (g) in bonytail fed five diets over 131 days. Weights were taken on a subsample of 30 fish at day 0, 37 and 71, and 60 fish at day 131.

Figure 3.— Representative photo of mesenteric fat level in bonytail at end of 131 day feed study. Fat levels were comparable in all diets.

Appendix A

BFTC Project Update - Interim Progress Report:
Improved Growth Performance of Bonytail (*Gila elgans*) through
Optimization of Diet, Temperature and Rearing Density

United States Department of the Interior

FISH AND WILDLIFE SERVICE
Mountain-Prairie Region

FISH TECHNOLOGY CENTER
4050 Bridger Canyon Road
Bozeman, MT 59715
(406) 587-9265/FAX 586-5942

DATE: December 16, 2010

TO: Manuel Ulibarri, Project Administrator

FROM: Kevin Kappenman, Fish Biologist, Bozeman Fish Technology Center

SUBJECT: Project Update - Interim Progress Report: Improved Growth Performance of Bonytail (*Gila elgans*) Through Optimization of Diet, Temperature and Rearing Density

Cc: Robert Muth, USFWS

Summary

The BFTC engaged in two bonytail studies beginning in 2008. The two studies were designed to 1) evaluate thermal requirements for bonytail propagation and 2) evaluate the effect ingredient sources and palatability in grower diets have on growth and survival of juvenile and sub-adult bonytail. The initial wet-laboratory investigation involving the trials with fish were completed in 2009. Data analyses and report development began in 2009. At years end of 2009 BFTC submitted an interim report that contained analyses and recommendations developed from the investigations. The 2009 report provided thermal guidelines for maximizing hatchery production. It also provided direction for future diet studies performed at Dexter Fish Technology Center. In this update we provide (below) the compiled results and finding from proximate analyses (fish body composition) that were not available for the interim report. Statistical analyses of the data will be completed by Dec. 31 2010 and provided in a final report.

Future Plans at BFTC

Two manuscripts are in development. One manuscript has the working title *The thermal requirements of Bonytail (Gila elgans); laboratory derived information with application to an altered riverscape and conservation propagation programs.* This manuscript is nearly complete and will be submitted by year end to the project administer as a deliverable (e.g. final report). A similar version will be submitted ASAP to a scientific journal. Our report for the thermal study in manuscript form will meet the deliverable date of December 31 as outlined in the original statement of work. The target journal for submission is The Southwestern Naturalist. We believe that the results have important application to both river management and aquaculture and think that this is an appropriate outlet for serving both venues. Current breakdown of manuscript as per completion is as follows: Introduction 95% complete, Methods 95% complete, Data Analyses and Results 80% (statistical analyses of proximate analyses have not been completed), Discussion and Recommendations 80%, Literature Search 95%. To date the recommendation from our interim report as per suggested temperature regime to optimize growth of bonytail has not changed and we anticipate no change to this recommendation in the final manuscript/report.

The second manuscript has the working title *Growth and Performance of Bonytail Fed Six Different Diets.* This manuscript is 60% complete. A final report will be delivered by December 31, 2010 as per the SOW. This report will contain the completed statistical results of the proximate analyses and incorporate all information from the interim report. This report will act as the deliverable to meet our obligations. A manuscript that is "journal ready" will not likely be available until later in the year. We intend to complete the manuscript and submit it to North American Journal of Aquaculture in 2011.

This update was prepared by Kevin M. Kappenman. Contributors to the project include Kevin M. Kappenman[1], Matt Toner[1], Jason Ilgen[1], Rick Barrows[2], Eli Cureton[1], William Fraser[1] and Greg Kindschi[1a]

[1]USFWS, Bozeman Fish Technology Center, Montana, [a] retired

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Acknowledgements and thanks to Molly Webb, Chris Hooley, Colleen Caldwell, Manuel Ulibarri and Yvette Converse

Proximate Analyses Results for Improved Growth Performance of Bonytail (*Gila elgans*) Through Optimization of Temperature

Proximate analysis.— Statistical analyses is not complete. A summary of the data is presented in table 1. A final report/manuscript will contain a table of proximate analyses data and comparisons among treatments. The body composition (protein, lipid, moisture, and ash) was calculated by means of standard proximate analysis. Body composition was measured at the end of 112 d from three randomly selected fish from each tank. Fish were pooled by temperature treatment (three fish per tank) and frozen until analysis. The treatment sample was partially thawed and homogenized with an equivalent weight of distilled water. Protein content was determined by thermal oxidation (Leco TruSpec, Leco

Corp., St. Joseph, Missouri) following the Association of Official Analytical Chemists method 992.15 (AOAC 1990). Lipid was measured using a petroleum ether extraction (Ankom XT 10; Ankom Technology, Macedan, New York). Tissue moisture was measured by freeze-drying a 2-g sample (Labconco Freezone 12; Labconco Corp., Kansas City, Missouri) until no mass change occurred, and ash content was determined by heating a 2-g subsample in a muffle furnace (Barnstead/Thermolyne 30400; Barnstead International, Dubuque, Iowa) at 555°C for 12 h.

Table 1. Weight gain, specific growth rate, and proximate analysis results of bonytail during 112-d temperature trials of 8-30°C based on three replicates per temperature treatment. Data are means ± SD for proximate analysis parameters.

Temp (°C)	Mean Weight (g/fish)	Specific Growth Rate	Protein (%)	Lipid (%)	Moisture (%)	Ash (%)
8	1.97	-0.15	15.6 ± 0.2	8.4 ± 1.1	71.4 ± 1.0	3.3 ± 0.0
10	2.15	-0.06	15.8 ± 0.2	8.3 ± 0.9	71.4 ± 0.7	3.4 ± 0.1
12	2.37	-0.06	16.7 ± 0.6	9.4 ± 1.2	70.0 ± 1.0	3.3 ± 0.1
14	2.64	-0.05	16.3 ± 0.2	9.1 ± 0.7	70.1 ± 0.9	3.4 ± 0.2
16	2.50	0.09	16.3 ± 0.2	9.4 ± 1.0	69.9 ± 1.0	3.4 ± 0.2
18	2.27	0.21	16.0 ± 0.3	9.4 ± 0.5	70.1 ± 0.4	3.5 ± 0.2
20	2.58	0.35	16.0 ± 0.3	9.1 ± 0.6	70.2 ± 0.4	3.4 ± 0.1
22	3.38	0.69	16.3 ± 0.3	9.5 ± 1.3	69.9 ± 1.1	3.5 ± 0.1
24	3.03	0.85	16.6 ± 0.3	8.3 ± 0.4	70.9 ± 0.3	3.6 ± 0.1
26	2.35	0.52	16.2 ± 0.4	10.1 ± 0.4	69.4 ± 0.3	3.8 ± 0.1
28	2.99	0.55	16.4 ± 0.1	10.1 ± 1.0	68.9 ± 0.6	3.8 ± 0.1
30	2.88	0.53	15.7 ± 0.3	12.3 ± 0.4	67.7 ± 0.4	3.6 ± 0.1

Initial Interpretation of Proximate Analyses

Body composition along with growth rate is affected by temperature. In short term thermal experiments the storage of energy reserves as reflected by lipid deposition can act as an indicator of metabolic thermal optimums and thresholds. In these types of studies percent composition of lipid is more readily effected than changes in protein composition which are generally observed over longer periods of suppressed growth. Though statistical analyses have not been performed our initial assessment is that the percent composition of lipid appears consistent with the effect we observed of temperature on growth of bonytail. In general, greater lipid reserves were present at temperatures greater than 14°C, and higher levels appear to occur at warmer temperatures. This corresponds with weight loss of fish held at 14°C or lower. Culturist should consider that fish exposed to temperatures below 14°C may suffer increased stress. The information we provide may allow culturists to develop hatchery practices that alleviate unnecessary stressors. The study demonstrates that bonytail can be cultured over a wide range of temperatures but do appear to have metabolic efficiencies over a range of temperatures. Culturists may wish to take advantage of those efficiencies. Culturists can use the information we provide to maximize growth by controlling temperatures or taking advantage of seasonal conditions to maximize growth.

Proximate Analyses results for Improving Growth Performance of Bonytail through Optimization of Diet

Proximate analysis.— Statistical analyses has not been completed. A summary of the fish body tissue composition data is provided in Table 1. The diet ingredients and proximate analyses for each diet are available in Table 2. A final report or manuscript will contain a table of proximate analyses data and comparisons among treatments and diet contents. The body composition (protein, lipid, moisture, and ash) was calculated by means of standard proximate analysis. Body composition was measured at the end of 123 d from three randomly selected fish from each tank. A sample of each diet was collected at the experiments end and processed using the same method as described for fish body composition. Fish were pooled by diet regime treatment (three fish per tank) and frozen until analysis. The treatment sample was partially thawed and homogenized with an equivalent weight of distilled water. Protein content was determined by thermal oxidation (Leco TruSpec, Leco Corp., St. Joseph, Missouri) following the Association of Official Analytical Chemists method 992.15 (AOAC 1990). Lipid was measured using a petroleum ether extraction (Ankom XT 10; Ankom Technology, Macedan, New York). Tissue moisture was measured by freeze-drying a 2-g sample (Labconco Freezone 12; Labconco Corp., Kansas City, Missouri) until no mass change occurred, and ash content was determined by heating a 2-g subsample in a muffle furnace (Barnstead/Thermolyne 30400; Barnstead International, Dubuque, Iowa) at 555°C for 12 h.

Initial Interpretation of Proximate Analyses

Body composition does appear to vary to some degree but results can not be explained until statistical analyses are complete.

Table 1. Summary of the percent body composition of bonytail in the six diet treatments during 123-d diet trials of based on three replicates per temperature treatment. Data are means ± SD for proximate analysis parameters.

Diet Treatment	Protein (%)	Lipid (%)	Moisture (%)	Ash (%)
7	15.8 ± 0.3	12.2 ± 0.7	68.0 ± 0.9	3.2 ± 0.0
8	15.9 ± 0.2	10.2 ± 1.7	69.5 ± 1.4	3.3 ± 0.1
9	16.1 ± 0.2	10.7 ± 1.3	69.3 ± 1.4	3.3 ± 0.1
10	16.0 ± 0.3	11.6 ± 2.2	68.4 ± 2.1	3.3 ± 0.1
11	16.3 ± 0.4	11.0 ± 1.6	68.7 ± 1.7	3.4 ± 0.0
12	16.0 ± 0.3	10.2 ± 1.1	69.3 ± 0.9	3.5 ± 0.1

Table 2. Ingredient Composition and Proximate Analyses (%) of Experimental Bonytail Diets

Ingredient	Diet 7	Diet 8	Diet 9	Diet 10	Diet 11	Diet 12
Menhaden meal, Special select	36.51	36.51	36.51	36.51	36.51	36.51
Corn gluten meal	27.21	27.21	27.21	9.23	9.23	9.23
Wheat gluten meal	4.93	4.93	4.93	14.93	14.93	14.93
Krill meal	---	---	---	4.98	4.98	4.98
Lecithin	3.00	3.00	3.00	3.00	3.00	3.00
Wheat flour	16.10	13.36	10.62	19.70	16.96	14.22
Di-calcium phosphate	---	2.74	5.48	---	2.74	5.48
Fish oil	9.85	9.85	9.85	9.85	9.85	9.85
Vitamin premix[1]	1.50	1.50	1.50	1.50	1.50	1.50
Choline Cl	0.60	0.60	0.60	0.60	0.60	0.60
Stay-C	0.20	0.20	0.20	0.20	0.20	0.20
Trace min premix[2]	0.10	0.10	0.10	0.10	0.10	0.10
<u>Proximate Analyses</u>						
Ash (%)	7.6	9.6	12.0	11.6	9.2	14.0
Protien (%)	48.1	48.2	46.6	46.7	47.1	45.3
Lipid (%)	17.0	17.2	16.5	17.3	16.7	16.4
Moisture (%)	4.5	4.4	4.3	3.0	3.4	4.0

[1] Contributed, per kg diet; vitamin A, 2880 IU; vitamin D, 2800 IU; vitamin E, 75 IU; vitamin K3, .5 mg; thiamin mononitrate, 3 mg; riboflavin 4.6 mg,; pyridoxine hydrochloride, 4.5 mg; pantothenate, DL-calcium, 23.3 mg; cyancobalamin, 0.01 mg; nicotinic acid, 10.9 mg; biotin, 0.16 mg; folic acid, 2.1

[2] Contributed in mg/kg of diet; zinc 37; manganese, 10; iodine, 5; copper, 1

Appendix B

USGS 2009 Interim Report

Diet Optimization and Growth Performance of Bonytail (*Gila elegans*)

Diet Optimization and Growth Performance of Bonytail (*Gila elgans*)

Colleen Caldwell[1], Rick Barrows[2], Manuel Ulibarri[3]

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New Mexico State University
[2]USDA, Agricultural Research Service, Hagerman Fish Culture Experiment Station, Idaho
[3]USFWS, Dexter National Fish Hatchery and Technology Center, New Mexico

2009 Interim Report
December 2009

Introduction

The bonytail (*Gila elegans*) was listed as federally endangered in 1980 (USFWS 1984). Native to the Colorado River Basin in the western U.S., the species is near extinction due to habitat loss, altered stream flows, hybridization, competition and predation by non-native fish species. The short-term recovery goal for the species is to prevent its immediate extinction. Although spawning occurs in wild populations of bonytail, these events are rare with recruitment extremely low. Thus, captive propagation is the only means to prevent extinction. Propagation efforts have been underway since 1990 at the USFWS Dexter National Fish Hatchery and Technology Center (Dexter NFHTC) with the spawning of bonytail broodstock for larval production and distribution to grow-out facilities. Each year, larval fish are distributed to state and federal facilities in Texas, Arizona, and Colorado for grow-out and eventual release at approximately 300 mm to prevent or reduce being predated upon by piscivorous non-native fishes. These facilities report a grow-out time frame of 8 months to 2 years to obtain the target size prior to release. Biologists reported that when the fish are reared in lower densities (500 fish/acre), the desired size was obtained sooner. This may reflect food availability and/or nutrition issue as well as a sensitivity of the species to elevated densities. Currently, the grow-out facilities are using a range of commercial diets and primarily rearing the fish in ponds.

Although diet and rearing density can be more easily manipulated in the hatchery, water temperature has a predominant influence on fish performance irrespective of diet and density. A thorough understanding of the thermal optima and thermal limits of bonytail will aid in the development of a species specific diet, and allow for more accurate projections of production capabilities at various facilities where water temperature cannot be manipulated.

Thus, the objective of this research was to optimize growth performance of bonytail reared indoors. By evaluating dietary preferences and requirements of bonytail, a species-specific feed formulation could be manufactured by commercial companies and made available to production and rearing facilities. Thus, a diet was needed that would consistently provide essential nutrients and palatability to obtain the needed size of 300 mm for augmentation of wild populations of bonytail. Since weight gain is directly dependant on the supply, balance, and quality of nutrients provided to the fish, development of a species-specific diet for the grower stage of production was deemed necessary. This work was conducted in accordance with the LCR MSCP Work Task C11 and addresses Management Actions identified in the Bonytail Recovery Plan (1984) to "Reintroduce hatchery-reared bonytail into the wild."

Methods

Fish.- Larval bonytail (approximately 3 days post-hatch; 7 May 2009) were obtained from the U.S. Fish and Wildlife Service (Service) Dexter National Fish Hatchery and Technology Center. Larvae were presented twice daily *ad libitum* with *Artemia* nauplii until day 30 post-hatch. At that time, larvae were supplemented with Razorback sucker diet (RSD) that had been ground and sifted to obtain particles ranging from 350 to 500 microns in size.

Experimental diets.- The grower diet (Razorback sucker grower diet; 0801) was the basis for formulation of the six grower diets and thus was included as the seventh diet fed as a reference in the feed trial. The six grower diets were modified to include two protein levels (approximately 48 and 42%) and three lipid levels (approximately 12, 17, 22%) in a 2 x 3 factorial treatment design. The experimental diets were manufactured in the Feeds and Nutrition Laboratory (USFWS, Fish Technology Center, Bozeman, Montana). All ingredients were ground using an air-swept pulverizer (Model 18H, Jacobsen, Minneapolis, Minnesota) to a particle size of <100 micron. Fish were fed a No. 2 crumble. Prior to the start of the feed study, approximately 30 fish and a sample of each diet were submitted for proximate analysis (crude protein, crude lipid, ash, moisture).

Experimental system.- The grower feed trial was conducted at the New Mexico State University Geothermal Fish Culture and Research Facility, Las Cruces, New Mexico (NMSU).

The facility at NMSU is an experimental greenhouse facility that uses treated water (NMSU water treatment plant) within a water reuse system to minimize water loss and optimal water quality for fish growth and maintenance (i.e., pH 8.0; conductivity 620 micromhos/cm; total dissolved solids 360 mg/L; chloride 60 mg/L; alkalinity 146 mg/L as $CaCO_3$; hardness 188 mg/L as $CaCO_3$). Geothermal water is used to heat the water throughout the winter. Because the facility is a greenhouse, the fish experience natural photoperiod and feeding schedules vary accordingly.

The feed study began September 24, 2008 and ended 19 December 2008. Two weeks prior to the feed trial, the young fish were graded to obtain comparable size throughout the study. All fish were fed the reference diet until the beginning of the feed trial. Every thirty days, length and weight were obtained on all fish throughout all diets. Aquaria will be siphoned daily of feces and uneaten food. At the end of the study, fish were euthanized and submitted to A&L Great Lakes Laboratories (Fort Wayne, IN) for mineral and proximate composition analysis.

Experimental design and performance measures.- The experimental design was a randomized complete block design with blocks as one of four locations on shelving units containing aquaria. Thus, we tested seven experimental feeds (treatments) randomly placed within each of the four blocks to obtain four replications (tanks) per diet for the experimental diets and six replications for the reference diet. Fish densities were 25 fish/74 L aquarium (0.4 g/L). Specific growth rate (SGRw), also referred to as instantaneous growth rate, was assessed at monthly intervals and was calculated as follows:

$$100 \text{ X } \{(\log_e[\text{final weight}] - \log_e[\text{initial weight}]) \div \text{ total experiment days}\}.$$

Temperature ($^{\circ}$C) and dissolved oxygen (mg/L) were monitored daily (HACH HQ40d for Luminescence; Hach Company, Loveland, Colorado). Ammonia (mg/L, NH_3-N, salicylate method) and nitrite (mg/L; NO_2-N, diazotization method) were measured using a HACH spectrophotometer. A Beckman 34 with an Orion probe was used to measure pH. Ammonia, nitrite and pH were characterized in all treatments weekly.

Results

Results of the proximate analysis of the six experimental diets indicate that the expected protein levels (48 and 42%) and lipid levels (12, 17, and 22%) were slightly less (1-2%) than the targeted levels (Table 1). The protein and lipid level of the reference diet (razorback sucker diet; RSD) was 45.7% and 17.9%, respectively, and overlapped with the percent protein and fat levels obtained for the experimental diets. An evaluation of growth (weight increase over the study) revealed no difference among the experimental diets. The greatest overall gain in weight occurred with the reference diet (Figure 1). Fish fed the RSD diet exhibited greater overall weight gain than fish fed the experimental diets within the first 30 days of the experiment. This weight gain continued throughout the end of the study. By December, growth declined in fish for all diets. The decline was due to photoperiod affecting feeding activity and not seasonal changes in temperature of the experimental units. Experimental temperature was maintained within 1°C of the target test temperature throughout the entire study.

Specific growth rate was similar among all experimental diets (0.3% per day) except in fish fed the reference diet (0.5% per day) (Table 2). Total weight gain (%) of initial body weight was low and varied from 20.4% (diet 1) to 25.0% (diet 4) among the six experimental diets (Table 3). Total weight gain was 34.8% in fish fed the reference diet.

No general trends were observed when comparing protein and fat levels between fish and respective experimental and reference feeds (Table 4). Despite the greater growth in bonytail fed the reference diet, protein (%) and fat (%) were not consistently greater in either the diet or the fish.

Water quality conditions were deemed acceptable throughout the feed trial at the New Mexico State University Geothermal Fish Culture and Research Facility in Las Cruces. The average dissolved oxygen concentration was 7.62 mg/L (standard error ± 0.023). Targeted water temperature of 21°C was obtained with a range from 20.3 to 21.1°C. This temperature was selected due to concurrent temperature studies taking place with the species at the U.S. Fish and Wildlife Service Bozeman Technology Center. The pH varied slightly from 8.30 to 8.35. Ammonia (mg/L TAN) varied from 0.02 to 0.07 mg/L and nitrite (mg/L- N) varied from 0.013 to 0.016 mg/L throughout the entire study.

Discussion

Growth performance was poor in juvenile bonytail fed a series of experimental diets and a reference diet varying in percentage of protein and fat. The juvenile fish obtained an average growth rate from 0.3 to 0.5% per day. All six of the experimental diets did not vary in ingredient composition while the percent protein and fat were altered. Despite the varying protein and fat levels, differences were not detected among the experimental diets. While the reference diet resulted in greater weight gain (35% by the end of the study), the overall growth is lower than anticipated for juvenile fish over an 86 day period. The decline in growth of the bonytail during the month of December in the trial reflects the effect of photoperiod associated with reduced day length. The research facility is a greenhouse and operated through the use of natural light. As day length decreased throughout the study, the hours of operation in which fish were fed and aquaria maintained were adjusted accordingly. However, the amount of feed and the frequency at which feed was offered to the fish was not altered. A similar decline in growth was also observed in juvenile Rio Grande silvery minnow during a series of diet trials at the NMSU/USGS experimental research facility in Las Cruces, New Mexico. The minnow achieved the greatest weight gains within the first 30 days of the study compared to the month of December (Caldwell et al. 2010).

Earlier research demonstrated the importance of protein source, energy content and protein level for juvenile bonytail (Henne et al. 2006). Commercial diets formulated for other fish species (for which the exact composition was unknown) were tested. All diets varied considerably in ingredient composition, protein and energy levels. During the 17 week trial (approximately 120 days), the authors fed juvenile bonytail three commercial diets and observed a 36% gain over initial weight compared to 85% gain over initial when fed a diet high in protein and energy, higher levels of invertebrate meals, and less fish meal and lower carbohydrate. The authors attributed the weight gain to the palatability of a diet that contained shrimp meal. This observation is comparable to preliminary results of feed studies with June suckers (*Chasmistes liorus*) (F.T. Barrows; manuscript in preparation). Feeding starter diets to June suckers containing high level of krill, and high protein and energy levels, resulted in higher survival and faster growth than other diets tested. This effect may have been due to nutritional factors rather than palatability alone as concluded by Henne et al. (2006).

The authors of this report are currently investigating the composition of the feed ingredients of fatty acid composition and proportion of protein represented by fish meal. Fatty acid content is expected to have played an important role in growth and survival of the June sucker (personal communication: F.T. Barrows). While fish meal represents an ideal nutritional source of dietary protein for carnivorous fish (Barrows and Lellis 1999), the bonytail is a catastomid that is considered omnivorous in its feeding habits. Elevated levels of fish meal resulted in reduced growth in an omnivorous cyprinid (Caldwell et al. 2010), thus it is likely that presence of fish meal in the diet may have had some deleterious effects on growth. Despite the effects of fish meal, overall growth in the juvenile fish offered the reference diet was less than expected. Whether the reduced growth rates were an effect of poor feed conversion due to fish meal, or, an artifact of the experimental system (i.e., conducting the study in aquaria), or, the cumulative effects of stress related to cleaning and monthly weight collections are important variables that need further evaluation in diet optimization. Current research is underway in which the cumulative effects of stress in the species is being characterized and should shed some light on these results.

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Table 1. Protein (%) and fat (%) of six experimental diets (wet weight) and one reference diet (RSD = Razorback sucker diet) fed to juvenile bonytail for 86 days. Values in parenthesis indicate difference in targeted nutrient level.

Diet	Protein (%)	Fat (%)
1	47.8 (0%)	21.2 (-1%)
2	46.6 (-2%)	15.3 (-2%)
3	47.0 (-1%)	10.6 (-1%)
4	41.7 (0%)	20.3 (-1%)
5	41.1 (-1%)	15.4 (-2%)
6	39.3 (-2%)	10.4 (-2%)
RSD	45.7	17.9

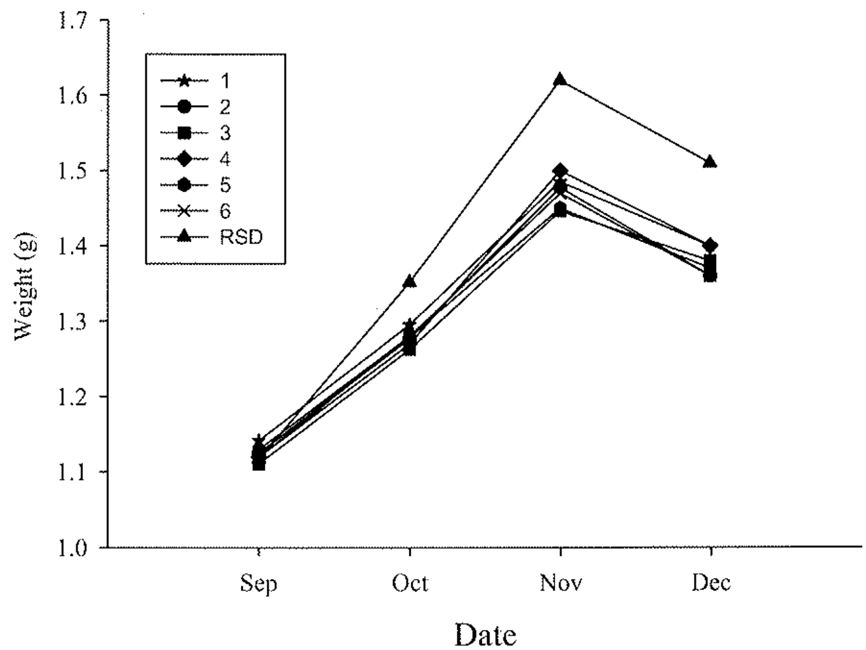

Figure 1. Weight gain (g) for juvenile bonytail fed six experimental diets and one reference for 86 d at the New Mexico State University Geothermal Fish Culture and Research Facility in Las Cruces. Values are means of four replicates per diet.

Table 2. Average specific growth rate (% per day; SGR_W) of juvenile bonytail fed six experimental diets and one reference diet (RSD) at New Mexico State University Geothermal Fish Culture and Research Facility in Las Cruces. Values are means of four replicates per diet.

	Diets						
	1	2	3	4	5	6	RSD
SGR_W (%/d)	0.3	0.3	0.3	0.3	0.3	0.3	0.5

$SGR_W = 100 \times \{(\log_e[\text{final weight}] - \log_e[\text{initial weight}]) \div \text{total experiment days}\}$.

Table 3. Weight changes between the beginning (Initial) and end of the feed trial (Final) for juvenile bonytail fed six experimental diets for 86 d at the New Mexico State University Geothermal Fish Culture and Research Facility in Las Cruces. Weight gain (% BW) is the total overall growth by the end of the feed trials. Values are mean weight (g) of four replicates per diet.

Diet	Weight (g)		Weight Gain[a]
	Initial	Final	(% BW)
1	1.14	1.40	22.8
2	1.13	1.36	20.4
3	1.11	1.38	24.3
4	1.12	1.40	25.0
5	1.13	1.37	21.2
6	1.12	1.36	21.4
RSD	1.12	1.51	34.8

[a] Weight gain or growth (%) expressed as percent of initial body weight (% BW).

Table 4. Protein (%) and fat (%) of bonytail (wet weight) fed six experimental diets and one reference diet (RSD = Razorback sucker diet) for 86 days. Protein (%) and fat (%) of respective diets (wet weight) are presented for comparison purposes.

Diet	Feed Protein (%)	Fish Protein (%)	Feed Fat (%)	Fish Fat (%)
1	47.8	15.81	21.2	12.92
2	46.6	16.14	15.3	11.13
3	47.0	16.57	10.6	12.71
4	41.7	15.98	20.3	12.47
5	41.1	14.84	15.4	12.44
6	39.3	14.77	10.4	11.95
RSD	45.7	15.06	17.9	11.04