

SELECTED ASPECTS OF THE NATURAL HISTORY AND CULTURE OF GILA CHUB

by

Andrew Alan Schultz

A Dissertation Submitted to the Faculty of the

COLLEGE OF AGRICULTURE

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

WITH A MAJOR IN NATURAL RESOURCE STUDIES
AND A MINOR IN ECOLOGY AND EVOLUTIONARY BIOLOGY

In the Graduate College

THE UNIVERSITY OF ARIZONA

2009

THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

As members of the Dissertation Committee, we certify that we have read the dissertation prepared by Andrew A. Schultz entitled, "Selected aspects of the natural history and culture of Gila chub" and recommend that it be accepted as fulfilling the dissertation requirement for the degree of Doctor of Philosophy.

Scott A. Bonar

Date

Courtney Conway

Date

Kevin Fitzsimmons

Date

Peter N. Reinthal

Date

Cecil Schwalbe

Date

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copies of the dissertation to the Graduate College. I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

Dissertation Director: Scott Bonar

Date

STATEMENT BY AUTHOR

This dissertation has been submitted in partial fulfillment of requirements for an advanced degree at the University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the library.

Brief quotations from this dissertation are allowable without special permission, provided that accurate acknowledgement of the source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: _____ Andrew Schultz

ACKNOWLEDGMENTS

I gratefully acknowledge my major advisor and mentor Dr. Scott Bonar for his enduring effort to help me grow as a professional, and my committee members Dr. Courtney Conway, Dr. Kevin Fitzsimmons, Dr. Peter Reinthal, and Dr. Cecil Schwalbe for their guidance, insightful criticism, and continued support. I thank Dr. Robert Frye and Dr. Mitch McClaran for their belief in me as a scientist and person. I thank Jeff Simms of Bureau of Land Management (BLM) for his tireless support on numerous levels. I thank all the professionals at the BLM in Safford and Tucson, Arizona for their support, especially Heidi Blasius. I thank all the staff and professionals at the Arizona Game and Fish Department (AGFD) including Robyn Beck, Robert Bettaso, Chris Cantrell, Mike Childs, Dean Foster (former AGFD), Melissa Kreighbaum, Don Mitchell, John Romero, Jeff Sorensen, David Ward, and many others. I thank Dr. Paul Barrett, Roger Hamman, and Manuel Ulibarri of the United States Fish and Wildlife Service (USFWS); Rob Clarkson from the Bureau of Reclamation, Dr. David Propst from the New Mexico Game and Fish Department and all the other professionals and staff associated with the Central Arizona Project (CAP) Transfer Fund. I thank Joshua Taiz of the United States Forest Service (USFS) and the staff associated with the USFS Santa Catalina Ranger District. I thank Craig Ivanyi and Ken Wintin of the Arizona Sonora Desert Museum. I thank all staff and faculty at the University of Arizona that assisted in any respect during this study, especially Anne Hartley, Linda Lee, Dee Simons, Cecily Westphal, and Carol Yde. A special thanks goes to Alison Iles of the Arizona Cooperative Fish and Wildlife Research Unit, and Dr. Patrick Reid and Dr. Malcolm Zwolinski of the University of Arizona's School of Renewable Natural Resources. I thank Dr. Mark Borgstrom and Dr. Robert Steidl for statistical advice. Thanks to Hoori Ajami, Andrew Honaman, Mickey Reed, and Gina Schultz for technical support. Special thanks to Fluo Mark and Paul Janssen for supplying fluorescent grit, providing advice, and sharing information on fish marking. Thanks also to the professionals at the Washington Department of Fish and Wildlife for their guidance and assistance regarding marking equipment and techniques. Thanks to Emmet McGuire of the USGS for providing discharge data. Thanks to Dr. William Matter for design support and his role in my development as a scientist. Thanks to Yashica Anderson, Tom Archdeacon, Daniel Arevalo, Sachiko Aso, Devin Beck, Sheldon Caldwell-Meeks, Dr. Jeanette Carpenter, Corissa Carveth, Brian Chaszar, Stan Culling, Teresa Drury, Walker Thomas Eldridge, Andrea Francis, Jason Kline, Dan Koepke, Guillermo Ley, Naomi Lupe, Michelle Martin, Shawna Hehr, Jake Mundy, Abe Randolph, Michelle Riley, Gina Schultz, Jasmine Schultz, Skye Schultz, Erica Sontz, John Sottolare, Greg Stoeher, Sean Tackley, Cyndi Tuell, Matt Walton, David Ward, and Sebastian Zeltzer for their assistance in the field and laboratory. Thanks to Roger Sorensen and Kirk Young of AGFD, and anonymous persons for their review of material within this dissertation. Thanks to all the other individuals, government agencies, non-governmental groups, and businesses not mentioned here that assisted with the project.

This project was funded by the BLM, AGFD Heritage Fund, the CAP Transfer Fund, the University of Arizona, and the Arizona Cooperative Fish and Wildlife Research Unit and its cooperators.

DEDICATION

For my daughters, Skye, Jasmine, and Sydney.

TABLE OF CONTENTS

LIST OF TABLES.....	9
LIST OF FIGURES.....	11
ABSTRACT.....	12
INTRODUCTION.....	14
PRESENT STUDY	17
LITERATURE CITED.....	20
APPENDIX A: Selected aspects of the natural history of Gila chub.....	22
Abstract.....	24
Introduction.....	25
Study Areas.....	27
Methods.....	30
<i>Habitat Selection</i>	30
<i>Reproduction</i>	34
<i>Movement</i>	35
Results.....	37
<i>Habitat Selection</i>	37
<i>Reproduction</i>	45
<i>Movement</i>	48
Discussion.....	49
<i>Habitat</i>	49
<i>Reproduction</i>	53
<i>Movement</i>	55

TABLE OF CONTENTS (continued)

Management Implications.....	57
Acknowledgments.....	61
Literature Cited.....	62
Tables, Figures, and Appendices.....	68
APPENDIX B: Spawning and hatching of Gila chub.....	97
Abstract.....	99
Introduction.....	100
Methods.....	101
Results.....	102
Discussion.....	104
Acknowledgments.....	108
Literature Cited.....	109
Tables, Figures, and Appendices.....	112
APPENDIX C: Effect of feed type, water temperature, and rearing density on growth and survival of larval and juvenile Gila chub.....	113
Abstract.....	114
Introduction.....	116
Methods.....	116
<i>Feed Type</i>	116
<i>Temperature</i>	118
<i>Density</i>	119
Results.....	121

TABLE OF CONTENTS (continued)

<i>Feed Type</i>	121
<i>Temperature</i>	122
<i>Density</i>	123
Discussion.....	124
<i>Feed Type</i>	124
<i>Temperature</i>	126
<i>Density</i>	128
Acknowledgments.....	130
Literature Cited.....	131
Tables, Figures, and Appendices.....	135

LIST OF TABLES

Table A.1.	Comparison of habitat types and catch data of Gila chub <i>Gila intermedia</i> from Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County).....	77
Table A.2.	Mean length of Gila chub <i>Gila intermedia</i> per habitat type in Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County).....	78
Table A.3.	Comparison of total cover categories and catch data of Gila chub <i>Gila intermedia</i> from Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County).....	79
Table A.4.	Comparison of woody cover categories and catch data of Gila chub <i>Gila intermedia</i> from Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County).....	80
Table A.5.	Comparison of vegetative cover categories and catch data of Gila chub <i>Gila intermedia</i> from Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County).....	81
Table A.6.	Comparison of rock/boulder cover categories and catch data of Gila chub <i>Gila intermedia</i> from Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County).....	82
Table A.7.	Comparison of algal cover categories and catch data of Gila chub <i>Gila intermedia</i> from Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County).....	83
Table A.8.	Comparison of mean depth categories and catch data of Gila chub <i>Gila intermedia</i> from Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County).....	84
Table A.9.	Comparison of maximum depth categories and catch data of Gila Chub <i>Gila intermedia</i> from Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County).....	85
Table A10.	Mean total length of Gila chub <i>Gila intermedia</i> per maximum depth category in Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County).....	86
Table A11.	Comparison of mean flow velocity (cm/s) categories and catch data of Gila chub <i>Gila intermedia</i> from Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County).....	87

LIST OF TABLES - Continued

Table A12.	Comparison of substrate categories and catch data of Gila chub <i>Gila intermedia</i> from Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County).....	88
Table A13.	Summary of physical/chemical/hydraulic measures taken in Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County).....	89
Table A14.	Identification, movement, and growth data on PIT-tagged Gila chub <i>Gila intermedia</i> recaptured in Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County).....	90
App. A.1.	Identification and location information for PIT-tagged Gila chub <i>Gila intermedia</i> from Bonita Creek, Arizona (Graham County).....	91
App. A.2.	Identification and location information for PIT-tagged Gila chub <i>Gila intermedia</i> from Cienega Creek, Arizona (Pima County).....	95
Table C.1.	Nutrient analysis of 2 natural diets and 7 prepared/commercial diets fed to three size classes of Gila chub <i>Gila intermedia</i>	135
Table C.2.	Mean weight and length gains per feed type for larval, small juvenile and large juvenile Gila chub <i>Gila intermedia</i>	136
Table C.3.	Mean weight and length gains per test temperature for larval, small juvenile and large juvenile Gila chub <i>Gila intermedia</i>	137
Table C.4.	Mean weight and length gains per rearing density for larval, small juvenile and large juvenile Gila chub <i>Gila intermedia</i>	138

LIST OF FIGURES

Figure A.1.	Map of sampling population (N=34) of approximate 100-m reaches and randomly chosen reaches (N=15) with sampling date in upper Cienega Creek, Arizona (Pima County).....	68
Figure A.2.	Map of sampling population (N=49) of approximate 100-m reaches and randomly chosen reaches (N=13) with sampling date in lower Cienega Creek, Arizona (Pima County).....	69
Figure A.3.	Map of sampling population (N=40) of approximate 100-m reaches and randomly chosen reaches (N=19) with sampling date in Bonita Creek, Arizona (Graham County).....	70
Figure A.4.	Mean gonadosomatic index (GSI) values (\pm SEM) per month for female Gila chub <i>Gila intermedia</i> in Bonita Creek, Arizona (Graham County).....	71
Figure A.5.	Mean gonadosomatic index (GSI) values (\pm SEM) per month for female Gila chub <i>Gila intermedia</i> in Cienega Creek, Arizona (Pima County).....	72
Figure A.6.	Total catch of small juvenile Gila chub <i>Gila intermedia</i> per month in Bonita Creek, Arizona (Graham County).....	73
Figure A.7.	Total catch of small juvenile Gila chub <i>Gila intermedia</i> per month in Cienega Creek, Arizona (Pima County).....	74
Figure A.8.	Frequency of occurrence for Gila chub <i>Gila intermedia</i> having moderate, strong, or very strong spawning colors, and/or expressing gametes/tubercles per month in Bonita Creek, Arizona (Graham County).....	75
Figure A.9.	Frequency of occurrence for Gila Chub <i>Gila intermedia</i> having moderate, strong, or very strong spawning colors, and/or expressing gametes/tubercles per month in Cienega Creek, Arizona (Pima County).....	76
Figure B.1.	Time to hatch plotted against mean incubation temperature (with linear regression fit) for larval Gila chub <i>Gila intermedia</i>	112

ABSTRACT

I studied habitat preferences of Gila chub in a canyon-bound system (Bonita Creek) and a marsh system (Cienega Creek). Gila chub in Bonita Creek, frequently occurred in a broader range of habitat types and conditions than Gila chub in Cienega Creek. Gila chub in Cienega Creek were highly pool oriented. In contrast, Gila chub in Bonita Creek generally preferred, or used in proportion, swifter shallower habitat types. Segregation between size classes in relation to habitat variables was noted, but was less than expected. I studied other life-history characteristics as well and found reproduction commencing in February, peaking in spring, and dropping off as summer begins. Spawning in the fall is suggested by the presence of small YOY and gonad development.

I also evaluated methods to spawn and rear Gila chub. Following initial spawning, Gila chub spawned consistently in the laboratory without hormonal, chemical, photoperiod, or drastic temperature and substrate manipulation, during all times of the year. Spawns were noted at temperatures ranging from about 15 to 26°C but spawning above 24°C occurred infrequently.

Larval Gila chub fed a commercial diet grew the same or slightly better than those fed thawed *Artemia* sp. nauplii, and significantly better than those fed chicken *Gallus domesticus* egg-yolk powder, but survived significantly better when fed *Artemia*. Despite the latter, observations suggest *Artemia* nauplii may be difficult for first-feeding larval Gila chub to handle. Thawed chironomid sp. larvae clearly outperformed prepared commercial feeds for small and large juvenile Gila chub with respect to growth. Growth of larval Gila chub was highest at 28°C and lowest at 32°C, while survival of larval Gila chub was highest at 24°C and lowest at 20°C. Spinal deformities were common (about 47%) for larval Gila chub reared at 32°C but generally uncommon for those reared at lower temperatures. Water temperatures from 20-28°C appear

suitable for rearing larval Gila chub, with temperatures from 24-28°C more optimal. Water temperatures from 20-29°C appear suitable for rearing juvenile Gila chub. My data strongly support increasing rearing density having a negative effect on growth and survival (larval only) of Gila chub.

Although populations of Gila chub share many natural history traits, my data suggests habitat use can vary among systems. It is possible unique preferences and strategies exist between different populations of Gila chub. Thus managers should be cautious about applying information based on one population to others. The future of Gila chub may someday depend in part on hatchery propagation to provide specimens for restocking formerly occupied habitats and establishing refuge populations. Information from my study can aid future efforts to successfully spawn and rear Gila chub and related species.

INTRODUCTION

Gila chub *Gila intermedia* is a cyprinid endemic to the Gila River basin of central and southeast Arizona, southwest New Mexico, and northern Sonora, Mexico (Rinne 1976; Minckley and DeMarais 2000). Males are usually smaller than females which reach total lengths of 200 mm or more. Coloration is darker dorsally, lighter ventrally, and can be brown, olivaceous, silvery or golden. At its peak, spawning coloration is typified by deep reds and oranges. A nuchal hump develops in some adults.

Populations of Gila chub have been reduced or extirpated throughout their range due to loss and modification of aquatic habitats (Hendrickson and Minckley 1984; Vives 1990; Weedman et al. 1996) and the introduction of nonnative species (Minckley et al. 1977; Minckley and Deacon 1991; Dudley and Matter 2000). This species is currently limited to about 29 isolated streams, cienegas, and springs (USFWS 2005); only one of which contains a population that was considered stable and secure by Weedman et al. (1996). Gila chub is listed as endangered with critical habitat under the United States Endangered Species Act (USFWS 2005).

The natural-history characteristics of Gila chub are poorly understood (Weedman et al. 1996). The limited knowledge of Gila chub is a deterrent to the recovery of this species (Vives 1990). Previous knowledge of habitat use by Gila chub is limited and largely qualitative. Gila chub are thought to be a highly secretive species, preferring calm deep water, or remaining close to various cover types (Rinne and Minckley 1991). Adults are often found in deep pools and eddies, below areas with swift current. Small young-of-year (YOY) inhabit shallow water among plants or debris, while older juveniles are often found in higher velocity areas (Minckley

1973). In Sabino Creek, Arizona, Dudley (1995) found Gila chub to be highly reclusive in winter, occupying interstitial spaces, with activity increasing as water temperature increased.

Information on reproductive ecology of Gila chub is largely limited and qualitative. Outside of constant temperature springs, spawning is thought to occur from late spring into summer (Minckley 1973; Griffith and Tiersch 1989; Nelson 1993). Minckley (1973) observed suspected spawning behavior over submerged aquatic vegetation, with large females being followed by several smaller males. Gila chub may reach sexual maturity by the end of their first year but most mature in their second or third year of life (Griffith and Tiersch 1989).

I am unaware of any quantitative data on the movement patterns of Gila chub. Closure of potential immigration routes to preclude invasion of nonnative fishes is recognized as a management option to protect Gila chub (Weedman et al. 1996) and other species of fishes native to the Southwest. The construction of barriers to prevent nonnative fishes from invading upstream areas inhabited by native fishes has been proposed for numerous streams throughout Arizona, including Bonita Creek and Redfield Canyon.

Previous observations (Ken Wintin, personal communication, Arizona-Sonora Desert Museum; Jeanette Carpenter, personal communication, U.S. Geological Survey; and personal observation) confirm that Gila chub have the ability to spawn and rear in captivity but culture techniques and requirements are largely unknown. The limited information available on culture techniques and general life-history of Gila chub hampers their recovery (Vives 1990). The future of Gila chub may someday depend in part on hatchery propagation to provide specimens for restocking formerly occupied habitats and establishing refuge populations.

My objectives were to identify and compare habitat preferences, reproductive ecology, and movement patterns of Gila chub in geologically different stream systems (i.e., a

canyon-bound stream versus an interior marshland stream); and to develop spawning and rearing techniques for Gila chub, specifically investigating the effect of feed type, temperature and density on growth, survival and overt fish appearance/health of larval and juvenile Gila chub.

PRESENT STUDY

The methods, results, and conclusions of this study are presented in the chapters to be submitted for publication appended to this dissertation. The following is a summary of the most important findings in these chapters.

I studied the habitat preferences, reproductive ecology, and movement patterns of Gila chub in Bonita Creek and Cienega Creek, Arizona (Appendix A). Overall catch per unit effort (CPUE) of Gila chub was almost three times greater in Bonita Creek than Cienega Creek, and six times greater for adult chub. Gila chub in Bonita Creek, occurred in a broader range of habitat types and conditions than Gila chub in Cienega Creek. Gila chub in Cienega Creek were highly pool-oriented. In contrast, Gila chub in Bonita Creek generally preferred, or used in proportion, swifter, shallower habitat types. Segregation between size classes in relation to habitat was noted, but was less than expected. Gila chub started to reproduce in February and peak spawning occurred at the beginning of spring, dropping off as summer began. Fall spawning was suggested by the presence of fry and adult gonad development in the fall. Adult and juvenile Gila chub showed little movement.

I identified methods to spawn and hatch Gila chub in captivity (Appendix B). I also investigated techniques to rear Gila chub including the effect of feed type temperature, and density on growth, survival, and overt appearance/health of larval and juvenile Gila chub (Appendix C). Following initial spawning, Gila chub spawned consistently in the laboratory without hormonal, chemical, photoperiod, temperature or substrate manipulation, during all times of the year. Spawns were recorded at temperatures ranging from about 15 to 26°C; however, I noted that Gila chub spawned infrequently at temperatures above 24°C. Multiple spawning attempts per year per individual are likely. There was a strong, inverse relationship

between time to hatch and incubation temperature. Hatch rate of eggs averaged 99.43% and larval Gila chub accepted a variety of natural and formulated diets at first feeding.

Larval Gila chub fed a commercial larval fish diet grew the same or slightly better than those fed thawed *Artemia* sp. nauplii, and significantly better than those fed chicken *Gallus domesticus* egg-yolk powder, but survived significantly better when fed *Artemia*. Despite the latter, observations suggest *Artemia* nauplii may be difficult for first-feeding larval Gila chub to handle. Thawed chironomid sp larvae clearly outperformed prepared commercial feeds for small and large juvenile Gila chub with respect to growth; however, survival was 100% for all feed treatments. Overt appearance/health of larval and juvenile Gila chub remained largely unchanged during all experiments. First-feeding larval Gila chub may be reared on a natural or prepared diet but those fed natural feed exhibited higher survival. Based on diets tested, I recommend juvenile Gila chub be fed a natural diet if faster growth is paramount to objectives.

I tested the effect of four different water temperatures on growth, survival, and overt health/appearance of larval (20, 24, 28, and 32°C) and two sizes of juvenile (20, 23, 26, and 29°C) Gila chub. Growth of larval Gila chub was highest at 28°C and lowest at 32°C, while survival of larval Gila chub was highest at 24°C and lowest at 20°C. Spinal deformities were common (about 47%) for larval Gila chub reared at 32°C but generally uncommon for those reared at lower temperatures. Although growth of small (32-49 mm TL) and large (52-72 mm TL) juvenile Gila chub generally increased with temperature, differences were not statistically significant. Their survival was 100% in all experiments and I noticed no external abnormalities. Water temperatures from 20-28°C were suitable larval Gila chub growth and survival, while fish grew best within temperatures from 24-28°C. Water temperatures from 20-29°C appear suitable for rearing juvenile Gila chub.

I tested the following densities to rear Gila chub: 0.065 g/L (38.9 fish/L), 0.540 g/L (319.5 fish/L), and 1.343 g/L (795 fish/L) for larval chub (6.3-6.8 mm TL); 3.618 g/L (4.0 fish/L), 16.986 g/L (20.1 fish/L), and 60.145 g/L (68.3 fish/L) for small juveniles (36-47 mm TL); and 1.681 g/L (0.4 fish/L), 14.346 g/L (2.7 fish/L), and 53.942 g/L (8.4 fish/L) for large juveniles (57-95 mm TL). Mean length and weight gain of larval and large juvenile Gila chub were inversely related to rearing density. Survival of larval Gila chub was significantly greater for those groups reared at low densities. Juvenile Gila chub survival approached 100% for all density treatments. Few oddities in overt fish appearance/health were noted during the experiments and ontogeny was related to growth rates. My data strongly supports that increasing density has a negative effect on growth and survival (larval only) of Gila chub.

LITERATURE CITED

- Dudley, R.K. 1995. The effects of green sunfish on the distribution, abundance and habitat use of Gila chub in Sabino Creek, Arizona. Master's thesis. University of Arizona, Tucson, Arizona.
- Dudley, R.K., and W.J. Matter. 2000. Effects of small green sunfish (*Lepomis cyanellus*) on recruitment of Gila chub (*Gila intermedia*) in Sabino Creek, Arizona. The Southwestern Naturalist 45:24-29.
- Griffith, J.S., and T.R. Tiersch. 1989. Ecology of fishes in Redfield Canyon, Arizona, with emphasis on *Gila robusta intermedia*. Southwestern Naturalist 34:131-134.
- Hendrickson, D.A., and W.L. Minckley. 1984. Cienegas-vanishing climax communities of the American Southwest. Desert Plants 6:141-175.
- Minckley, W.L. 1973. Fishes of Arizona. Sims Printing Company, Inc., Phoenix, Arizona.
- Minckley, W.L., and J.E. Deacon, editors. 1991. Battle against extinction: native fish management in the American West. University of Arizona Press, Tucson, Arizona.
- Minckley, W.L., and B.D. DeMarias. 2000. Taxonomy of chubs (Teleostei, Cyprinidae, genus *Gila*) in the American Southwest with comments on conservation. Copeia 2000:251-256.
- Minckley, W.L., J.N. Rinne, and J.E. Johnson. 1977. Status of the Gila topminnow and its co-occurrence with mosquitofish. United States Department of Agriculture, Research paper RM-198, Fort Collins, Colorado.
- Nelson, B. 1993. Spawning characteristics of Gila chub (*Gila intermedia*) in Cienega creek, Pima County, Arizona. Report for USDI Bureau of Land Mangement, Tucson Resource Area, Arizona.

- Rinne, J.N. 1976. Cyprinid fishes of the genus *Gila* from the Lower Colorado River basin. *Wasmann Journal of Biology* 34:65-107.
- Rinne, J.N., and W.L. Minckley. 1991. Native fishes of arid lands: a dwindling resource of the desert Southwest. United States Department of Agriculture, General Technical Report RM-206, Ft. Collins, Colorado.
- U.S. Fish and Wildlife Service. 2005. Endangered and threatened wildlife and plants; listing the Gila chub as endangered with critical habitat; final rule. *Federal Register* 70(211):66664-66721.
- Vives, S.P. 1990. Element Stewardship Abstract for *Gila intermedia*. Tucson: The Nature Conservancy, Arizona Field Office.
- Weedman, D.A., A.L. Girmendonk, and K.L. Young. 1996. Status review of Gila chub, *Gila intermedia*, in the United States and Mexico. Nongame and Endangered Wildlife Program, Arizona Game and Fish Department, Technical Report 91, Phoenix, Arizona.

APPENDIX A

Selected aspects of the natural history of Gila chub

SELECTED ASPECTS OF THE NATURAL HISTORY OF GILA CHUB

Andrew A. Schultz

Arizona Cooperative Fish and Wildlife Research Unit

United States Geological Survey

School of Natural Resources

University of Arizona

104 Biological Sciences East

Tucson, AZ 85721

ABSTRACT

Little is known about the natural history of Gila chub *Gila intermedia*, a cyprinid endemic to the Gila River Basin of Arizona, New Mexico, and Mexico and recently listed as endangered under the U.S. Endangered Species Act. I studied habitat preferences, reproductive ecology, and movement patterns of Gila chub in Bonita Creek and Cienega Creek, Arizona. Total CPUE of Gila chub was almost three times greater in Bonita Creek than Cienega Creek, and six times as many adults were caught in Bonita Creek. Gila chub in Bonita Creek occupied a broader range of habitat types than those in Cienega Creek. Gila chub in Cienega Creek were highly pool-oriented. In contrast, Gila chub in Bonita Creek generally preferred, or used in proportion to their availability, swifter shallower habitat types. There was some segregation in habitat between different size classes, but it was less than expected. Reproduction appeared to start in February and peak at the beginning of spring, declining as summer began. However, I captured fry and noted some Gila chub gonad development in fall, suggesting that fall spawning occurs. Adult and juvenile Gila chub showed little movement. Although populations of Gila chub share many natural history traits, my data suggest habitat use can vary among systems. It is possible unique preferences and strategies exist between different populations of Gila chub. Thus managers should be cautious about applying information based on one population to others.

INTRODUCTION

Gila chub *Gila intermedia* is a cyprinid endemic to the Gila River basin of central and southeast Arizona, southwest New Mexico, and northern Sonora, Mexico (Rinne 1976; Minckley and DeMarais 2000). Males are usually smaller than females which reach total lengths of 200 mm or more. Coloration is darker dorsally, lighter ventrally, and ranges from brown, olivaceous, silvery, and golden. At its peak, spawning coloration is typified by deep reds and oranges. A nuchal hump develops in some adults.

Populations of Gila chub have been reduced or extirpated throughout their range due to loss and modification of aquatic habitats (Hendrickson and Minckley 1984; Vives 1990a; Weedman et al. 1996) and the introduction of nonnative species (Minckley et al. 1977; Minckley and Deacon 1991; Dudley and Matter 2000). This species is currently limited to about 29 isolated streams, cienegas, and springs (USFWS 2005); only one of which contains a population that was considered stable and secure by Weedman et al. (1996). Gila chub is listed as endangered with critical habitat under the United States Endangered Species Act (USFWS 2005).

The natural-history characteristics of Gila chub are poorly understood (Weedman et al. 1996), which hampers their recovery (Vives 1990a). Previous knowledge of habitat use by Gila chub is limited and largely qualitative. Gila chub are thought to be highly secretive, preferring calm deep water, or remaining close to cover (Rinne and Minckley 1991). Adults are often found in deep pools and eddies, below areas of swift current. Small young-of-year (YOY) inhabit shallow water among plants or debris, while older juveniles are often found in higher velocity areas (Minckley 1973). In Sabino Creek, Arizona, Dudley (1995) found Gila chub to be

highly reclusive in winter, occupying interstitial spaces, with activity increasing as water temperature increased.

Spawning characteristics have yet to be defined. Outside of constant temperature springs, spawning is thought to occur from late spring into summer (Minckley 1973; Griffith and Tiersch 1989; Nelson 1993). Minckley (1973) observed suspected spawning behavior over beds of submerged aquatic vegetation, with large females being followed by several smaller males. Gila chub may reach sexual maturity by the end of their first year, but most mature in their second or third year of life (Griffith and Tiersch 1989).

I am unaware of any quantitative data on the movement patterns of Gila chub. Closure of potential immigration routes to preclude invasion of nonnative fishes is recognized as a current management option for populations of Gila chub (Weedman et al. 1996) and other native southwestern fishes. The construction of barriers to prevent nonnative fishes from invading upstream areas inhabited by native fishes has been proposed for numerous streams throughout Arizona, including Bonita Creek and Redfield Canyon. Information on Gila chub movement could be used to help determine if barrier construction is appropriate for specific streams.

My primary objectives for the study were to identify and compare habitat preferences, reproductive ecology, and movement patterns of Gila chub, in geologically different stream systems (i.e., a canyon-bound stream versus an interior marshland stream).

STUDY AREAS

Bonita Creek is a first-order tributary (Graham County, Arizona) of the Gila River. The stream flows southeast from the Nantac Rim between the Gila Mountains before joining the Gila River east of Safford, Arizona. Bonita Creek drains an area of about 958 km² and its elevation ranges from 1,580 m at the headwaters to 960 m at its confluence with Gila River.

Much of Bonita Creek is canyon-bound, especially downstream of its headwaters on the San Carlos Apache Indian Reservation. Within my study reaches downstream of the reservation boundary, habitat was highly heterogeneous consisting of riffles, runs, chutes, cascades, and pools of various depths generally formed by obstructions, bedrock/channel deflection, and beaver (*Castor canadensis*) activity. The stream was lined by a gallery of riparian trees which were mostly Sycamores *Platanus wrightii*, cottonwoods *Populus* spp., ash *Fraxinus* spp., willows *Salix* spp., *Baccharis* spp., and a variety of other vegetation, including some aquatic species. Vegetation of nearby floodplains and uplands included mesquite *Prosopis* spp. and desert scrub.

Perennial flow in Bonita Creek began on the San Carlos Apache Indian Reservation and continued from the reservation boundary about 17 km downstream before going underground then resurfacing about 6 km from the Gila River. This ephemeral reach was tied to intake structures the city of Safford uses to supply water for municipal uses. Gila chub inhabit most of perennial Bonita Creek, but were considered rare from the confluence with the Gila River upstream to the aforementioned ephemeral reach (this section not included in my study scope). In contrast, nonnative fishes were abundant below, yet absent above, this reach (Weedman et al. 1996; Jeff Simms, Bureau of Land Management [BLM], personal communication). Four other native fishes (above the Safford withdrawal) are currently extant in Bonita Creek (i.e., longfin

dace *Agosia chrysogaster*, speckled dace *Rhinichthys osculus*, Sonora sucker *Catostomus insignis*, and desert sucker *Catostomus clarkii*.

Stream flow in Bonita Creek is elevated in the winter and early spring, decreases in May-June and then increases again during the monsoon season (mid July-September). Flash flooding is common, occurring here more often than in Cienega Creek because of its canyon-bound nature. Annual mean discharge in Bonita Creek is about 0.34 m³/s, and annual maximum discharge averages about 86.83 m³/s with the largest maximum discharge of 552.18 m³/s occurring in January 1993 (period of record 1981-2005). Larger peak discharges have likely occurred outside this period in recent times (Hadley et al. 1993).

The BLM owns and manages most of the land along the perennial sections of Bonita Creek below the reservation boundary. Bonita Creek is included within the Gila Box Riparian National Conservation Area.

Cienega Creek is a third-order tributary of the Santa Cruz River (Pima and Santa Cruz County, Arizona). It flows north through a valley lying between the Santa Rita Mountains and Empire Mountains on the west and the Whetstone Mountains on the east, and joins Pantano Wash near Vail, Arizona (Pima County). Cienega Creek drains an area of about 749 km² and its elevation ranges from about 1,520 m at the headwaters to about 1,070 m at its confluence with Pantano Wash.

Although portions of Cienega Creek are largely incised and bound by high earthen banks, largely due to human impacts beginning in the 1890's (see Hendrickson and Minckley [1984] among others), most of the creek is not canyon bound. Cienega Creek is marshy, sometimes boggy (especially in its upper reaches) often containing dense emergent and submergent vegetation characteristic of its namesake. Deep pools are often connected by shallow runs or

marsh, most of which are dominated by fine particle and organic substrates. Riparian woodlands, mostly cottonwoods and willows, vary in density and magnitude throughout the stream course. Vegetation of nearby floodplains and uplands includes mesquite, desert scrub, and semi-desert grassland (e.g., big sacaton *Sporobolus wrightii*). A detailed description of cienega habitats and their characteristics is in Hendrickson and Minckley (1984).

Approximately 13.6 km of Cienega Creek, including Mattie Canyon and Empire Gulch, starting at the headwaters near Gardner Canyon to downstream of “the narrows” is inhabited by Gila chub (Jeff Simms, BLM, personal communication). Two other species of native fishes are extant in Cienega Creek (i.e., longfin dace *A. chrysogaster* and Gila topminnow *Poeciliopsis occidentalis occidentalis*). Stream flow is stable in the winter and early spring with noticeable decreases in May-June, eventually increasing during the monsoon season (mid July-September). Flooding occurs regularly, especially during the monsoon season. Annual mean discharge in Cienega Creek averages about 0.03 m³/s, and annual maximum discharge averages about 6.53 m³/s with the largest maximum discharge of 12.15 m³/s occurring in August 2005 (period of record 2001-2005).

The BLM manages the Las Cienegas National Conservation Area (LCNCA), which encompasses all of the perennial section of Cienega Creek in which Gila chub occur south of U.S. Interstate 10. The LCNCA is managed to conserve aquatic, riparian, and associated wildlife values. Restoration projects are ongoing including grazing restrictions, road closures, and channel restoration. Cienega Creek is the only stream within the Gila chub's range that was listed as stable and secure by Weedman et al. (1996). Nonnative fishes have not been recorded from Cienega Creek and the threat of contamination from migration or surreptitious introduction

from local sources is low (Weedman et al. 1996). Beaver formerly occurred within the Cienega Creek valley (Hendrickson and Minckley 1984) but have long been extirpated.

Due to the presence of native fish communities, and relatively natural flow regimes and channels, Cienega Creek and Bonita Creek provide ideal settings to study the biology of Gila chub in different habitats (i.e., a largely canyon-bound stream versus an interior marshland stream).

METHODS

Habitat Selection

Sampling design. – I used a random stratified design (Brown and Austen 1996) to select stream reaches in which to sample Gila chub from 3-29-02 to 5-17-05 during daylight hours. I stratified both streams by upper and lower sections. These sections were divided into 100-m stream reaches ($N = 34$ for upper Cienega Creek and 49 for lower Cienega Creek; $N = 20$ for upper Bonita Creek and 20 for lower Bonita Creek) (Figures 1, 2, and 3, respectively). Each randomly chosen reach was subdivided into habitat types using criteria in McMahon et al. (1996) with the addition of a marsh habitat type.

Capture of Gila chub. – I captured Gila chub within designated habitats using a Smith-Root Model 12-B POW backpack electrofisher set at a frequency of 60-Hz pulsed DC and a 6-ms pulse width. Voltage usually remained at 300 V. Care was taken to thoroughly sample each habitat type with equal effort per unit area. Time permitting, if no Gila chub were captured within a chosen reach, or the reach was dry, an adjacent reach was sampled on the same day. I held captured chub in aerated buckets treated with Stress Coat™ (Aquarium Pharmaceuticals,

Chalfont, PA). I measured total length (TL) for all captured Gila chub. Following measurements, I returned Gila chub to the area from which they were captured.

Physical features. – Prior to instream sampling activity I visually quantified stream cover, substrate, canopy cover, bank stability, water clarity, and cattle use for each habitat type. I defined stream cover, recorded as a percent area of each habitat type, as the physical features of stream relief that could provide cover to fish (i.e., woody, vegetative, rock/boulder, and algal cover), which were instream or closely overhanging (< 0.5 m) the stream surface. I classified substrate according to a modified Wentworth scale (McMahon et al. 1996) (boulder > 256 mm, cobble 64-256 mm, pebble 32-64 mm, gravel 8-32 mm, sand/gravel 0.5-8 mm, fines < 0.5 mm, bedrock, and organic material [i.e., algae/detritus/vegetation]) and recorded it as a percent area of each habitat type. I defined canopy cover as that cover directly above the stream and wetted stream bank, and categorized it as sparse (0-25%), moderate (25-50%), dense (50-75%), and very dense (75-100%). Bank stability was categorized as poor, fair, or good. Poor bank stability was characterized by sloughing banks with little or no vegetative or other stabilizing support. Good bank stability was characterized as having no sloughing banks and well established bank support. Fair bank stability was a moderate condition between poor and good bank stability. I categorized water clarity as clear (water is largely transparent with stream bottom in clear view), slightly to moderately stained (although most submerged features are seen by an observer, dissolved and/or suspended material reduces instream visibility), or highly stained to opaque (a high concentration of dissolved and/or suspended material greatly reduces or eliminates instream visibility). I defined cattle use as the presence/absence of cattle feces within the riparian channel. I eliminated observer bias by using the same observer for all visual estimates.

Physico-chemical measures. – Within each designated habitat type I measured four physico-chemical factors during mid-day (usually between 1300 and 1500 hrs): (1) dissolved oxygen (Hydrolab Quanta [Hach Company, Loveland, CO] and YSI 58 Dissolved Oxygen Meter [Yellow Springs, OH]), (2) temperature, (3) conductivity (Hydrolab Quanta and YSI 30 Salinity, Conductivity, and Temperature Meter), and (4) pH (Hydrolab Quanta and Eutech Instruments pH Testr-2 [(Vernon Hills, IL)]). All instruments were calibrated according to manufacturers' recommendations.

Hydraulic variables. – Following electrofishing I used a point-transect method to quantify depth and velocity of each habitat type. Transects were spaced every 2 m if the habitat length was < 20 m and every 5 m if the length was > 20 m. Habitats 5 m or less were bisected. I recorded stream width at each transect and calculated surface area for each habitat type by summing the area of trapezoidal planes between width measurements. I recorded stream depth at three equidistant points along each transect (sensu Platts et al. 1983). At each point, a Global Flow Probe model FP101 was moved slowly from the surface to the stream bottom and automatically calculated the average velocity. I recorded maximum depth for each habitat type. Discharge information was taken from United States Geological Survey (USGS) online records for measurement stations 0947800 (Bonita Creek) and 0948455 (Cienega Creek).

Data management and analysis. – Habitat and catch data for locations where there may be water, but no stream flow, were not included in the analysis of habitat preference. I used Jacob's electivity index (Jacobs 1974):

$$D = (r - p) (r + p - 2rp)^{-1}$$

where D is the index of electivity, r is the proportion of the resource used by fish, and p is the proportion available in the environment, to measure Gila chub's preference for habitat

type, cover, depth, velocity, and substrate. The index produces values between -1 and $+1$, where -1 indicates total avoidance and $+1$ total preference. As summarized in Baltz (1990), I subdivided the range of the electivity index to better describe habitat selection: -1.00 to -0.50 (strong avoidance), -0.49 to -0.26 (moderate avoidance), -0.25 to $+0.25$ (neutral selection), $+0.26$ to $+0.49$ (moderate preference), $+0.50$ to $+1.00$ (strong preference).

For analysis of substrate preference I used the dominant substrate type of each habitat sampled. Due to the rarity of certain substrate classes I modified some of the categories of the modified Wentworth substrate scale. For Bonita Creek, boulder and cobble were combined into a single substrate category named coarse; pebble, gravel and sand/gravel were combined into a single category named moderate; fines and organic material remained as before. A new category called varied included the habitats where the dominant substrate type was not above 30% of the area. For Cienega Creek, the substrate categories fines and organic material remained unchanged. Due to the lack of other substrate types in Cienega Creek, a category called mixed was included, which was defined as 10% or more of the substrate consisting of something other than fines or organic material.

I used a Chi-Square Goodness of Fit Test to compare observed and expected catch among different levels and categories of habitat variables. I used one-way analysis of variance (ANOVA) with a Tukey-Kramer Multiple Comparisons Procedure, or when appropriate a Kruskal-Wallis ANOVA with a Dwass-Steel-Critchlow-Fligner Multiple Comparisons Procedure, to compare catch per unit effort (CPUE) of Gila chub among different levels and categories of habitat variables.

Reproduction

Behavior. – On each survey trip, I noted any instances of spawning and associated behavior (Vives 1990b). I used direct observation and a video camera to record spawning and associated behavior in the laboratory.

Temporal range. – I removed gonads from sacrificed Gila chub taken throughout the year, on about a monthly basis, in Bonita Creek and Cienega Creek. I divided gonad weight by total fish weight minus the gastrointestinal tract and gonad weight, and multiplied this value by 100 to determine the Gonadosomatic Index (GSI). I plotted mean GSI values by month to estimate the temporal range of the spawning season (Vives 1987a, 1987b). I also used presence of small YOY Gila chub (< 50 mm TL) as a relative index of spawning events. I recorded stream temperature and information about the type of habitat each fish was occupying for those Gila chub taken for GSI measurements outside of habitat selection sampling.

Physical characteristics. – I estimated fecundity of Gila chub by multiplying counts of weighed subsamples of ova from sacrificed chub with eggs near full maturity ($\geq 90\%$ diameter) to the total weight of the ova collected (Crim and Glebe 1990) and counting eggs from spawns in the laboratory. Maturity of eggs was based on egg diameters of newly deposited Gila chub eggs in the laboratory. Ovum diameters were measured using an ocular micrometer and an average diameter was determined from a subsample of ova.

I recorded coloration patterns, and other secondary sexual characteristics, of captured Gila chub during all field activities and in the laboratory. Gila chub were placed into six categories related to spawning coloration: (1) none, (2) very slight (very slight coloring at base of ventral fins including pectoral), (3) slight (as prior but slightly more intense with some coloring present in ventral areas), (4) moderate (as prior but more intense and definite color in ventral

areas), (5) strong (strong colors noted around mouth, opercle, base of ventral fins, and other ventral body areas both anterior and posterior of the vent, moving up toward the midline; dark lateral bands may also be present), and (6) very strong (as prior but extremely vivid; dark lateral bands also present). Slight and very slight spawning colors were typified by muted yellows, oranges and reds. Strong and very strong spawning colors were typified by vivid oranges and reds.

Movement

Adult Gila chub. – From 3-10-02 to 5-26-05 I estimated movement patterns of Gila chub > 90 mm TL through the recapture of tagged fish using electrofishing (as prior) and baited (canned dog food) hoop-nets (3 hoops with \approx 5 mm mesh). I injected sterilized Passive Integrated Transponder (PIT) tags (Biomark, Boise, ID) into the left post-dorsal musculature of adult Gila chub and sealed the wound with an antibacterial ointment. A mini portable reader (Destron Fearing Corporation, St. Paul, MN) was used to scan all captured Gila chub for previously injected PIT-tags.

Juvenile Gila chub. – From 1-4-05 to 3-27-05 I marked juvenile and sub-adult Gila chub (40-80 mm TL) with fluorescent grit pigment (Flou Mark, Paul Janssen, former employee Idaho Fish and Game Department). The flour-sized grit is a dyed polymer. Gila chub were captured using baited hoop-nets and occasionally minnow traps. I used a portable mini-sandblasting unit powered by compressed gas coming from a scuba-sized tank secured in a backpack harness to apply the grit. Pressure during spraying remained near 120 psi. Groups of fish ranging from 1 to 121 individuals were marked within a modified 5-gallon bucket. The procedure consisted of suspending a group of Gila chub above the waterline using a collapsible strainer in the bucket to

a pre-designated height. The sandblasting gun was then inserted into a protective plastic shield approximately 49 cm away from the fish and turned on for 2 seconds. The marked Gila chub were then transferred to an instream enclosure for observation before release, or released immediately into the stream. I marked Gila chub captured in Cienega Creek upstream of the EC 903 road input, from just upstream of the USGS gauging station to the confluence with Mattie Canyon, and near the EC 910-D road crossing (Figures 1 and 2) with chartreuse, red, and orange colored grit, respectively. I marked Gila chub in Bonita Creek within a 1-km of Red Knoll's Canyon Road crossing and Lee Trail Road with chartreuse and red colored grit, respectively (Figure 3). From 4-6-05 to 5-26-05 I searched for marked Gila chub within, upstream, and downstream, of previous marking locations using baited hoop-nets. I examined Gila chub for grit marks in an on-site portable dark room (consisting of a polyvinyl chloride frame and black felt cloth) with a hand-held blue-light-emitting diode flashlight (470-nm-wavelength). Gila chub were considered marked if at least one granule was readily recognizable and unmovable when touched. Detection was aided with the use of amber glasses. I measured total length (mm) and noted location of all granules on recaptured marked Gila chub before returning them to the stream.

Larval Gila chub. – I estimated time of emergence and drift patterns of larval Gila chub using drift nets (about 46 cm wide, 99 cm long, and 0.5 mm mesh) (Kelso and Rutherford 1996). I also opportunistically sampled larval fish using fine-meshed dip-nets in areas lacking significant flow. I set drift nets in Cienega Creek from 6-4-04 to 5-13-05 (N = 33) and in Bonita Creek from 10-9-04 to 5-19-05 (N = 24). Nets were set during the day and during the same night in both streams. In Cienega Creek drift-nets were usually set in a stream reach directly upstream,

and about 400 m upstream, of a natural stream barrier located near a USGS stream gauge (#09484550).

RESULTS

Habitat Selection

A total of 110 habitats (e.g., pool, runs, riffles) and 8,192.2 m² of stream area (1,932 m of linear stream length) were sampled in Bonita Creek. A total of 118 habitats and 9,749.9 m² of stream area (2,431 m of linear stream length) were sampled in Cienega Creek. Overall CPUE of Gila chub was almost three times greater in Bonita Creek (0.046/m²) than in Cienega Creek (0.017/m²), while CPUE of adult chub was six times greater in Bonita Creek (0.031/m²) than Cienega Creek (0.005/m²).

Habitat types. – Runs and pools accounted for over 83% of the habitat area sampled in Bonita Creek (Table 1). Chutes and riffles largely accounted for the rest of the sampled area, with cascades being less than one percent of the total. Gila chub were captured in all five habitat types (i.e., pool, run, riffle, chute, and cascade) in Bonita Creek.

Observed and expected catch of all size classes of Gila chub differed substantially among habitat types in Bonita Creek (Table 1). Small (20-49 mm TL) and large (50-79 mm TL) juveniles in Bonita Creek preferred cascades and runs (Table 1). Adult Gila chub (> 80 mm TL) preferred chutes. Adult Gila chub were frequently found in pools and runs but did not exhibit preference for these habitat types. There was some evidence that mean CPUE of small and large juvenile Gila chub differed among habitat types in Bonita Creek (Table 1). Mean CPUE of adult Gila chub did not differ among habitat types in Bonita Creek.

Pools accounted for almost half of the area sampled in Cienega Creek, with runs and marsh making up the bulk of the remaining half (Table 1). Chutes and riffles comprised little of the area sampled. Only 2 of the 150 Gila chub captured in Cienega Creek for habitat analysis were in habitats other than pools (Table 1). There was strong evidence observed and expected catch of all size classes of Gila chub differed among habitat types in Cienega Creek (Table 1). All size classes of Gila chub in Cienega Creek strongly preferred pools (Table 1). There was some evidence that mean CPUE of small juvenile, large juvenile, and adult Gila chub differed among habitat types in Cienega Creek (Table 1).

Gila chub captured during my habitat selection study ranged from 21-222 mm TL. In Bonita Creek, the largest Gila chub were found in chutes and riffles, and the smallest were captured in cascades (one-way ANOVA = 5.74; $df = 4, 385$; $P < 0.001$, Table 2). In Cienega Creek, the mean total length of Gila chub was about 72 mm. Mean TL of Gila chub captured in Bonita Creek (101 mm) was about 30 mm greater (95% confidence interval: 24 to 37 mm TL) than that in Cienega Creek (71 mm; two-sample t -test = 8.88; $df = 538$; $P < 0.001$).

Total cover. – Habitats with moderate (25-50%) or low (0-25%) total cover dominated the area sampled (83%) in Bonita Creek (Table 3). There was evidence of a difference between the observed and expected catch of all size classes of Gila chub among total cover categories in Bonita Creek (Table 3). Small and large juveniles in Bonita Creek avoided areas with low cover and preferred areas with high cover (75-100%; Table 3). Adult Gila chub did not show preference for habitats based on total cover. Mean CPUE of small juvenile Gila chub in Bonita Creek did not differ among habitats with different levels of total cover (Table 3). There was slight evidence of a difference in mean CPUE of large juvenile and adult Gila chub in Bonita Creek among total cover categories.

In contrast to Bonita Creek, habitats with high total cover, often marsh habitats, were common in Cienega Creek (Table 3). All size classes of Gila chub in Cienega Creek showed a strong preference for habitats with 25-50% cover (Table 3). In contrast to Bonita Creek, juveniles in Cienega Creek did not appear to select habitats with the highest amount of cover. Despite the very low occurrence of Gila chub in habitats with high cover, mean CPUE of small juvenile, large juvenile, and adult Gila chub in Cienega Creek did not significantly differ with respect to total cover (Table 3).

Woody cover. – The majority of habitats in Bonita Creek had 0-10% woody cover (Table 4). There was a significant difference between the observed and expected catch of small juvenile Gila chub among woody cover categories in Bonita Creek (Table 4). Small juvenile Gila chub in Bonita Creek preferred habitats with the highest level of woody cover and avoided habitats with the least cover (Table 4). Large juvenile and adult Gila chub showed no preference for differing amounts of woody cover. Mean CPUE of all size classes of Gila chub in Bonita Creek did not differ with respect to woody cover (Table 4).

Habitats with low (0-10%) woody cover accounted for 67% of the area sampled in Cienega Creek (Table 4). There was strong evidence of a difference between the observed and expected catch of large juvenile Gila chub among woody cover categories in Cienega Creek (Table 4). Selection of habitats in Cienega Creek with regard to woody cover varied among size classes (Table 4). Despite the absence of all size classes of Gila chub from some of the habitats with high woody cover, no significant difference in mean CPUE with respect to woody cover was found (Table 4).

Vegetative cover. – Habitats with low (0-15%) vegetative cover accounted for over half of the area sampled in Bonita Creek (Table 5). There was evidence of a difference between the

observed and expected catch of all size classes of Gila chub among vegetative cover categories in Bonita Creek (Table 5). Small and large juveniles avoided habitats with low vegetative cover and large juvenile and adult chub preferred habitats with the highest proportion (45% +) of vegetative cover (Table 5). Mean CPUE of all size classes of Gila chub in Bonita Creek did not differ among areas containing varying amounts of vegetative cover (Table 5).

In marked contrast to Bonita Creek, a large proportion of Cienega Creek (38.53%) had high vegetative cover (Table 5). However, all size classes of Gila chub in Cienega Creek strongly avoided these habitats (Table 5). There was strong evidence of a difference between the observed and expected catch of all size classes of Gila chub among vegetative cover categories in Cienega Creek (Table 5). In contrast to Bonita Creek, small juvenile Gila chub preferred habitats with the lowest proportion of vegetative cover. There was no evidence mean CPUE of Gila chub in Cienega Creek differed with respect to vegetative cover (Table 5).

Rock/boulder cover. – Habitats with the lowest proportion (0-10%) of rock/boulder cover accounted for over half of the area sampled in Bonita Creek, however, rock/boulder cover was still much more prevalent than in Cienega Creek (Table 6). There was strong evidence of a difference between the observed and expected catch of all size classes of Gila chub among rock/boulder cover categories in Bonita Creek (Table 6). All size classes of Gila chub in Bonita Creek strongly preferred habitats with the lowest proportion (0-10%) of rock/boulder cover and most avoided habitats with high levels of rock/boulder cover (Table 6). There was no difference in mean CPUE of any size class of Gila chub in Bonita Creek with respect to rock/boulder cover (Table 6).

Most habitats sampled in Cienega Creek had little or no rock/boulder cover (Table 6). All size classes of Gila chub in Cienega Creek preferred habitats with at least some rock/boulder

cover. There was evidence mean CPUE of large juvenile and adult Gila chub in Cienega Creek differed with respect to rock/boulder cover, being highest in habitats with 0-5% and 5% + rock/boulder cover, respectively (Table 6). Mean CPUE of adult Gila chub in Cienega Creek did not differ with respect to rock/boulder cover.

Algal cover. – Most habitats sampled in Bonita Creek had a low proportion (0-5%) of algal cover (Table 7). There was moderate evidence of a difference between the observed and expected catch of large juvenile and adult Gila chub among algal cover categories in Bonita Creek (Table 7). All size classes of Gila chub preferred habitats with the lowest proportion of algal cover and largely avoided habitats with higher levels of algal cover (Table 7). Mean CPUE of any size class of Gila chub in Bonita Creek did not differ with respect to algal cover (Table 7).

As with Bonita Creek, most of Cienega Creek had the lowest proportion of algal coverage (Table 7). All size classes of Gila chub in Cienega Creek strongly avoided habitats with the highest level of algal cover but varied in selection of habitats with lower levels of algal cover (Table 7). Mean CPUE of any size class of Gila chub in Cienega Creek did not differ with respect to algal cover (Table 7).

Mean depth. – Mean depths of 0-25 cm accounted for over half of the area sampled in Bonita Creek (Table 8). These shallow habitats were not highly selected by any size class of Gila chub in Bonita Creek (Table 8). All size classes of Gila chub preferred habitats with mean depths of 25-75 cm. Mean CPUE of all size classes of Gila chub in Bonita Creek did not differ with respect to mean depth (Table 8).

Similar to Bonita Creek, mean depths of 0-25 cm accounted for over half of the area sampled in Cienega Creek (Table 8). Habitats with mean depths in this range were avoided by all size classes of Gila chub in Cienega Creek as well, and all size classes preferred habitats with

moderate mean depths (Table 8). There was strong evidence of a difference between the observed and expected catch of all size classes of Gila chub among mean depth categories in Cienega Creek (Table 8). There was strong evidence of a difference in mean CPUE of all size classes of Gila chub with respect to mean depth in Cienega Creek (Table 8). In addition, mean CPUE of all size classes of Gila chub in habitats with a mean depth of 50-75 cm was at least twice that of other depths. Mean CPUE of all size classes of Gila chub was lowest in habitats with a mean depth of 0-25 cm.

Maximum depth. – Habitats with the lowest (0-50 cm) maximum depths accounted for almost half of the area sampled in Bonita Creek (Table 9). There was evidence of a difference between the observed and expected catch of adult Gila chub among maximum depth categories in Bonita Creek (Table 9). Most commonly, all size classes of Gila chub in Bonita Creek used habitats with regard to maximum depth proportionate to their availability (Table 9). There was moderate evidence that mean CPUE of small juvenile Gila chub differed with respect to maximum depth in Bonita Creek being highest in habitats with a maximum depth of 50-100 cm (Table 9). There was no evidence that mean CPUE of large juvenile and adult Gila chub differed with respect to maximum depth in Bonita Creek.

Cienega creek had more habitats with the least (0-50 cm) maximum depths and almost twice as many habitats with the greatest (200 + cm) maximum depths than Bonita Creek (Table 9). There was strong evidence of a difference between the observed and expected catch of all size classes of Gila chub among maximum depth categories in Cienega Creek (Table 9). In general, all size classes of Gila chub in Cienega Creek strongly avoided habitats with the least and greatest maximum depths and preferred habitats with moderate maximum depths (Table 9). Mean CPUE of all size classes of Gila chub differed with respect to maximum depth in Cienega

Creek (Table 9). Gila chub of all size classes were sparse in habitats with the least (0-50 cm) and greatest (> 200 cm) maximum depths, and most dense in habitats having moderate maximum depths (100-150 cm).

Mean TL of Gila chub in Bonita Creek differed with respect to maximum depth of habitats sampled (one-way ANOVA = 2.69; $df = 4$, 385; $P = 0.030$) being greatest in habitat types with maximum depths of 100-150 cm and least in habitats with the greatest (≥ 200 cm) maximum depths (Table 10). Mean TL of Gila chub in Cienega Creek differed with respect to maximum depth of habitats sampled (one-way ANOVA = 4.64; $df = 3$, 146; $P = 0.004$) being greatest in habitat types with maximum depths ≥ 200 cm (Table 10).

Flow velocity. – In Bonita Creek, habitats with no flow velocity accounted for 31.10% of the area sampled, while habitats with mean flow velocity of > 0 -50 cm/s accounted for about half of the area sampled (Table 11). Small juveniles strongly avoided habitats with the greatest mean flow velocities (100 + cm/s), but in general, juvenile size classes of Gila chub in Bonita Creek varied in selection of habitats with respect to flow velocity (Table 11). Adult Gila chub avoided habitats with no flow velocity but used all other mean flow velocity categories roughly in proportion to their availability. Mean CPUE of all size classes of Gila chub in Bonita Creek did not significantly differ among habitats with respect to velocity (Table 11).

In Cienega Creek, there was no discernable flow in roughly two-thirds of the area sampled (Table 11). Habitats with low (> 0 -25 cm/s) mean flow velocities accounted for most of the remaining area sampled. There was evidence of a difference between the observed and expected catch of large juvenile and adult Gila chub among mean velocity categories in Cienega Creek (Table 11). In slight contrast to Bonita Creek, Gila chub of all size classes preferred habitats with no discernable flow (Table 11). Despite the lack of Gila chub caught in habitats

with higher (> 50 cm/s) mean flow velocities, mean CPUE of all size classes of Gila chub in Cienega Creek did not statistically differ among habitats with respect to flow velocity (Table 11).

Substrate. – In Bonita Creek, fines and organic material were the dominant substrate type (Table 12). Although not always dominant on a whole habitat scale, substrates with diameters between sand and boulder size were much more common in Bonita than Cienega Creek. There was evidence of a difference between the observed and expected catch of large juvenile and adult Gila chub, respectively, among substrate categories in Bonita Creek (Table 12). Small juvenile Gila chub appeared to have a more similar pattern of selection for dominant substrate types with adults than with larger juveniles (Table 12). Small juvenile and adult Gila chub preferred habitats dominated by organic substrate, whereas larger juveniles preferred habitats dominated by coarser substrate (Table 12). Mean CPUE of all size classes of Gila chub in Bonita Creek did not differ among habitats with respect to dominant substrate type (Table 12).

Habitats with mostly organic substrate (e.g., plants, detritus, and algae) accounted for 62% of the area sampled in Cienega Creek, followed by habitats dominated by fines, then mixed substrates (Table 12). Catch of small and large juvenile Gila chub in Cienega Creek was more than expected in habitats dominated by fine substrates (Table 12). Gila chub in Cienega Creek appeared to avoid habitats with mixed substrates (Table 12). Mean CPUE of all size classes of Gila chub in Cienega Creek did not significantly differ among habitats with respect to dominant substrate type (Table 12).

Physical/chemical/hydraulic measures. – Stream banks were more stable, cattle sign was less prevalent, and water was clearer more often in Bonita Creek than Cienega Creek (Table 13). Canopy cover was more dense overall in Bonita Creek than Cienega Creek (Table 13). Water temperature, dissolved oxygen, and conductivity had a greater range in Cienega Creek than

Bonita Creek (Table 13). Gila chub were captured within the majority of the range of measured water quality parameters. Bonita Creek had a more dependable stream flow (only Cienega Creek and Mattie Canyon had intermittent reaches within study area boundaries), and greater annual mean and maximum discharge (Table 13). Average annual mean discharge and maximum discharge for Bonita Creek was much greater for the twenty years prior to my study.

Reproduction

Behavior. – Gila chub (Sabino Creek, Arizona stock) spawned regularly in the laboratory during all times of the year at temperatures ranging from about 15 to 26°C. Temperatures in the upper part of the range did not appear to be preferred for spawning. Spawning behavior of Gila chub (those acclimated to the laboratory) was observed several times in the laboratory and behavior appeared little affected by observers. Before spawning one to several presumed males chased what appeared to be a lone female. Presumed males usually had more vivid spawning colors than females. Strong, dark, lateral banding was noted on the most active fish. Nudging and nipping of the female posteriorly by males was noted. The actual release of gametes was often immediately preceded by a slight upward turn and then a light to violent shudder by the female, especially when against a rough surface or wedged between in-tank structures. Roughly 30 eggs were released during each act. Following the act, nearby fish, including those involved in the act, immediately began eating any available eggs. Such spawning acts were repeated several times by what appeared to be the same female. Video footage taken in the laboratory confirmed the behavior. Spawning often lasted over an hour.

Chasing behavior similar to that observed in the laboratory during spawning was noted in Bonita Creek on 4-13-02 during the day but further activity was not witnessed during the study.

The specific area where the activity was observed was a flowing section of a larger run with mixed substrate of mostly cobble, pebble, gravel, and fines, and low cover. The flow velocity of the run averaged 36 cm/s and depth averaged 15.8 cm with mixed substrates dominated by fines, and a water temperature of 21.7 °C. The downstream and upstream adjacent habitat types were riffles. The level of reproductive activity occurring in the area appeared to be high, because of 26 Gila chub captured, 7 had very strong spawning colors, 9 had strong spawning colors, 7 had tubercles, and 4 were expressing gametes.

Temporal range. – Although sample sizes were not large ($n = 55$ Bonita Creek and 31 Cienega Creek), a relatively clear pattern of gonad growth was observed when the mean monthly GSI values were graphed (Figures 4 and 5). Gonadosomatic Index values rose quickly in the late winter and spring in both streams, were lowest during the late summer and rose again in the fall. Of the four captured Gila chub having eggs near maturity (all in Bonita), three were captured in late February and the other in mid-September.

Gila chub 40-49 mm TL were captured during ten months of the year including the winter months of December, January, and February (Figures 6 and 7). Gila chub 30-39 mm TL were captured during seven months of the year including September, October, January, and February. Gila chub 20-29 mm TL were captured in February, April, May, and August.

Physical characteristics. – In both streams Gila chub exhibited moderate spawning colors throughout the year, with the highest frequency of occurrence in late winter and spring (Figures 8 and 9). Gila chub in Bonita Creek had strong or very strong spawning colors from February-May. Gila chub in Cienega Creek exhibited strong or very strong spawning colors from March-June, and then again in August and December. Spawning colors were noted throughout the year in the laboratory; however, spawning colors in the laboratory rarely achieved

the intensity of colors in the field. Gila chub with strong and very strong spawning colors were found in water temperatures from about 13-22 °C in Bonita Creek and 12-28 °C in Cienega Creek.

Coloration for individuals expressing gametes or tubercles ranged from moderate to very strong. The expression of gametes ($N = 6$ Bonita Creek and 2 Cienega Creek) or tubercles ($N = 15$ Bonita Creek and 1 Cienega Creek) was only noted from March-June (Figures 8 and 9). Tubercles were observed mostly on the head. Of those Gila chub that were expressing tubercles and could also be sexed, all were males ($N = 4$). Males also dominated the catch of Gila chub that had strong and very strong spawning coloration. Gila chub presumed to be males (due to spawning behavior and growth in the laboratory) expressed a greater intensity in spawning coloration than other captive Gila chub.

Thirty-three of 37 Gila chub (captured during habitat selection work) in Bonita Creek exhibiting strong-very strong spawning colors and/or expressing gametes or tubercles were captured in habitat types other than pools. In contrast, all but one of the Gila chub in Cienega Creek having strong-very strong spawning colors and/or expressing gametes were found in pools.

Eggs of Gila chub from laboratory spawns (Sabino Creek, Arizona stock) were demersal, adhesive, ovoid, and translucent with about the inner 80-90% of the egg a light yellow cream color and the rest clear. Eggs < 1 d old measured 2 mm or less (mean = 1.93 mm; $n = 5$) and hatched in about 6 days at 22 °C. Newborn Gila chub are about 6.5 mm TL ($n = 20$) and swim-up by some individuals was noted within the first 48 hours. Fecundity estimated from spawns in the laboratory ranged from roughly 300 to over 2,000 for Gila chub about

110-170 mm TL. Mean fecundity estimated from sacrificed Gila chub (mean TL = 164 mm) captured in Bonita Creek was 10,392 ($n = 4$).

The mean TL of adult female Gila chub in Bonita Creek was over 13 mm greater than the mean TL of adult male chub (two-sample t-test = 2.84; $df = 84$; $P = 0.005$; 95% confidence interval = 3.9 to 22.6 mm). The mean TL of adult female Gila chub in Cienega Creek was 9 mm greater than the mean TL of adult male chub but the difference was not statistically significant (two-sample t-test = 1.55; $df = 65$; $P = 0.125$). The largest confirmed female Gila chub during my study was 222 mm TL and the largest confirmed male was 205 mm TL (both from Bonita Creek).

Movement

Adult Gila chub. – One hundred sixty Gila chub were tagged in Bonita Creek. I recaptured nine of these chub (5.6%) once and one twice (Table 14). Nine of the 10 recapture events were < 200 m, with one of 1,675 m. No clear pattern was present for direction of movements.

Forty-one Gila chub were tagged in Cienega Creek. Five Gila chub were recaptured once (12.2 %) and one twice (Table 14). Only 1 of the 5 recaptured chub had moved out of the sample reach in which it was previously caught and the lone movement was about 11 m. The lone double-recapture was captured in the same location each time (Table 14).

Growth of recaptured Gila chub varied from 0-42 mm TL. The highest growth rate of any recaptured Gila chub was 0.125 mm/d (Table 14).

Juvenile Gila chub. – A total of 793 Gila chub were marked in Cienega Creek. Of a total 2,044 Gila chub checked for marks, 26 were identified as recaptures. None of the recaptures

were from areas outside the zones they were marked. The mean length of recaptured Gila chub was about 70 mm TL (range = 46-93 mm TL). Areas where the grit was retained was highly variable and included the head, opercles, mouth, eyes, nearly all the fins, and most body locations. The number of grit specks identified on recaptured Gila chub ranged from one to a number too high to count. All recaptured Gila chub appeared healthy. A total of 64 Gila chub were marked in Bonita Creek. No marked Gila chub were recaptured in Bonita Creek.

Larval Gila chub. – Drift nets were deployed for a total of 4,372 minutes. Forty-six larval longfin dace *A. chrysogaster* and three larval catostomids were identified from drift net and dip-net samples. No larval Gila chub were identified in any of the drift net or dip-net samples from either stream.

DISCUSSION

Habitat

The chance of accurately characterizing an animal's habitat preferences may vary greatly depending on what spatial and temporal scale is being studied and the methods employed. Following a pilot survey I chose not to use a micro approach to define habitat preferences of Gila chub in Cienega Creek and Bonita Creek. Microhabitat approaches usually measure use at the scale of where an individual fish is observed (although it has also been used for fish shoals [Nykänen and Huusko 2003]). Such techniques are best used where fish are easy to observe and habitat features do not restrict observation. Since microhabitat techniques depend on a very specific three-dimensional point in the stream, any disturbance to an individual fish's normal behavior will affect the accuracy of the data collected. Even where visibility is acceptable, observations of particular fish (those apparent to the observer) may not be proportional to the

abundance of fish in all habitat types examined, especially in complex habitats (Heggenes et al. 1990). Because microhabitat approaches measure use at a particular point in time and at a particular place in time, they usually do not account for normal spatial variation in movement, and thus may overestimate the importance of values of habitat variables at a particular observation point. Macrohabitat approaches may reduce the bias by putting importance on the entire nearby area surrounding an observation. However, the homogenization of use data may lead an analyst to overlook particular values of habitat variables for which a fish is responding. In either case, bias does occur with both approaches and numerous confounding variables are usually in effect. Thus caution is warranted when interpreting the data collected based on methods and limitations therein. In addition, statistical analyses and type or grouping of data used therein can differentially affect interpretation of results of one particular method (e.g., as in some of my Chi-Square versus ANOVA tests). The use of multiple approaches at multilevel scales to define habitat preference (Bozek and Rahel 1991), and when appropriate, the use of experimental techniques (Sugiyama and Goto 2002) show promise in further reducing bias in characterizing habitat preference.

When my data for both streams are taken as a whole, the results of habitat use versus availability suggest Gila chub are generalists. However, within each stream, habitat use was more defined for some variables and size classes, and between-stream differences were apparent.

Gila chub, especially adults, have often been regarded as preferring calm-water and pools. While there is no doubt that large Gila chub are often conspicuous in clear deep pools, my data and experiences from Bonita Creek have shown that large Gila chub may at times show preference, or at least use in proportion to abundance, habitats comprising moderate-shallow water with significant current. Some of these individuals were in spawning or pre-spawn

condition and thus the use of such habitats by adults may be seasonally linked to reproduction. As stated prior, 89% of the Gila chub in Bonita Creek having a high level of primary or secondary sexual characteristics were captured in habitats other than pools. Of the four chub in Bonita Creek expressing gametes after capture (among those captured during habitat selection work), two were found in shallow runs and two in riffles. In contrast, all but one of the Gila chub captured in Cienega Creek having a high level of primary or secondary sexual characteristics were found in pools.

Small streams are often divided into habitat types at the macro level (i.e. pool, run, riffle, etc.). While no two habitat types are exactly alike, they share physical/hydraulic characteristics. Correspondingly, fish species and size classes often relate to habitat types in a predictable way. My data do not allow for specific analysis on how Gila chub may have been affected, but signs of recent beaver activity were commonplace during my study, and beaver appeared to have a significant influence on the flow and channel dynamics of Bonita Creek and likely the distribution and frequency of certain habitat types.

Run and marsh habitats made up almost half the area I sampled in Cienega Creek, yet only 2 of the 149 Gila chub captured over a three-year period were captured in habitat types other than pools. In stark contrast, Gila chub in Bonita Creek preferred, or used in proportion, habitat types that were avoided in Cienega Creek, including swift shallow habitats. Adult Gila chub in Bonita Creek preferred chutes, and used pools, runs, and riffles in proportion to availability. Catch per effort was higher in chutes and runs than in pools. Some deep pools and areas of high cover were difficult to sample effectively with a backpack electrofisher which may have lessened capture efficiency in these areas. The generality in habitat preference for Gila

chub has been noted by others. Griffith and Tiersch (1989) found Gila chub within Redfield Canyon, Arizona using riffle habitats as much as pools at one sampling site but not another.

Gila chub have often been associated with cienega-type habitats (i.e., marsh stream systems; Hendrickson and Minckley 1984). However, my data from Cienega Creek shows Gila chub avoided marsh habitats, and were found mostly in pools. Pools and runs located near marsh habitat were also selected less than pools and runs away from marsh habitat. However, it should be noted that marsh habitats were often some of the most difficult to sample effectively. Pools within marsh-dominated reaches were also extremely difficult to sample due to their deep nature, steep banks, algae/macrophyte growth, and unsolidified substrates. Thus, a lowered sampling efficiency may have partially accounted for the lower CPUE in these areas.

In contrast to Bonita Creek, juvenile Gila chub in Cienega Creek did not appear to select habitats with high cover. In Sabino Creek (Pima County, Arizona) Dudley (1995) found subadults were in closer proximity to cover than adult Gila chub.

Dudley (1995) found sub-adult Gila chub in Sabino Creek commonly used areas in, or near riffles, exposing them to faster currents, larger substrates, and shallower depths. I found juvenile Gila chub in Bonita Creek avoided riffles and chutes, whereas adults preferred or used in proportion these habitat types. In contrast, all size classes of Gila chub in Cienega Creek preferred pools. If habitat segregation between juvenile Gila chub and adults in Cienega Creek did occur on a micro scale it was likely based on use of specific cover types or water depth, but not velocity.

Intra and interspecies segregation in habitat use between size classes is well known for fishes (Matthews 1998) and is often influenced by predation and competition. Gila chub would have likely been the only member of either fish assemblage to prey on fish larger than

larval-sized. In Cienega Creek the predatory influence of adults over juveniles may be lower than in Bonita Creek as the CPUE of adults in Bonita Creek was six times greater ($0.031/\text{m}^2$ vs. $0.005/\text{m}^2$) and mean length of adults in Bonita Creek was much greater (123 mm TL vs. 98 mm TL) than in Cienega Creek. The lower CPUE of adults, especially large adults, in Cienega Creek was linked to a large fish die-off in 2000 from a disease outbreak that occurred before my study (Jeff Simms and Heidi Blasius, BLM, personal communication). The extent competition from other fish species affected Gila chub habitat use is unknown.

Griffith and Tiersch (1989) found large Gila chub in Redfield Canyon, Arizona consumed speckled dace up to 73 mm TL. I also found fish in the stomachs of large Gila chub. One Gila chub (205 mm TL) from Bonita Creek had 3 juvenile chub (largest = 55 mm TL) in its stomach and two other unidentifiable fish. Dudley (1995) suggested predation threat or competition from adult Gila chub may be causing sub-adult chub in Sabino Creek to utilize different habitats than adults.

Due to low sample sizes and often moderate temperatures I did not examine seasonal variation in habitat selection analyses but did note that during the coldest times of the year, Gila chub decreased activity, often holding close to or within cover. This coincides with that found by Dudley (1995).

Reproduction

Much of the existing information on Gila chub reproductive ecology is qualitative. Suspected spawning behavior of Gila chub in a pond was described by Minckley (1973). My results show the breadth of spawning habitat, temporal range, and temperature may be greater than once thought, especially for Gila chub occurring outside of constant temperature springs.

Nelson (1993) reported on some aspects of the reproductive biology of Gila chub in Cienega Creek. The author used a spawning coloration scale similar to ours. Nelson (1993) found the most intense coloration when water temperatures were $> 17^{\circ}\text{C}$. Based on spawning coloration patterns the author hypothesized Gila chub in Cienega Creek greater than 75 mm could spawn. I found a very plump female Gila chub that was 69 mm TL in Cienega Creek releasing eggs. However, the eggs appeared smaller than normal and it is unknown if they were viable or not. All other Gila chub releasing gametes or showing heightened secondary sexual characteristics were much larger. Although spawning coloration is undoubtedly related to the reproductive cycle it is not clear if a definitive relationship exists between intensity of spawning colors and time before spawning. During my study, Gila chub releasing gametes ranged in color from moderate to very strong. The most intensely colored Gila chub (\geq strong spawning colors) were found at daytime water temperatures from about $13\text{--}22^{\circ}\text{C}$ in Bonita Creek and $12\text{--}28^{\circ}\text{C}$ in Cienega Creek.

The GSI, coloration, tubercle, ripeness, catch, and observation data suggest that spawning begins earlier (late February to early March) than previously thought. Although less pronounced, the same data suggest a smaller fall spawning, probably following monsoon rains. Small YOY Gila chub (20-39 mm TL) were captured during the fall and winter, and Gila chub with eggs near full maturity were captured in the fall. The GSI values were high in February and March, and rose again to a lesser extent in the fall. Prior monitoring of Bonita Creek and Cienega Creek also found small YOY Gila chub (20-39 mm TL) during the fall and winter (Jeff Simms, unpublished data). A bi-modal spawning season has been found in other cyprinids native to the Southwest (John 1963; Vives and Minckley 1990; Rinne 1995).

There was a marked disparity between estimates of fecundity from the enumeration of actual spawns in the laboratory and extrapolation of total ova from ovaries of sacrificed Gila chub that could not be explained by size differences. The actual production of viable oocytes (functional fecundity) may differ from true reproductive potential due to incomplete spawning or degeneration and resorption of oocytes (Crim and Glebe 1990).

Movement

Gila chub moved little during my study, agreeing largely with results for movement of other native southwestern fishes in Bestgen et al. (1987), Williams (1991), and Schultz (2000). Bestgen et al. (1987) observed little movement of recaptured fishes in the Gila and San Francisco rivers along the Arizona-New Mexico border, despite two major floods. The authors hypothesized that the primary reasons for the lack of movement were the relatively large habitats and comparatively cool thermal regime of the study area. Schultz (2000) found little movement of PIT-tagged fish between fixed sampling stations within an isolated 7-km section of Sonoita Creek, Arizona (Santa Cruz, County). However, sampling efforts outside of these stations were limited. In contrast Siebert (1980) found significant movement of most native fishes (Gila chub were not present) in Aravaipa Creek, Arizona. Fish moved seasonally away from a central gorge in winter and toward it in summer. The author hypothesized that fish moved to avoid high summer temperatures outside the gorge. Williams (1991) results did not agree in large part with Siebert (1980) despite studying the same system.

Tag return rates for adult Gila chub in both streams (5.6% Bonita Creek and 12.2% Cienega Creek) were similar to those for southwestern Cypriniformes of Schultz (2000), Williams (1991), and Siebert (1980), but higher than that of Bestgen et al. (1987). The latter

recaptured only 48 of 1,754 fishes tagged (2.7%). The stream systems of Bestgen et al. (1987) were much larger than ours and others mentioned.

The disparity in the number of adult Gila chub tagged between the two streams was related directly to the low catch of suitable-sized chub in Cienega Creek. Despite the low number of Gila chub tagged in Cienega Creek, return rate was fairly high (12.2%) in Cienega Creek when compared to Bonita Creek (5.6%). The disparity in recapture rates may be related to a higher prevalence of natural barriers, intermittency, and other factors in Cienega Creek that may concentrate adult Gila chub in certain areas.

It was not unexpected that recaptures of marked juvenile Gila chub occurred only in Cienega creek as many more juvenile chub were marked here than in Bonita (817 versus 64). My lack of finding juvenile Gila chub of a suitable marking size at Bonita was likely related to numerous factors, such as differential habitat use, gear efficiency, and predation/agonistic interactions from adults. The latter may have been augmented by the attraction of large adults to my baited hoop-nets.

Mass marking of fish using fluorescent grit has been used for some time (Jackson 1959). Most of the subsequent published work using the technique involved salmonids (Phinney et al. 1967; Phinney and Mathews 1973; Pauley and Troutt 1988; Nielson 1990). I believe the method of mass-marking using a fluorescent grit is promising and likely underused for native southwestern fishes. Nielson (1990) characterized marking using fluorescent grit as an acceptable, permanent, long-term mark (up to 12 years in some cases) that was cost effective with no apparent negative effects on the fish.

Experimental work is needed on mark retention and survival in relation to pigment color, fish size, and delivery force for native poeciliids, cyprinids, and catostomids. Mass marking

using a fluorescent grit appears more time efficient than most other marking techniques and is perhaps comparable to, or better than, marking small fish using many other commonly employed techniques with respect to retention and survival. Comparison studies do exist (Pauley and Troutt 1988; Nielson 1990) but not for cyprinids and more are needed.

Back-calculation from scale analysis of Gila chub in Redfield Canyon estimated average lengths of 90, 135, 160, and 183 mm TL in the first four years (Griffith and Tiersch 1989). My limited growth data shows Gila chub in Bonita and Cienega Creek ranging in growth from a comparable to much slower rate. PIT-tags do not have adverse effects on survival, growth, swimming speed, or stamina (Jenkins and Smith 1990; Prentice et al. 1990). Tagging wounds of recaptured Gila chub appeared to heal well with scarring of the entry point sometimes apparent.

MANAGEMENT IMPLICATIONS

The benefits obtained from an accurate knowledge of the habitat requirements of Gila chub are as follows: (1) Areas critical to the continued survival of the species can be quantitatively defined in terms of functional importance. (2) In the event that it becomes necessary or desirable to restore habitat, or create additional habitat, these activities can be undertaken with knowledge of how it should affect this species. (3) Over time, changes in the habitat can be evaluated to determine whether current management activities are benefiting or impacting this species. (4) Critical needs can be identified for the species and use patterns designed to avoid conflicts during limiting periods (e.g. spawning).

Although populations of Gila chub share many natural history traits, my data and other works suggest habitat use and perhaps other natural history strategies can vary among systems. It is possible unique preferences and strategies exist between different populations of Gila chub.

Thus managers should be cautious about applying information based on one population to others. This was quite apparent in Bonita Creek as Gila chub CPUE, frequency of occurrence, and corresponding habitat preference data differed with certain long-perceived notions about the species (e.g., Minckley 1973). This was also true for reproductive ecology. While some long-held suspicions were strengthened (e.g., a longer and possibly bi-modal spawning season) other data were surprising. The use of swift-flowing relatively shallow habitats (often with coarser substrate) during times of reproduction is worth further study.

Although not strongly quantified, morphological and coloration differences between Gila chub in Bonita Creek and Cienega Creek have been noted by us and others (Jeff Simms, BLM, personal Communication). Bonita Creek and Cienega Creek are separated by hundreds of stream miles within two drainage basins (i.e., Gila River basin and Santa Cruz River basin) long connected only during flood flows. The two populations have been evolving with differing environmental conditions and disturbances. Natural gene flow between these two populations and many other populations of fishes within the Gila River basin and Santa Cruz River basin have likely not occurred within recent times. While recent analyses of genetic data by Schwemm (2006) did not allow for discrimination among the *robusta* complex (*G. robusta*, *G. nigra*, and *G. intermedia*), unique genetic characteristics and differences were found between certain populations of Gila chub. Based on his findings Schwemm (2006) recommended ten different management units for *Gila intermedia*, with the chub population in Bonita Creek included in a management unit with East and Upper Eagle, Harden-Cienega, and Turkey creeks, and Cienega Creek as a separate management unit on its own.

Closure of potential immigration routes to preclude invasion of nonnative fishes is recognized as a current management option to protect populations of Gila chub (Weedman et al.

1996) and other species of fishes native to the Southwest. The construction of barriers to prevent nonnative fishes from invading upstream areas inhabited by native fishes has been proposed for numerous streams throughout Arizona. My knowledge of how the movement patterns of Gila chub and other native southwestern fishes with regard to barriers may affect populations of these fishes is largely incomplete. Most Gila chub in my study were rather sedentary or made small movements more akin to typical home range use. While study of other populations may reveal marked differences in movement patterns, and more evaluation is warranted, Gila chub in my study appeared to maintain viable populations in areas above natural barriers.

While my use of the fluorescent grit marking method has not been commonly employed in southwestern streams, it is by no means new. It is a proven technique that can be tailored to meet the logistical constraints of a project. The system I used was portable enough to facilitate travel to destinations where vehicle access is limited, yet provided enough capacity to mark thousands of fish if necessary. Access in many of the streams in the Southwest is lacking, therefore light, highly portable equipment, is often necessary. The apparent efficiency, low-cost, and safe nature of the method (Nielson 1990) make it a powerful tool for managers when it becomes necessary to track fish that are too small for tagging.

A report by USFWS (2005) stated approximately 85-90 % of the Gila chub's habitat has been degraded or destroyed with much unrecoverable. Although able to withstand harsh conditions, without adequate surface water Gila chub and other native fishes cannot survive. As the frequency of intermittence within a stream increases, the chances of even normal drought periods eliminating population segments increases greatly. In Cienega Creek and Bonita Creek such areas of increased intermittence often looked as though they should hold fish, but did not. These same areas were often dry during an above average drought period. Such cycles probably

preclude permanent residence of certain species in these areas. Thus it would seem evolutionarily adaptive for fishes to develop strategies and senses that enable them to avoid such areas. No threat to aquatic species in arid climates is likely greater than the continual drying of my remaining streams. Protection of instream flow during critical periods, such as summer drought, and spawning, should be a focus of management plans.

Bonita Creek and Cienega Creek are important areas of aquatic biodiversity. Both streams currently lack nonnative fishes for the majority of their length but the threat is omnipresent. Other deleterious nonnatives (i.e., bullfrog *Rana catesbeiana*) have already invaded Cienega Creek. Stock tanks and private waters can be sources of nonnatives. Pathogens brought in by animals or people can wreak havoc on fishes. Gila chub co-occurring with nonnative fishes downstream of the Safford water withdrawal in Bonita Creek were heavily parasitized by anchor worm *Lernea cyprinacea* (USFWS 2005). Large Gila chub were once common in certain areas of Cienega Creek where current densities of adults are now low (personal observation; Jeff Simms, personal communication). As mentioned prior, this condition was linked to a disease outbreak that occurred before my study (Jeff Simms and Heidi Blasius, BLM, personal communication). Management for Bonita Creek and Cienega Creek should include plans to quickly identify and block or eliminate potential sources of nonnative species. This is especially true given the often overwhelming difficulty, economic costs, and controversy surrounding strategies to control or eliminate established deleterious nonnative species. Preventative measures against nonnatives, instream flow management, crises and proactive planning, cooperative efforts, and habitat conservation, are vital to the continued survival of Gila chub and other aquatic organisms in Bonita Creek and Cienega Creek.

ACKNOWLEDGMENTS

I must first thank Gina Schultz for her enduring technical support. I thank Jeff Simms of BLM for his tireless support on numerous levels. I thank BLM for funding this study and BLM employee Janell Reifel for her administrative assistance. I thank my Ph.D. committee members Dr. Scott Bonar (major advisor), Dr. Courtney Conway, Dr. Kevin Fitzsimmons, Dr. Peter Reinthal, and Dr. Cecil Schwalbe. I thank the professionals at the BLM in Safford, Arizona for their support, especially Heidi Blasius. I thank all staff and faculty at the University of Arizona that assisted in any respect during this study, especially Anne Hartley, Linda Lee, Dee Simons, Cecily Westphal, and Carol Yde. A special thanks goes to Dr. Mark Borgstrom and Dr. Robert Steidl for statistical advice. Thanks to Andrew Honaman, Mickey Reed, and Hoori Ajami for technical support. Special thanks to Fluo Mark and Paul Janssen for supplying fluorescent grit, providing advice, and sharing information. Thanks also to the professionals at the Washington Department of Fish and Wildlife for their guidance and assistance regarding marking equipment and techniques. Thanks to Emmet McGuire of the USGS for providing discharge data. Thanks to Dr. William Matter for logistical and design support. Thanks to Tom Archdeacon, Daniel Arevalo, Sachiko Aso, Devin Beck, Sheldon Caldwell-Meeks, Corissa Carveth, Brian Chaszar, Stan Culling, Walker Thomas Eldridge, Andrea Francis, Jason Kline, Dan Koepke, Naomi Lupe, Michelle Martin, Shawna Minor, Abe Randolph, Michelle Riley, Gina Schultz, Jasmine Schultz, Skye Schultz, Erica Sontz, John Sottolare, Greg Stoehr, Sean Tackley, Cyndi Tuell, Matt Walton, David Ward, and Sebastian Zeltzer for their assistance in the field and laboratory. Thanks to all the other individuals, government agencies, non-governmental groups, and businesses not mentioned here that assisted with the project.

LITERATURE CITED

- Baltz, D.M. 1990. Autecology. Pages 585-607 in C.B. Schreck and P.B. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland.
- Bestgen, K.R., D.A. Hendrickson, D.M. Kubly, and D.L. Propst. 1987. Movements and growth of fishes in the Gila River drainage, Arizona and New Mexico. The Southwestern Naturalist 32:351-356.
- Bozek, M.A., and F.J. Rahel. 1991. Assessing habitat requirements of young Colorado River cutthroat trout by use of macrohabitat and microhabitat analyses. Transactions of the American Fisheries Society 120:571-581.
- Brown, M.L., and D.J. Austen. 1996. Data management and statistical techniques. Pages 17-62 in B.R. Murphy and D.W. Willis, editors. Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.
- Crim, L.W., and B.D. Glebe. 1990. Reproduction. Pages 529-553, in C.B. Schreck and P.B. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland.
- Dudley, R.K. 1995. The effects of green sunfish on the distribution, abundance and habitat use of Gila chub in Sabino Creek, Arizona. Master's thesis. University of Arizona, Tucson, Arizona.
- Dudley, R.K., and W.J. Matter. 2000. Effects of small green sunfish (*Lepomis cyanellus*) on recruitment of Gila chub (*Gila intermedia*) in Sabino Creek, Arizona. The Southwestern Naturalist 45:24-29.
- Griffith, J.S., and T.R. Tiersch. 1989. Ecology of fishes in Redfield Canyon, Arizona, with emphasis on *Gila robusta intermedia*. Southwestern Naturalist 34:131-134.

- Hadley, D., R.V.N. Ahlstrom, and S. Mills. 1993. El rio bonito: an ethnological study of the Bonita Creek watershed, southeastern Arizona. Arizona State Office of the U.S. Bureau of Land Management, Cultural Resource Series No. 8. Phoenix, Arizona.
- Heggenes, J., Å. Brabrand, and S.J. Saltveit. 1990. Comparison of three methods for studies of stream habitat use by young brown trout and Atlantic salmon. Transactions of the American Fisheries Society 119:101-111.
- Hendrickson, D.A., and W.L. Minckley. 1984. Cienegas-vanishing climax communities of the American Southwest. Desert Plants 6:141-175.
- Jackson, C.F. 1959. A technique for mass marking fish by means of compressed air. New Hampshire Fish and Game Department, Technical Circular 17:1-8. Concord, New Hampshire.
- Jacobs, J. 1974. Quantitative measurement of food selection: a modification of the forage ration and Ivlev's index. Oecologia 14:413-417.
- Jenkins, W.E., and T.I.J. Smith. 1990. Use of PIT tags to individually identify striped bass and red drum brood stocks. Pages 341-345 in N.C. Parker, A.E. Giorgi, R. C. Heidinger, D.B. Jester, Jr., E.D. Prince, and G.W. Winans, editors. Fish-marking techniques. American Fisheries Society Symposium 7, Bethesda, Maryland.
- John, K.R. 1963. The Effect of Torrential Rains on the Reproductive Cycle of *Rhinichthys osculus* in the Chiricahua Mountains, Arizona. Copeia 2:286-291.
- Kelso, W.E., and D.A. Rutherford. 1996. Collection, preservation, and identification of fish eggs and larvae. Pages 255-302 in B.R. Murphy and D.W. Willis, editors. Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda Maryland.

- Matthews, W.J. 1998. Patterns in freshwater fish ecology. Kluwer Academic Publishers, Norwell, Massachusetts.
- McMahon, T.E., A.V. Zale, and D.J. Orth. 1996. Aquatic habitat measurements. Pages 83-120 in L.A. Nielsen and D.L. Johnson, editors. Fisheries techniques. Southern Printing Company, Inc., Blacksburg, Virginia.
- Minckley, W.L. 1973. Fishes of Arizona. Sims Printing Company, Inc., Phoenix, Arizona.
- Minckley, W.L., and J.E. Deacon, editors. 1991. Battle against extinction: native fish management in the American West. University of Arizona Press, Tucson, Arizona.
- Minckley, W.L., and B.D. DeMarias. 2000. Taxonomy of chubs (Teleostei, Cyprinidae, genus *Gila*) in the American Southwest with comments on conservation. Copeia 2000:251-256.
- Minckley, W.L., J.N. Rinne, and J.E. Johnson. 1977. Status of the Gila topminnow and its co-occurrence with mosquitofish. United States Department of Agriculture, Research paper RM-198, Fort Collins, Colorado.
- Nelson, B. 1993. Spawning characteristics of Gila chub (*Gila intermedia*) in Cienega creek, Pima County, Arizona. Report for USDI Bureau of Land Management, Tucson Resource Area, Arizona.
- Nielson, B.R. 1990. Twelve-year overview of fluorescent grit marking of cutthroat trout in Bear Lake, Utah-Idaho. American Fisheries Society Symposium 7:42-46.
- Nykänen, M., and A. Huusko. 2003. Size-related changes in habitat selection by larval grayling (*Thymallus thymallus* L.). Ecology of Freshwater Fish 12 (2):127-133.
- Pauley, G.B., and D.A. Troutt. 1988. Comparison of three methods of fluorescent dye application for marking juvenile steelhead. Transactions of the American Fisheries Society 117:311-313.

- Phinney, D.E., and S.B. Mathews. 1973. Retention of fluorescent pigment by coho salmon after two years. *Progressive Fish-Culturist* 35:161-163.
- Phinney, D.E., D.M. Miller, and M.L. Dahlberg. 1967. Mass marking young salmonids with fluorescent pigment. *Transactions of the American Fisheries Society* 96:157-162.
- Platts, W.S., W.F. Megahan, and G.W. Minshall. 1983. Methods for evaluating stream, riparian, and biotic conditions. United States Department of Agriculture, General technical report INT-138, Fort Collins, Colorado.
- Prentice, E.F., T.A. Flagg, and C.S. McCutcheon. 1990. Feasibility of using implantable passive integrated transponder (PIT) tags in salmonids. *American Fisheries Society Symposium* 7:317-322.
- Rinne, J.N. 1976. Cyprinid fishes of the genus *Gila* from the Lower Colorado River basin. *Wasmann Journal of Biology* 34:65-107.
- Rinne, J.N. 1995. Reproductive biology of the Rio Grande Chub *Gila pandora* (teleostomi: cypriniformes), in a montane stream, New Mexico. *The Southwestern Naturalist* 40 (1):107-110.
- Rinne, J.N., and W.L. Minckley. 1991. Native fishes of arid lands: a dwindling resource of the desert Southwest. United States Department of Agriculture, General Technical Report RM-206, Ft. Collins, Colorado.
- Schultz, A.A. 2000. The Structure and Dynamics of the Fish Assemblage in Lower Sonoita Creek, Arizona. Master's Thesis. University of Arizona, Tucson, Arizona.
- Schwemm, M.R. 2006. Genetic variation in the *Gila robusta* complex (Teleostei: Cyprinidae) in the lower Colorado River. Master's Thesis. Arizona State University, Tempe, Arizona.

- Siebert, D.J. 1980. Movements of fishes in Aravaipa Creek, Arizona. Master's Thesis. Arizona State University, Tempe, Arizona.
- Sugiyama, H., and A. Goto. 2002. Habitat selection by larvae of a fluvial lamprey, *Lethenteron reissneri*, in a small stream and an experimental aquarium. *Ichthyological Research* 49(1):62 – 68.
- U.S. Fish and Wildlife Service. 2005. Endangered and threatened wildlife and plants; listing the Gila chub as endangered with critical habitat; final rule. *Federal Register* 70(211):66664-66721.
- Vives, S.P. 1987a. Aspects of the life history of the slender madtom *Noturus exilis* in northeastern Oklahoma (Pisces: Ictaluridae). *The American Midland Naturalist* 117:167-176.
- Vives, S.P. 1987b. Life history of *Etheostoma punctulatum* (Pisces: Percidae) in northeastern Oklahoma. *The Southwestern Naturalist* 32:439-447.
- Vives, S.P. 1990a. Element Stewardship Abstract for *Gila intermedia*. Tucson: The Nature Conservancy, Arizona Field Office.
- Vives, S.P. 1990b. Nesting ecology and behavior of hornyhead chub, *Nocomis biguttatus*, a keystone species in Allequash Creek, Wisconsin. *American Midland Naturalist* 124:46-56.
- Vives, S.P., and W.L. Minckley. 1990. Autumn spawning and other reproductive notes on loach minnow, a threatened cyprinid fish of the American Southwest. *The Southwestern Naturalist* 35 (4):451-454.

- Weedman, D.A., A.L. Girmendonk, and K.L. Young. 1996. Status review of Gila chub, *Gila intermedia*, in the United States and Mexico. Nongame and Endangered Wildlife Program, Arizona Game and Fish Department, Technical Report 91, Phoenix, Arizona.
- Williams, C.M. 1991. Fish movement relative to physical environment in a Sonoran desert stream. Master's Thesis. Arizona State University, Tempe, Arizona.

FIGURE 1.—Map of sampling population (N=34) of approximate 100-m reaches and randomly chosen reaches (N=15) in upper Cienega Creek, Arizona (Pima County). Locations of reaches sampled are shaded with date sampled in parentheses.

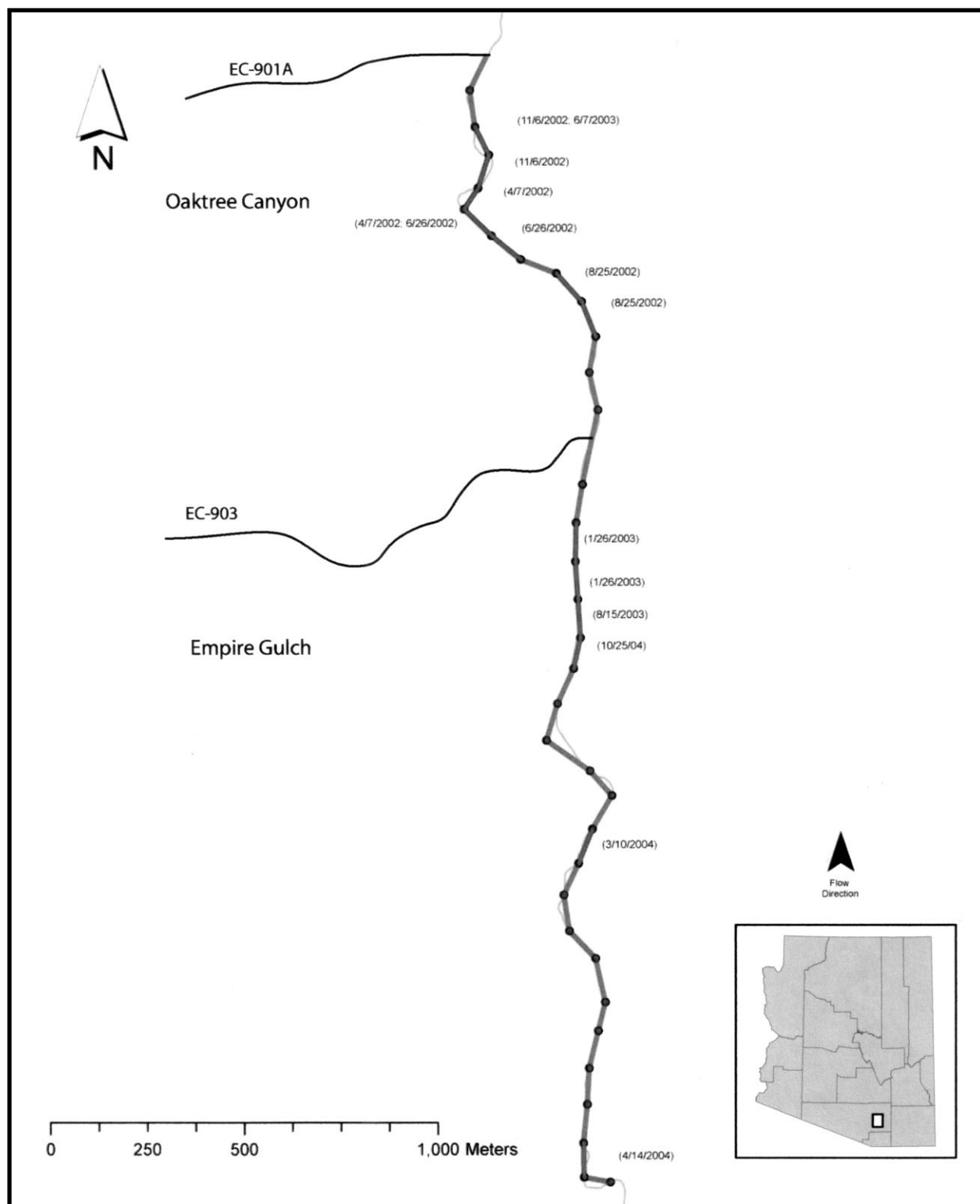


FIGURE 2.—Map of sampling population (N=49) of approximate 100-m reaches and randomly chosen reaches (N=13) in lower Cienega Creek, Arizona (Pima County). Locations of reaches sampled are shaded with date sampled in parentheses.

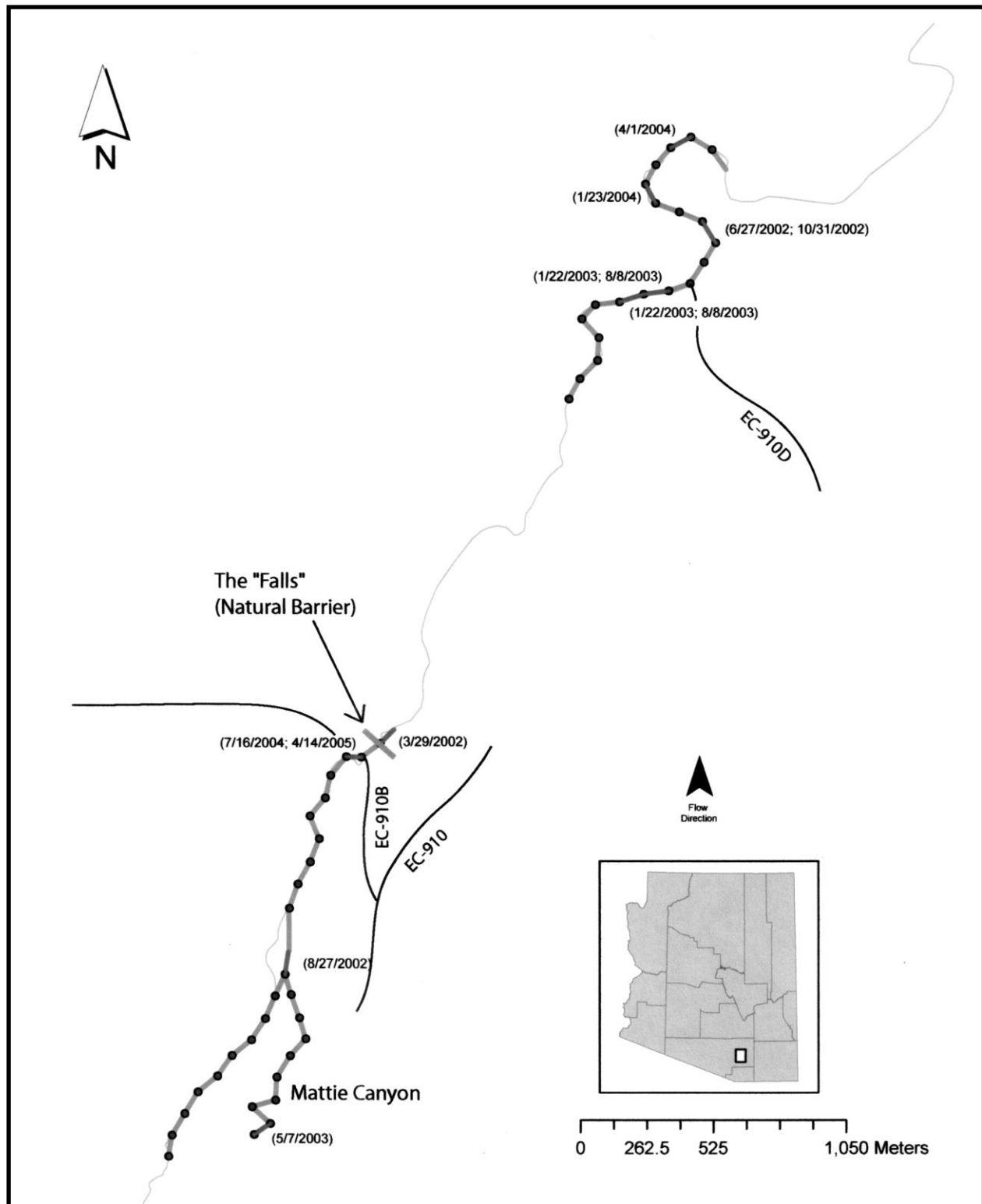


FIGURE 3.—Map of sampling population (N=40) of approximate 100-m reaches and randomly chosen reaches (N=19) in Bonita Creek, Arizona (Graham County). Locations of reaches sampled are shaded with date sampled in parentheses.

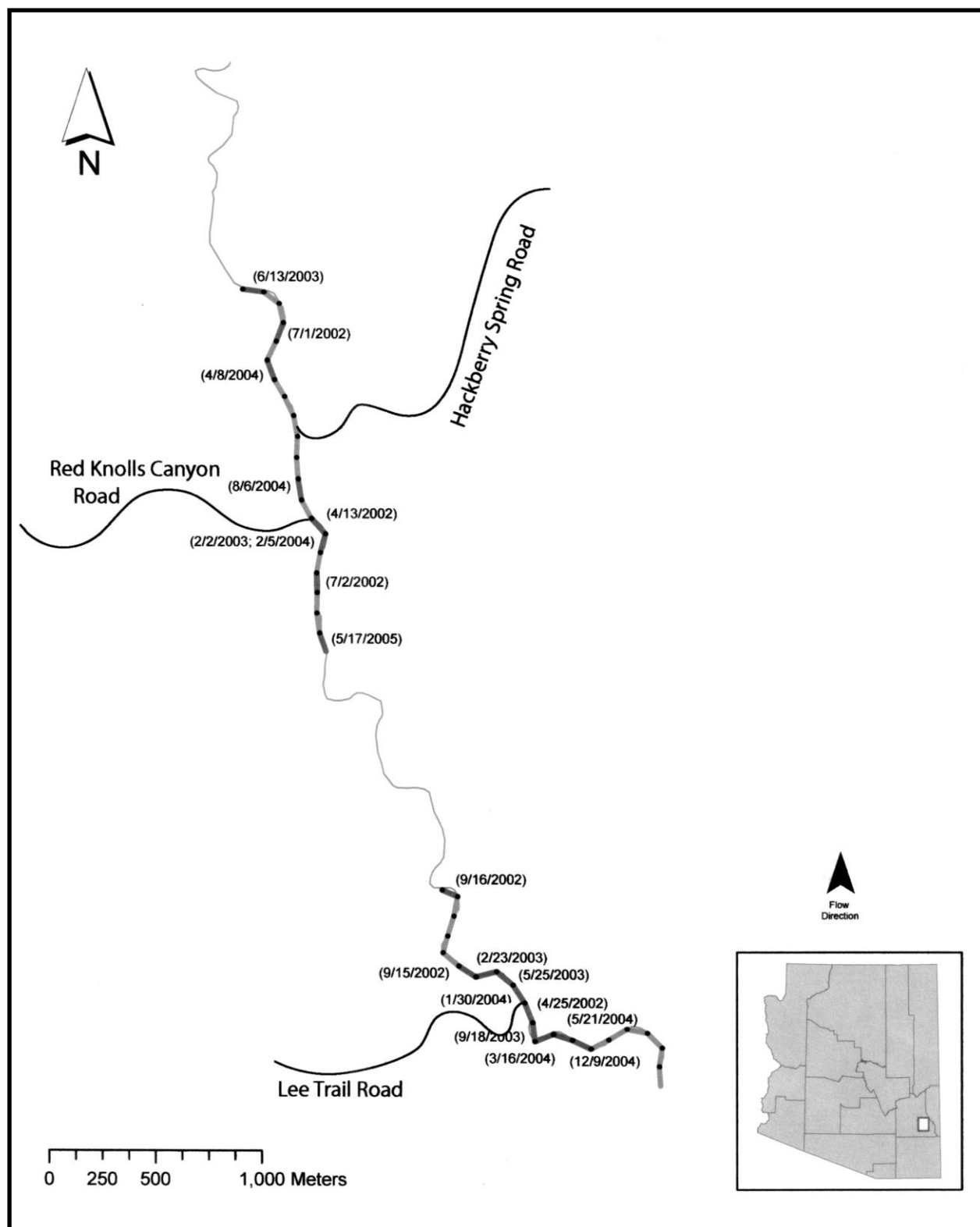


FIGURE 4.—Mean gonadosomatic index (GSI) values (\pm SEM) per month for female Gila chub *Gila intermedia* in Bonita Creek, Arizona (Graham County).

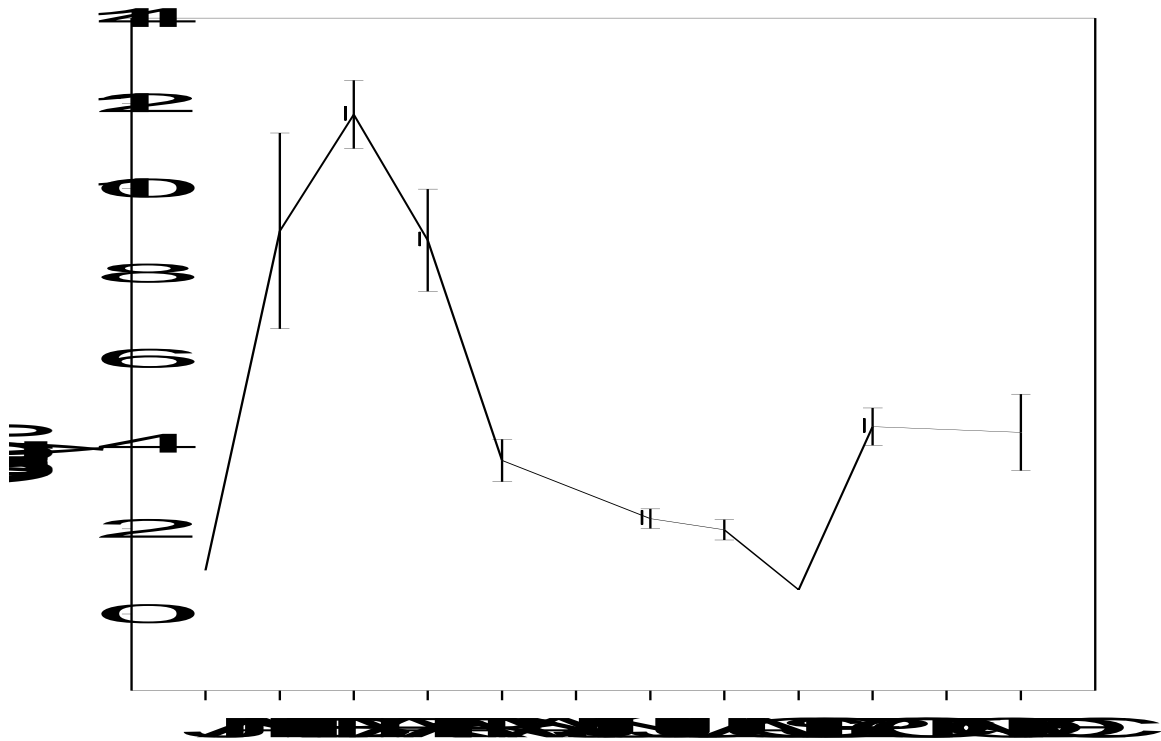


FIGURE 5.—Mean gonadosomatic index (GSI) values (\pm SEM) per month for female Gila chub *Gila intermedia* in Cienega Creek, Arizona (Pima County).

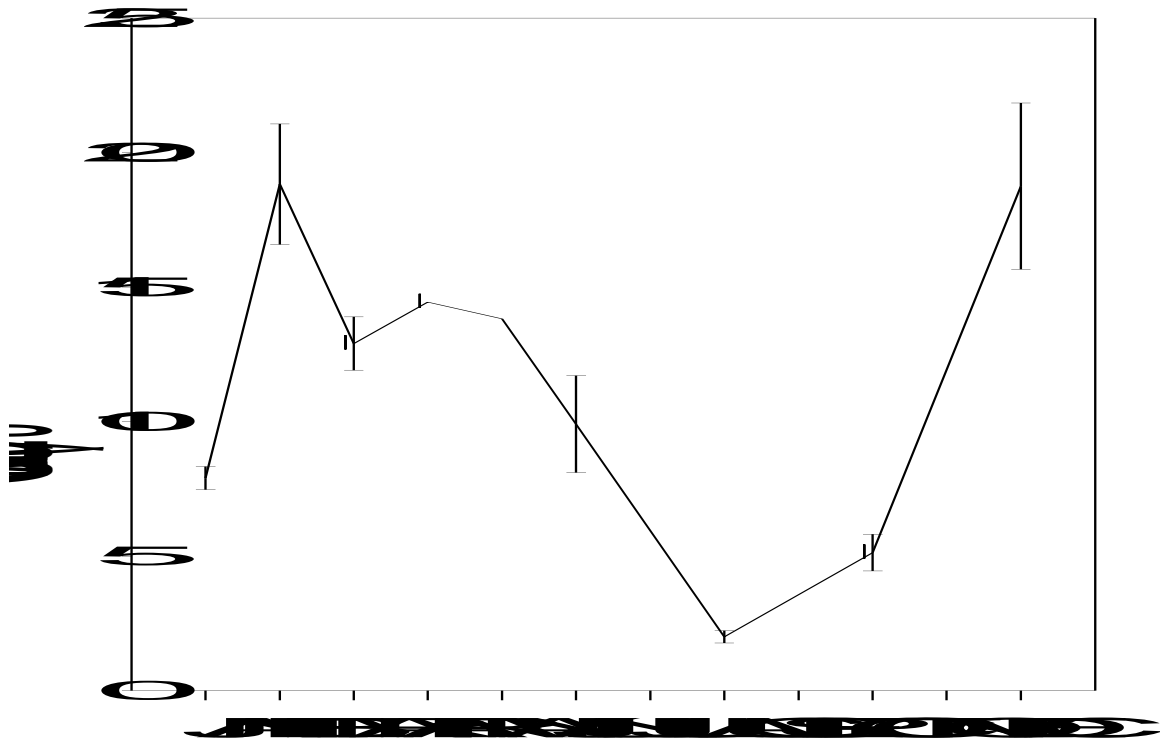


FIGURE 6.—Total catch of small juvenile Gila chub *Gila intermedia* per month in Bonita Creek, Arizona (Graham County).

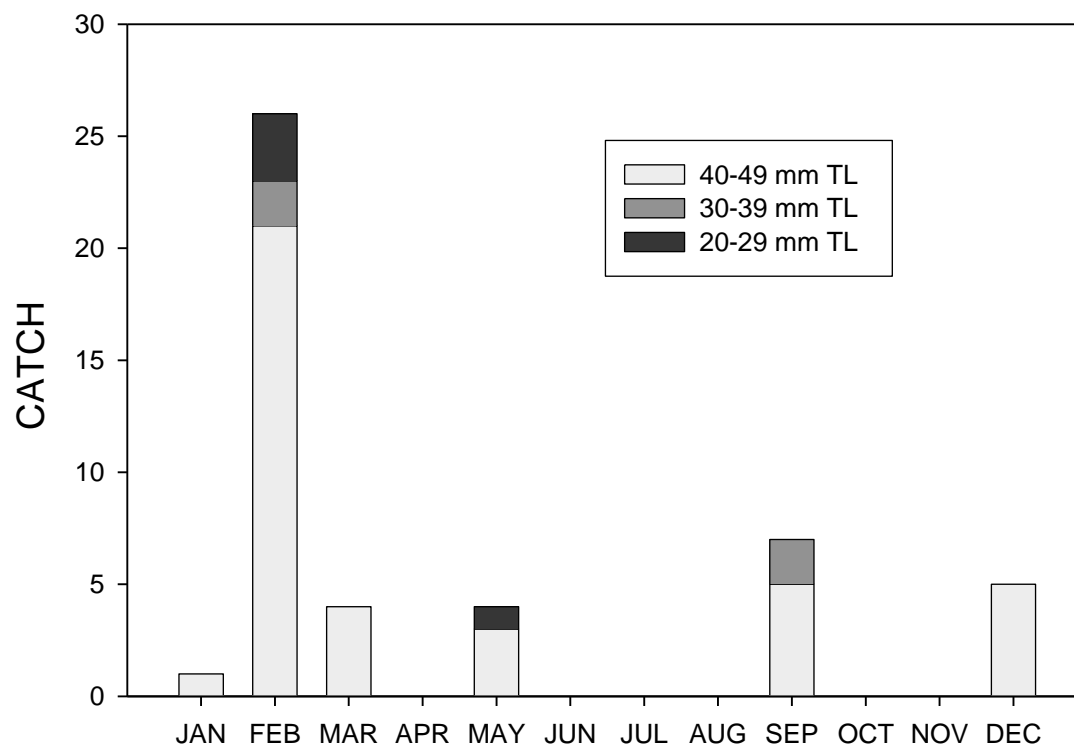


FIGURE 7.—Total catch of small juvenile Gila chub *Gila intermedia* per month in Cienega Creek, Arizona (Pima County).

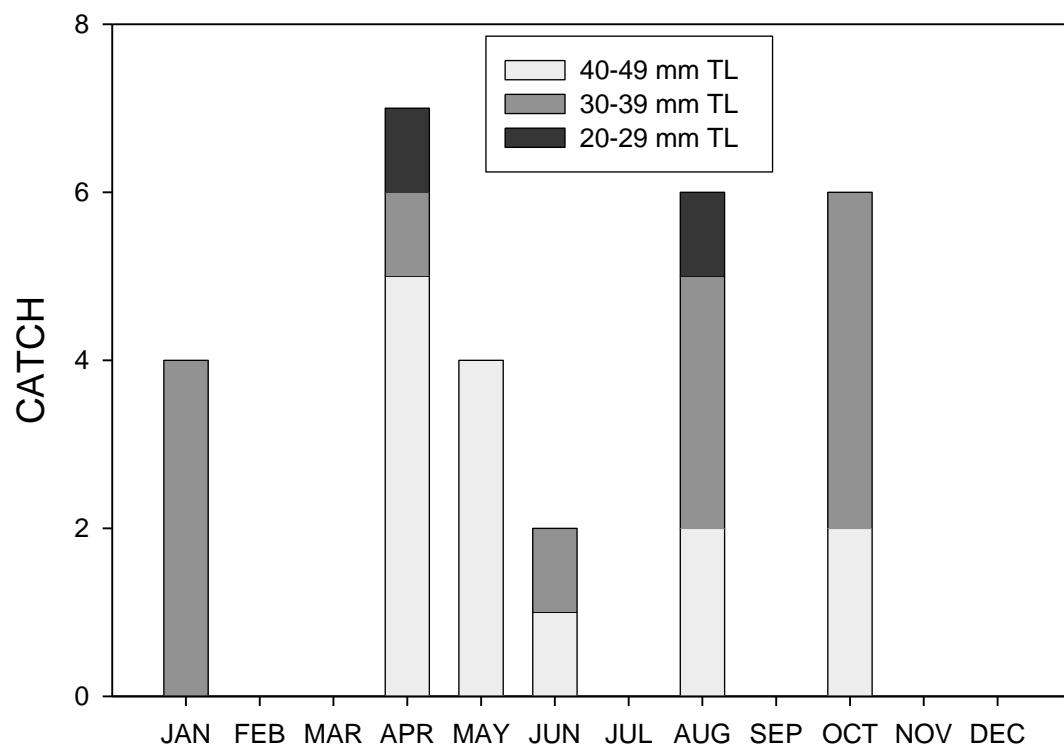


FIGURE 8.—Frequency of occurrence for Gila chub *Gila intermedia* having moderate, strong, or very strong spawning colors, and/or expressing gametes/tubercles per month in Bonita Creek, Arizona (Graham County).

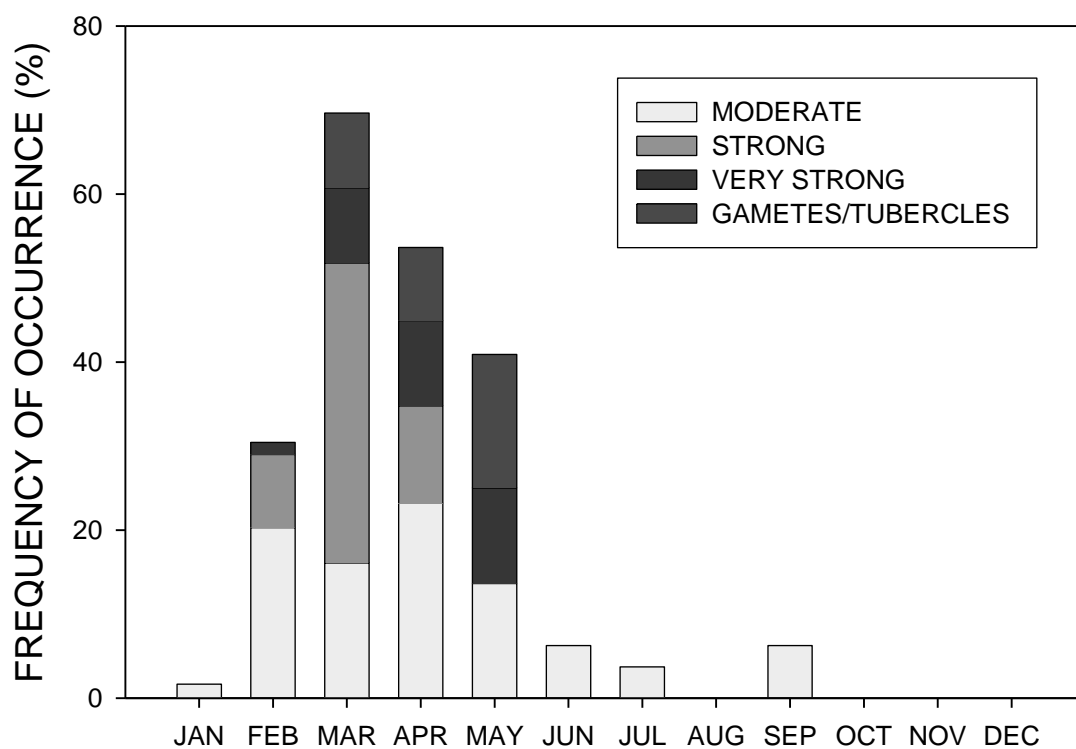


FIGURE 9.—Frequency of occurrence for Gila chub *Gila intermedia* having moderate, strong, or very strong spawning colors, and/or expressing gametes/tubercles per month in Cienega Creek, Arizona (Pima County).

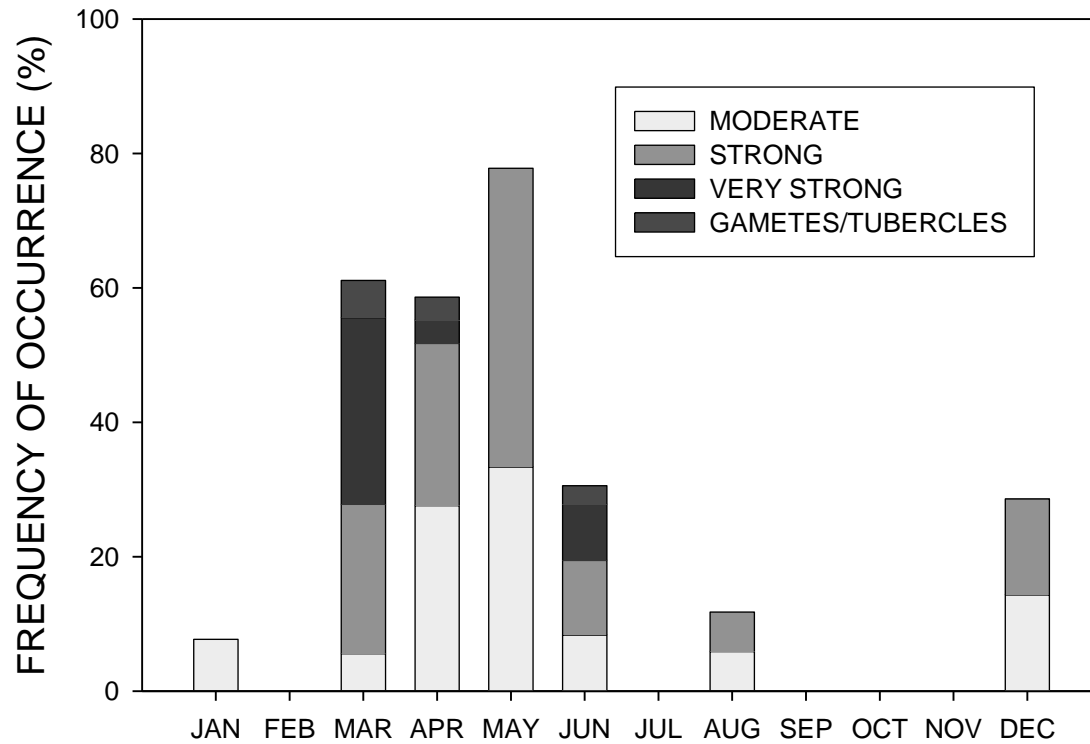


TABLE 1.—Comparison of habitat types and catch data of Gila chub *Gila intermedia* from Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County), for years 2002 – 2005. We used a chi-square goodness-of-fit test (χ^2) and one-way analysis of variance or Kruskal Wallis analysis of variance (F) to test for differences among observed and expected catch, and mean catch per unit effort (CPUE) of Gila chub, respectively. Selection is based on electivity index (EI) values, which are derived from equations detailed in Jacobs (1974). Mean CPUE values sharing a letter are not significantly different ($P \leq 0.05$).

Habitat Type	<i>n</i>	Relative Area	Catch	χ^2 ^a	EI Value	Selection ^b	Mean CPUE (per 100 m ²)	SD	<i>F</i> ^a
Bonita Creek									
Size Class 20 - 49 mmTL									
Pool	22	41.46%	17	***	-0.111	Neutral	0.495 xy	1.349	*
Run	54	41.93%	28		0.342	Moderate +	1.352 xy	4.199	
Riffle	22	10.70%	0		-1.000	Strong –	0.000 x	0.000	
Chute	10	5.43%	0		-1.000	Strong –	0.000 xy	0.000	
Cascade	2	0.49%	2		0.802	Strong +	2.755 y	3.896	
Size Class 50 - 79 mmTL									
Pool	22	41.46%	23	***	-0.290	Moderate –	0.816 x	1.420	**
Run	54	41.93%	54		0.455	Moderate +	3.748 x	9.514	
Riffle	22	10.70%	1		-0.813	Strong –	0.063 y	0.297	
Chute	10	5.43%	3		-0.204	Neutral	1.128 xy	3.567	
Cascade	2	0.49%	1		0.434	Moderate +	1.377 xy	1.948	
Size Class 80+ mmTL									
Pool	22	41.46%	99	**	-0.074	Neutral	5.581	7.950	NS
Run	54	41.93%	113		0.028	Neutral	7.680	14.620	
Riffle	22	10.70%	21		-0.156	Neutral	2.302	2.965	
Chute	10	5.43%	28		0.354	Moderate +	9.986	14.619	
Cascade	2	0.49%	0		-1.000	Strong –	0.000	0.000	
Cienega Creek									
Size Class 20 - 49 mmTL									
Pool	59	49.90%	28	***	0.931	Strong +	0.705	2.556	NS
Run	38	23.89%	1		-0.796	Strong –	0.031	0.194	
Riffle	2	1.73%	0		-1.000	Strong –	0.000	0.000	
Marsh	9	23.72%	0		-1.000	Strong –	0.000	0.000	
Chute	10	0.76%	0		-1.000	Strong –	0.000	0.000	
Size Class 50 - 79 mmTL									
Pool	59	49.90%	71	***	0.972	Strong +	1.148	2.935	*
Run	38	23.89%	1		-0.914	Strong –	0.045	0.277	
Riffle	2	1.73%	0		-1.000	Strong –	0.000	0.000	
Marsh	9	23.72%	0		-1.000	Strong –	0.000	0.000	
Chute	10	0.76%	0		-1.000	Strong –	0.000	0.000	
Size Class 80+ mmTL									
Pool	59	49.90%	49	***	1.000	Strong +	1.041 x	3.121	**
Run	38	23.89%	0		-1.000	Strong –	0.000 y	0.000	
Riffle	2	1.73%	0		-1.000	Strong –	0.000 xy	0.000	
Marsh	9	23.72%	0		-1.000	Strong –	0.000 xy	0.000	
Chute	10	0.76%	0		-1.000	Strong –	0.000 xy	0.000	

^a NS = not significant.

* $P \leq 0.05$.

** $P \leq 0.01$.

*** $P \leq 0.001$.

^b + = preference.

– = avoidance.

TABLE 2.—Mean length of Gila chub *Gila intermedia* per habitat type in Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County). Mean length values sharing a letter are not significantly different ($P \leq 0.05$).

Habitat Type	Mean Length (mm TL)	SD	<i>n</i>
Bonita Creek			
Pool	107.36 xy	41.78	139
Run	94.62 x	37.77	195
Riffle	116.00 y	23.92	22
Chute	112.48 y	27.90	31
Cascade	50.67 x	6.43	3
Cienega Creek			
Pool	71.51	2.04	148
Run	50.50	12.50	2
Riffle	0.00	--	0
Marsh	0.00	--	0
Chute	0.00	--	0

TABLE 3.—Comparison of total cover categories and catch data of Gila chub *Gila intermedia* from Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County), for years 2002 – 2005. We used a chi-square goodness-of-fit test (χ^2) and one-way analysis of variance or Kruskal Wallis analysis of variance (F) to test for differences among observed and expected catch, and mean catch per unit effort (CPUE) of Gila chub, respectively. Selection is based on electivity index (EI) values, which are derived from equations detailed in Jacobs (1974).

Total Percent Cover	<i>n</i>	Relative Area	Catch	χ^2 ^a	EI Value	Selection ^b	Mean CPUE (per 100 m ²)	SD	<i>F</i> ^a
Bonita Creek									
Size Class 20 - 49 mmTL									
0% - 25%	43	37.54%	9	***	-0.435	Moderate –	0.253	0.957	NS
25% - 50%	46	45.89%	22		0.018	Neutral	0.809	2.885	
50% - 75%	19	16.03%	9		0.107	Neutral	1.026	3.037	
75% - 100%	2	0.53%	7		0.941	Strong +	10.903	15.420	
Size Class 50 - 79 mmTL									
0% - 25%	43	37.54%	18	***	-0.363	Moderate –	0.949	2.521	NS
25% - 50%	46	45.89%	38		0.009	Neutral	1.792	4.278	
50% - 75%	19	16.03%	22		0.315	Moderate +	5.266	14.511	
75% - 100%	2	0.53%	4		0.811	Strong +	6.231	8.811	
Size Class 80+ mmTL									
0% - 25%	43	37.54%	77	*	-0.179	Neutral	3.567	6.848	NS
25% - 50%	46	45.89%	142		0.169	Neutral	7.812	9.767	
50% - 75%	19	16.03%	41		-0.012	Neutral	9.061	21.810	
75% - 100%	2	0.53%	1		-0.162	Neutral	1.558	2.203	
Cienega Creek									
Size Class 20 - 49 mmTL									
0% - 25%	36	37.95%	12	***	0.071	Neutral	0.662	3.011	NS
25% - 50%	36	18.81%	15		0.644	Strong +	0.419	1.295	
50% - 75%	20	7.16%	2		-0.020	Neutral	0.194	0.753	
75% - 100%	25	36.08%	0		-1.000	Strong –	0.000	0.000	
Size Class 50 - 79 mmTL									
0% - 25%	36	37.95%	13	***	-0.470	Moderate –	0.331	1.873	NS
25% - 50%	36	18.81%	47		0.781	Strong +	1.254	3.128	
50% - 75%	20	7.16%	8		0.237	Neutral	0.427	1.425	
75% - 100%	25	36.08%	4		-0.811	Strong –	0.140	0.700	
Size Class 80+ mmTL									
0% - 25%	36	37.95%	14	***	-0.209	Neutral	0.196	0.860	NS
25% - 50%	36	18.81%	28		0.704	Strong +	0.858	2.633	
50% - 75%	20	7.16%	7		0.368	Moderate +	1.135	4.015	
75% - 100%	25	36.08%	0		-1.000	Strong –	0.000	0.000	

^a NS = not significant.

* $P \leq 0.05$.

** $P \leq 0.01$.

*** $P \leq 0.001$.

^b + = preference.

– = avoidance.

TABLE 4.—Comparison of woody cover categories and catch data of Gila chub *Gila intermedia* from Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County), for years 2002 – 2005. We used a chi-square goodness-of-fit test (χ^2) and one-way analysis of variance or Kruskal Wallis analysis of variance (F) to test for differences among observed and expected catch, and mean catch per unit effort (CPUE) of Gila chub, respectively. Selection is based on electivity index (EI) values, which are derived from equations detailed in Jacobs (1974).

Percent Woody Cover	<i>n</i>	Relative Area	Catch	χ^2 ^a	EI Value	Selection ^b	Mean CPUE (per 100 m ²)	SD	<i>F</i> ^a
Bonita Creek									
<i>Size Class 20 - 49 mmTL</i>									
0 - 10%	58	50.37%	16	*	-0.326	Moderate –	0.309	1.053	NS
10 - 20%	24	25.85%	13		0.046	Neutral	1.094	2.895	
20 - 30%	16	17.64%	11		0.176	Neutral	1.600	4.512	
30%+	11	6.13%	7		0.456	Moderate +	1.982	6.575	
<i>Size Class 50 - 79 mmTL</i>									
0 - 10%	58	50.37%	40	NS	-0.032	Neutral	1.088	2.594	NS
10 - 20%	24	25.85%	21		-0.006	Neutral	1.450	2.159	
20 - 30%	16	17.64%	13		-0.064	Neutral	2.980	6.463	
30%+	11	6.13%	8		0.246	Neutral	7.996	19.139	
<i>Size Class 80+ mmTL</i>									
0 - 10%	58	50.37%	156	NS	0.188	Neutral	6.254	9.422	NS
10 - 20%	24	25.85%	36		-0.371	Moderate –	2.140	3.141	
20 - 30%	16	17.64%	54		0.098	Neutral	5.596	7.147	
30%+	11	6.13%	15		-0.035	Neutral	14.552	28.183	
Cienega Creek									
<i>Size Class 20 - 49 mmTL</i>									
0 - 10%	76	67.36%	20	NS	0.037	Neutral	0.459	2.250	NS
10 - 20%	25	21.94%	8		0.151	Neutral	0.250	0.630	
20 - 30%	10	8.28%	0		-1.000	Strong –	0.000	0.000	
30%+	7	2.42%	1		0.180	Neutral	0.242	0.641	
<i>Size Class 50 - 79 mmTL</i>									
0 - 10%	76	67.36%	30	***	-0.486	Moderate –	0.572	2.693	NS
10 - 20%	25	21.94%	37		0.580	Strong +	1.177	3.082	
20 - 30%	10	8.28%	1		-0.730	Strong –	0.171	0.540	
30%+	7	2.42%	4		0.407	Moderate +	0.500	1.323	
<i>Size Class 80+ mmTL</i>									
0 - 10%	76	67.36%	33	NS	< 0.000	Neutral	0.432	1.879	NS
10 - 20%	25	21.94%	16		0.266	Moderate +	0.796	1.865	
20 - 30%	10	8.28%	0		-1.000	Strong –	0.000	0.000	
30%+	7	2.42%	0		-1.000	Strong –	0.000	0.000	

^a NS = not significant.

* $P \leq 0.05$.

** $P \leq 0.01$.

*** $P \leq 0.001$.

^b + = preference.

– = avoidance.

TABLE 5.—Comparison of vegetative cover categories and catch data of Gila chub *Gila intermedia* from Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County), for years 2002 – 2005. We used a chi-square goodness-of-fit test (χ^2) and one-way analysis of variance or Kruskal Wallis analysis of variance (F) to test for differences among observed and expected catch, and mean catch per unit effort (CPUE) of Gila chub, respectively. Selection is based on electivity index (EI) values, which are derived from equations detailed in Jacobs (1974).

Percent Vegetative Cover	<i>n</i>	Relative Area	Catch	χ^2 ^a	EI Value	Selection ^b	Mean CPUE (per 100 m ²)	SD	<i>F</i> ^a
Bonita Creek									
<i>Size Class 20 - 49 mmTL</i>									
0 - 15%	62	51.66%	17	**	-0.307	Moderate –	0.465	2.394	NS
15 - 30%	32	28.60%	13		-0.023	Neutral	0.865	1.757	
30 - 45%	14	17.74%	17		0.449	Moderate +	2.502	6.455	
45%+	2	2.00%	0		-1.000	Strong –	0.000	0.000	
<i>Size Class 50 - 79 mmTL</i>									
0 - 15%	62	51.66%	29	*	-0.323	Moderate –	1.348	3.887	NS
15 - 30%	32	28.60%	33		0.254	Neutral	3.727	11.364	
30 - 45%	14	17.74%	17		0.096	Neutral	1.605	3.498	
45%+	2	2.00%	3		0.300	Moderate +	4.110	5.812	
<i>Size Class 80+ mmTL</i>									
0 - 15%	62	51.66%	117	*	-0.136	Neutral	4.229	6.890	NS
15 - 30%	32	28.60%	87		0.110	Neutral	8.792	17.549	
30 - 45%	14	17.74%	47		0.009	Neutral	6.605	11.617	
45%+	2	2.00%	10		0.322	Moderate +	10.764	11.900	
Cienega Creek									
<i>Size Class 20 - 49 mmTL</i>									
0 - 15%	30	39.81%	17	***	0.364	Moderate +	0.914	3.293	NS
15 - 30%	30	13.43%	9		0.487	Moderate +	0.343	1.359	
30 - 45%	24	8.24%	1		-0.431	Moderate –	0.050	0.244	
45%+	34	38.53%	2		-0.789	Strong –	0.114	0.580	
<i>Size Class 50 - 79 mmTL</i>									
0 - 15%	30	39.81%	24	***	-0.139	Neutral	0.660	2.228	NS
15 - 30%	30	13.43%	40		0.779	Strong +	1.386	3.353	
30 - 45%	24	8.24%	0		-1.000	Strong –	0.000	0.000	
45%+	34	38.53%	8		-0.667	Strong –	0.602	3.088	
<i>Size Class 80+ mmTL</i>									
0 - 15%	30	39.81%	19	***	-0.022	Neutral	0.386	1.054	NS
15 - 30%	30	13.43%	23		0.702	Strong +	0.888	2.849	
30 - 45%	24	8.24%	0		-1.000	Strong –	0.000	0.000	
45%+	34	38.53%	7		-0.580	Strong –	0.317	1.152	

^a NS = not significant.

* $P \leq 0.05$.

** $P \leq 0.01$.

*** $P \leq 0.001$.

^b + = preference.

– = avoidance.

TABLE 6.—Comparison of rock/boulder cover categories and catch data of Gila chub *Gila intermedia* from Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County), for years 2002 – 2005. We used a chi-square goodness-of-fit test (χ^2) and one-way analysis of variance or Kruskal Wallis analysis of variance (F) to test for differences among observed and expected catch, and mean catch per unit effort (CPUE) of Gila chub, respectively. Selection is based on electivity index (EI) values, which are derived from equations detailed in Jacobs (1974). Mean CPUE values sharing a letter are not significantly different ($P \leq 0.05$).

Percent Rock/Boulder Cover	<i>n</i>	Relative Area	Catch	χ^2 ^a	EI Value	Selection ^b	Mean CPUE (per 100 m ²)	SD	<i>F</i> ^a
Bonita Creek									
Size Class 20 - 49 mmTL									
0 - 10%	87	50.37%	37	***	0.569	Strong +	0.799	3.170	NS
10 - 20%	9	25.85%	7		-0.332	Moderate -	1.660	4.105	
20 - 30%	8	17.64%	1		-0.816	Strong -	0.201	0.569	
30%+	6	6.13%	2		-0.190	Neutral	0.918	2.249	
Size Class 50 - 79 mmTL									
0 - 10%	87	50.37%	66	***	0.605	Strong +	2.400	7.710	NS
10 - 20%	9	25.85%	2		-0.866	Strong -	0.458	1.375	
20 - 30%	8	17.64%	7		-0.393	Moderate -	0.979	1.850	
30%+	6	6.13%	7		0.176	Neutral	2.574	3.230	
Size Class 80+ mmTL									
0 - 10%	87	50.37%	232	***	0.775	Strong +	6.624	12.896	NS
10 - 20%	9	25.85%	14		-0.720	Strong -	4.123	4.589	
20 - 30%	8	17.64%	11		-0.659	Strong -	3.269	4.669	
30%+	6	6.13%	4		-0.615	Strong -	3.638	5.670	
Cienega Creek									
Size Class 20 - 49 mmTL									
0%	101	85.21%	15	***	-0.686	Strong -	0.286	1.838	NS
0 - 5%	11	9.61%	8		0.564	Strong +	0.459	0.857	
5%+	5	5.18%	6		0.654	Strong +	1.429	3.194	
Size Class 50 - 79 mmTL									
0%	101	85.21%	31	***	-0.768	Strong -	0.440 x	2.218	*
0 - 5%	11	9.61%	32		0.765	Strong +	2.579 y	4.137	
5%+	5	5.18%	9		0.446	Moderate +	2.143 xy	4.792	
Size Class 80+ mmTL									
0%	101	85.21%	25	***	-0.694	Strong -	0.235 x	0.983	*
0 - 5%	11	9.61%	12		0.506	Strong +	0.992 xy	1.609	
5%+	5	5.18%	12		0.712	Strong +	2.857 y	6.389	

^a NS = not significant.

* $P \leq 0.05$.

** $P \leq 0.01$.

*** $P \leq 0.001$.

^b + = preference.

- = avoidance.

TABLE 7.—Comparison of algal cover categories and catch data of Gila chub *Gila intermedia* from Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County), for years 2002 – 2005. We used a chi-square goodness-of-fit test (χ^2) and one-way analysis of variance or Kruskal Wallis analysis of variance (F) to test for differences among observed and expected catch, and mean catch per unit effort (CPUE) of Gila chub, respectively. Selection is based on electivity index (EI) values, which are derived from equations detailed in Jacobs (1974).

Percent Floating Algal Cover	<i>n</i>	Relative Area	Catch	χ^2 ^a	EI Value	Selection ^b	Mean CPUE (per 100 m ²)	SD	<i>F</i> ^a
Bonita Creek									
Size Class 20 - 49 mmTL									
0 - 5%	104	86.96%	44	NS	0.375	Moderate +	0.868	3.165	NS
5 - 10%	3	5.14%	0		-1.000	Strong -	0.000	0.000	
10%+	3	7.90%	3		-0.114	Neutral	0.441	0.764	
Size Class 50 - 79 mmTL									
0 - 5%	104	86.96%	79	*	0.596	Strong +	2.234	7.123	NS
5 - 10%	3	5.14%	1		-0.629	Strong -	0.114	0.197	
10%+	3	7.90%	2		-0.549	Strong -	0.294	0.255	
Size Class 80+ mmTL									
0 - 5%	104	86.96%	241	*	0.287	Moderate +	6.238	11.994	NS
5 - 10%	3	5.14%	7		-0.326	Strong -	0.798	1.382	
10%+	3	7.90%	13		-0.242	Neutral	2.204	3.442	
Cienega Creek									
Size Class 20 - 49 mmTL									
0 - 10%	104	88.74%	28	NS	0.561	Strong +	0.403	1.951	NS
10 - 20%	7	6.81%	0		-1.000	Strong -	0.000	0.000	
20 - 30%	3	1.83%	1		0.313	Moderate +	0.278	0.481	
30%+	4	2.62%	0		-1.000	Strong -	0.000	0.000	
Size Class 50 - 79 mmTL									
0 - 10%	104	88.74%	47	***	-0.615	Strong -	0.580	2.455	NS
10 - 20%	7	6.81%	9		0.323	Moderate +	1.172	2.032	
20 - 30%	3	1.83%	16		0.877	Strong +	4.441	7.692	
30%+	4	2.62%	0		-1.000	Strong -	0.000	0.000	
Size Class 80+ mmTL									
0 - 10%	104	88.74%	43	*	-0.047	Neutral	0.420	1.736	NS
10 - 20%	7	6.81%	3		-0.057	Neutral	0.403	1.067	
20 - 30%	3	1.83%	3		0.555	Strong +	0.833	1.442	
30%+	4	2.62%	0		-1.000	Strong -	0.000	0.000	

^a NS = not significant.

* $P \leq 0.05$.

** $P \leq 0.01$.

*** $P \leq 0.001$.

^b + = preference.

- = avoidance.

TABLE 8.—Comparison of mean depth categories and catch data of Gila chub *Gila intermedia* from Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County), for years 2002 – 2005. We used a chi-square goodness-of-fit test (χ^2) and one-way analysis of variance or Kruskal Wallis analysis of variance (F) to test for differences among observed and expected catch, and mean catch per unit effort (CPUE) of Gila chub, respectively. Selection is based on electivity index (EI) values, which are derived from equations detailed in Jacobs (1974). Mean CPUE values sharing a letter are not significantly different ($P \leq 0.05$).

Mean Depth (cm)	<i>n</i>	Relative Area	Catch	χ^2 ^a	EI Value	Selection ^b	Mean CPUE (per 100 m ²)	SD	<i>F</i> ^a
Bonita Creek									
Size Class 20 - 49 mmTL									
0 - 25	71	51.42%	13	**	-0.469	Moderate –	0.355	1.213	NS
25 - 50	26	20.52%	18		0.412	Moderate +	2.049	5.805	
50 - 75	7	12.88%	8		0.162	Neutral	1.194	2.253	
75 - 100	5	12.80%	8		0.166	Neutral	0.506	0.694	
100+	1	2.39%	0		-1.000	Strong –	0.000	--	
Size Class 50 - 79 mmTL									
0 - 25	71	51.42%	36	*	-0.150	Neutral	1.920	7.846	NS
25 - 50	26	20.52%	29		0.359	Moderate +	3.396	5.801	
50 - 75	7	12.88%	9		-0.091	Neutral	0.450	0.593	
75 - 100	5	12.80%	8		-0.152	Neutral	1.605	2.306	
100+	1	2.39%	0		-1.000	Strong –	0.000	--	
Size Class 80+ mmTL									
0 - 25	71	51.42%	107	***	-0.207	Neutral	5.343	12.903	NS
25 - 50	26	20.52%	80		0.263	Moderate +	9.170	10.701	
50 - 75	7	12.88%	40		0.101	Neutral	6.137	8.364	
75 - 100	5	12.80%	32		-0.025	Neutral	5.250	6.990	
100+	1	2.39%	2		-0.520	Strong –	1.023	--	
Cienega Creek									
Size Class 20 - 49 mmTL									
0 - 25	72	54.53%	1	***	-0.942	Strong –	0.017 x	0.141	***
25 - 50	22	12.75%	8		0.446	Moderate +	1.012 xy	3.832	
50 - 75	12	12.49%	14		0.735	Strong +	1.024 y	2.120	
75 - 100	7	12.69%	4		0.048	Neutral	0.270 xy	0.714	
100+	5	7.54%	2		-0.048	Neutral	0.465 xy	1.040	
Size Class 50 - 79 mmTL									
0 - 25	72	54.53%	1	***	-0.977	Strong –	0.024 x	0.201	***
25 - 50	22	12.75%	21		0.476	Moderate +	1.155 y	2.819	
50 - 75	12	12.49%	37		0.762	Strong +	2.816 y	4.735	
75 - 100	7	12.69%	8		-0.075	Neutral	0.391 y	0.880	
100+	5	7.54%	5		-0.044	Neutral	1.163 xy	2.600	
Size Class 80+ mmTL									
0 - 25	72	54.53%	0	***	-1.000	Strong –	0.000 x	0.000	***
25 - 50	22	12.75%	8		0.144	Neutral	1.169 y	3.923	
50 - 75	12	12.49%	29		0.821	Strong +	2.344 y	4.100	
75 - 100	7	12.69%	8		0.146	Neutral	0.413 y	0.563	
100+	5	7.54%	4		0.043	Neutral	0.930 y	2.080	

^a NS = not significant.

* $P \leq 0.05$.

** $P \leq 0.01$.

*** $P \leq 0.001$.

^b + = preference.

– = avoidance.

TABLE 9.—Comparison of maximum depth categories and catch data of Gila chub *Gila intermedia* from Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County), for years 2002 – 2005. We used a chi-square goodness-of-fit test (χ^2) and one-way analysis of variance or Kruskal Wallis analysis of variance (F) to test for differences among observed and expected catch, and mean catch per unit effort (CPUE) of Gila chub, respectively. Selection is based on electivity index (EI) values, which are derived from equations detailed in Jacobs (1974). Mean CPUE values sharing a letter are not significantly different ($P \leq 0.05$).

Maximum Depth (cm)	<i>n</i>	Relative Area	Catch	χ^2 ^a	EI Value	Selection ^b	Mean CPUE (per 100 m ²)	SD	<i>F</i> ^a
Bonita Creek									
Size Class 20 - 49 mmTL									
0 - 50	71	44.76%	18	NS	-0.133	Neutral	0.640	2.818	*
50 - 100	26	25.01%	12		0.014	Neutral	1.272	4.179	
100 - 150	9	15.59%	8		0.053	Neutral	1.059	2.060	
150 - 200	3	6.95%	5		0.229	Neutral	0.892	0.772	
200 +	2	7.70%	4		0.054	Neutral	0.425	0.602	
Size Class 50 - 79 mmTL									
0 - 50	71	44.76%	37	NS	0.007	Neutral	2.153	8.006	NS
50 - 100	26	25.01%	24		0.108	Neutral	2.533	5.331	
100 - 150	9	15.59%	12		-0.037	Neutral	1.307	2.052	
150 - 200	3	6.95%	2		-0.498	Moderate –	0.372	0.343	
200 +	2	7.70%	7		0.056	Neutral	0.950	0.462	
Size Class 80+ mmTL									
0 - 50	71	44.76%	113	**	-0.030	Neutral	6.080	13.667	NS
50 - 100	26	25.01%	55		-0.111	Neutral	5.176	6.100	
100 - 150	9	15.59%	61		0.246	Neutral	9.531	9.581	
150 - 200	3	6.95%	18		-0.004	Neutral	3.408	3.135	
200 +	2	7.70%	14		-0.191	Neutral	1.899	0.923	
Cienega Creek									
Size Class 20 - 49 mmTL									
0 - 50	67	54.54%	0	***	-1.000	Strong –	0.000 x	0.000	***
50 - 100	28	11.45%	10		0.606	Strong +	0.916 y	3.416	
100 - 150	12	14.85%	13		0.647	Strong +	1.197 y	2.141	
150 - 200	5	5.51%	6		0.635	Strong +	0.843 y	1.164	
200 +	6	13.65%	0		-1.000	Strong –	0.000 xy	0.000	
Size Class 50 - 79 mmTL									
0 - 50	67	54.54%	1	***	-0.977	Strong –	0.026 x	0.209	***
50 - 100	28	11.45%	21		0.522	Strong +	1.258 xy	3.950	
100 - 150	12	14.85%	37		0.717	Strong +	2.993 y	4.765	
150 - 200	5	5.51%	11		0.512	Strong +	1.729 y	2.592	
200 +	6	13.65%	2		-0.694	Strong –	0.062 xy	0.152	
Size Class 80+ mmTL									
0 - 50	67	55.52%	0	***	-1.000	Strong –	0.000 x	0.000	***
50 - 100	28	11.66%	10		0.321	Moderate +	0.484 xy	1.499	
100 - 150	12	13.33%	29		0.808	Strong +	2.425 z	4.069	
150 - 200	5	5.60%	6		0.403	Moderate +	1.119 yz	2.016	
200 +	6	13.89%	4		-0.290	Moderate –	0.124 yz	0.305	

^a NS = not significant.

* $P \leq 0.05$.

** $P \leq 0.01$.

*** $P \leq 0.001$.

^b + = preference.

– = avoidance.

TABLE 10.—Mean total length of Gila chub *Gila intermedia* per maximum depth category in Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County). Mean length values sharing a letter are not significantly different ($P \leq 0.05$).

Maximum Depth (cm)	Mean Length (mm TL)	SD	<i>n</i>
Bonita Creek			
0-50	99.52	35.02	168
50-100	97.10	39.95	91
100-150	112.89	40.62	81
150-200	103.68	46.28	25
200+	90.88	38.46	25
Cienega Creek			
0-50	63.00	--	1 ^a
50-100	68.54 x	23.49	41
100-150	73.43 x	24.78	79
150-200	61.30 x	19.84	23
200+	100.00 y	29.20	6

^a Not included in statistical comparison.

TABLE 11.—Comparison of mean flow velocity (cm/s) categories and catch data of Gila chub *Gila intermedia* from Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County), for years 2002 – 2005. We used a chi-square goodness-of-fit test (χ^2) and one-way analysis of variance or Kruskal Wallis analysis of variance (F) to test for differences among observed and expected catch, and mean catch per unit effort (CPUE) of Gila chub, respectively. Selection is based on electivity index (EI) values, which are derived from equations detailed in Jacobs (1974).

Velocity (cm/s)	<i>n</i>	Relative Area	Catch	χ^2 ^a	EI Value	Selection ^b	Mean CPUE (per 100 m ²)	SD	<i>F</i> ^a
Bonita Creek									
Size Class 20 - 49 mmTL									
0	12	31.10%	14	*	-0.031	Neutral	0.775	1.699	NS
> 0 - 25	28	21.50%	18		0.388	Moderate +	1.546	4.730	
25 - 50	30	27.43%	11		-0.106	Neutral	0.967	3.406	
50 - 75	12	6.32%	1		-0.513	Strong -	0.097	0.336	
75 - 100	11	5.05%	3		0.123	Neutral	0.602	1.661	
100+	17	8.59%	0		-1.000	Strong -	0.000	0.000	
Size Class 50 - 79 mmTL									
0	12	31.10%	13	NS	-0.411	Moderate -	0.327	0.409	NS
> 0 - 25	28	21.50%	29		0.333	Moderate +	4.055	12.162	
25 - 50	30	27.43%	23		0.015	Neutral	1.772	3.397	
50 - 75	12	6.32%	9		0.292	Moderate +	1.869	3.460	
75 - 100	11	5.05%	3		-0.167	Neutral	2.355	6.946	
100+	17	8.59%	5		-0.182	Neutral	0.988	2.846	
Size Class 80+ mmTL									
0	12	31.10%	46	NS	-0.357	Moderate -	1.424	1.633	NS
> 0 - 25	28	21.50%	77		0.209	Neutral	9.369	18.275	
25 - 50	30	27.43%	86		0.131	Neutral	5.886	9.518	
50 - 75	12	6.32%	11		-0.211	Neutral	4.106	7.913	
75 - 100	11	5.05%	14		0.032	Neutral	5.195	8.507	
100+	17	8.59%	27		0.103	Neutral	7.388	10.349	
Cienega Creek									
Size Class 20 - 49 mmTL									
0	57	65.05%	24	NS	0.294	Moderate +	0.642	2.535	NS
> 0 - 25	34	24.91%	5		-0.294	Moderate -	0.182	0.875	
25 - 50	15	5.20%	0		-1.000	Strong -	0.000	0.000	
50 - 75	3	0.20%	0		-1.000	Strong -	0.000	0.000	
75 - 100	2	2.55%	0		-1.000	Strong -	0.000	0.000	
100+	7	2.08%	0		-1.000	Strong -	0.000	0.000	
Size Class 50 - 79 mmTL									
0	57	65.05%	57	**	0.184	Neutral	1.285	4.855	NS
> 0 - 25	34	24.91%	15		-0.184	Neutral	0.918	3.572	
25 - 50	15	5.20%	0		-1.000	Strong -	0.000	0.000	
50 - 75	3	0.20%	0		-1.000	Strong -	0.000	0.000	
75 - 100	2	2.55%	0		-1.000	Strong -	0.000	0.000	
100+	7	2.08%	0		-1.000	Strong -	0.000	0.000	
Size Class 80+ mmTL									
0	57	65.05%	40	***	0.258	Moderate +	0.654	2.144	NS
> 0 - 25	34	24.91%	9		-0.258	Moderate -	0.344	1.315	
25 - 50	15	5.20%	0		-1.000	Strong -	0.000	0.000	
50 - 75	3	0.20%	0		-1.000	Strong -	0.000	0.000	
75 - 100	2	2.55%	0		-1.000	Strong -	0.000	0.000	
100+	7	2.08%	0		-1.000	Strong -	0.000	0.000	

^a NS = not significant.

* $P \leq 0.05$.

** $P \leq 0.01$.

*** $P \leq 0.001$.

^b + = preference.

- = avoidance.

TABLE 12.—Comparison of substrate categories and catch data of Gila chub *Gila intermedia* from Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County), for years 2002 – 2005. We used a chi-square goodness-of-fit test (χ^2) and one-way analysis of variance or Kruskal Wallis analysis of variance (F) to test for differences among observed and expected catch, and mean catch per unit effort (CPUE) of Gila chub, respectively. Selection is based on electivity index (EI) values, which are derived from equations detailed in Jacobs (1974).

Substrate	<i>n</i>	Relative Area	Catch	χ^2 ^a	EI Value	Selection ^b	Mean CPUE (per 100 m ²)	SD	<i>F</i> ^a
Bonita Creek									
Size Class 20 - 49 mmTL									
Organic	46	35.85%	26	NS	0.378	Moderate +	1.103	3.769	NS
Fines	47	54.85%	19		-0.285	Moderate –	0.705	2.791	
Moderate	3	3.78%	0		-1.000	Strong –	0.000	0.000	
Coarse	9	3.72%	2		0.071	Neutral	0.612	1.837	
Varied	2	1.71%	0		-1.000	Strong –	0.000	0.000	
Size Class 50 - 79 mmTL									
Organic	46	35.85%	24	**	-0.123	Neutral	1.029	2.706	NS
Fines	47	54.85%	42		-0.036	Neutral	3.395	10.053	
Moderate	3	3.78%	6		0.353	Moderate +	1.744	2.500	
Coarse	9	3.72%	7		0.432	Moderate +	1.716	2.860	
Varied	2	1.71%	0		-1.000	Strong –	0.000	0.000	
Size Class 80+ mmTL									
Organic	46	35.85%	125	***	0.272	Moderate +	6.853	10.301	NS
Fines	47	54.85%	113		-0.204	Neutral	6.555	14.753	
Moderate	3	3.78%	5		-0.322	Moderate –	1.337	1.385	
Coarse	9	3.72%	6		-0.227	Neutral	3.002	4.822	
Varied	2	1.71%	4		-0.039	Neutral	6.922	6.466	
Cienega Creek									
Size Class 20 - 49 mmTL									
Organic	54	62.48%	12	*	-0.287	Moderate –	0.235	1.080	NS
Fines	31	26.87%	12		0.430	Moderate +	0.368	1.046	
Mixed	17	10.65%	1		-0.482	Moderate –	0.070	0.290	
Size Class 50 - 79 mmTL									
Organic	54	62.48%	42	*	0.004	Neutral	0.762	2.537	NS
Fines	31	26.87%	25		0.237	Neutral	0.709	2.285	
Mixed	17	10.65%	0		-1.000	Strong –	0.000	0.000	
Size Class 80+ mmTL									
Organic	54	62.48%	26	NS	0.020	Neutral	0.484	2.104	NS
Fines	31	26.87%	15		0.222	Neutral	0.440	1.281	
Mixed	17	10.65%	0		-1.000	Strong –	0.000	0.000	

^a NS = not significant.

* $P \leq 0.05$.

** $P \leq 0.01$.

*** $P \leq 0.001$.

^b + = preference.

– = avoidance.

TABLE 13.--Summary and ranges of physical/chemical/hydraulic measures taken in Bonita Creek, Arizona (Graham County) and Cienega Creek, Arizona (Pima County), for years 2002 - 2005.

Discharge (m ³ /s)	Canopy Cover	Bank Stability	Cattle Sign	Water Clarity (Clear)	Temperature (°C)	pH	Dissolved Oxygen (mg/L)	Conductivity (µS/cm ³)
Bonita Creek								
Annual Mean	Sparse 31%	Good 46%	15%	84%	10.5 - 25.2	7.0 - 9.5	6.2 - 10.1	125 - 438
0.12	Moderate 29%	Fair 43%			(10.5 - 25.2)*	(7.0 - 9.5)*	(6.2 - 10.1)*	(125 - 438)*
(0.39)**	Dense 24%	Poor 11%						
Avg. Annual Max.	Very Dense 16%							
12.58								
(99.11)**								
Largest Max.								
48.70								
(552.18)**								
Cienega Creek								
Annual Mean	Sparse 35%	Good 4%	70%	72%	8.0 - 29.0	6.6 - 9.0	0.5 - 10.3	115 - 1,010
0.03	Moderate 52%	Fair 25%			(11.2 - 28.0)*	(6.6 - 8.9)*	(1.3 - 10.3)*	(469 - 760)*
Avg. Annual Max.	Dense 12%	Poor 71%						
8.11	Very Dense 1%							
Largest Max.								
12.15								

* = Range Gila chub were captured.

** = Historic values prior to study (water years 1981 - 2001).

TABLE 14.—Identification, movement, and growth data on PIT-tagged Gila chub *Gila intermedia* recaptured in Bonita Creek (Graham County), and Cienega Creek (Pima County), Arizona.

Tag and Recapture Date	PIT Code	Length (mmTL)	Growth ^a (mm)	Distance Travelled	Movement Direction
Bonita Creek					
4/25/2002	413933530B	103	--	--	--
4/10/2005	"	145	42	~109 m	Upstream
2/2/2003	413907671B	111	--	--	--
2/5/2004	"	120	9	No movement	No Movement
2/2/2003	4139292F0F	129	--	--	--
3/27/2005	"	153	24	~ 26 m	Upstream
5/25/2003	41391C0D5B	136	--	--	--
7/1/2004	"	153	17	~ 54 m	Upstream
1/23/2005	"	155	2	~146 m	Downstream
7/1/2004	4139175962	165	--	--	--
1/8/2005	"	168	3	No movement	No Movement
1/8/2005	41461C7069	212	--	--	--
1/23/2005	"	211	No growth	~148 m	Upstream
1/23/2005	4146253511	161	--	--	--
4/10/2005	"	164	3	~ 105 m	Upstream
3/6/2005	4146320C1A	138	--	--	--
3/27/2005	"	136	No growth	~182 m	Downstream
3/27/2005	41461E3A05	142	--	--	--
5/23/2005	"	136	No growth	~1,675 m	Downstream
Cienega Creek					
6/3/2004	414566694B	118	--	--	--
7/16/2004	"	115	No growth	~ 11 m	Upstream
8/17/2004	414630072C	114	--	--	--
5/26/2005	"	127	13	No Movement	No Movement
1/4/2005	41460B7543	130	--	--	--
2/21/2005	"	136	6	No Movement	No Movement
5/8/2005	"	127	No growth	No Movement	No Movement
2/21/2005	41461F013D	135	--	--	--
5/8/2005	"	128	No growth	No Movement	No Movement
2/21/2005	41461F1826	145	--	--	--
4/14/2005	"	144	No growth	No Movement	No Movement

^a A negative change in length was considered a measurement error or fin erosion and is listed as "no growth".

Appendix A.1. Identification and location information for PIT-tagged Gila chub *Gila intermedia* from Bonita Creek, Arizona (Graham County).

Tag Date	PIT Code	PIT (R for recapture)	GPS Coordinates (UTM)		GPS Coordinate System	Length (mm TL)
1/23/2005	414412278		0637193	3647504	WGS 84	175
4/13/2002	4138510828		0636343	3649560	WGS 84	117
5/25/2003	4138662647		0637222	3647459	WGS 84	130
9/15/2002	4138691959		0637048	3647496	WGS 84	124
6/13/2003	4138692356		0636054	3650687	WGS 84	134
2/2/2003	4139111853		0636319	3649472	WGS 84	165
9/15/2002	4139114012		0637048	3647496	WGS 84	112
4/13/2002	4139134362		0636343	3649560	WGS 84	123
5/25/2003	4139175962		0637222	3647459	WGS 84	162
4/25/2002	4139191927		0637315	3647284	WGS 84	130
7/2/2002	4139231530		0636303	3649284	WGS 84	126
9/16/2002	4139235244		0636963	3647870	WGS 84	136
4/13/2002	4139243362		0636343	3649560	WGS 84	134
4/25/2002	4139247701		0637315	3647284	WGS 84	136
5/25/2003	4139273840		0637222	3647459	WGS 84	103
2/2/2003	4139280170		0636319	3649472	WGS 84	158
4/25/2002	4139295806		0637315	3647284	WGS 84	100
4/13/2002	4139305378		0636343	3649560	WGS 84	152
9/18/2003	4139314005		0637325	3647194	WGS 84	120
2/2/2003	4139324155		0636319	3649472	WGS 84	126
6/13/2003	4139385038		0636054	3650687	WGS 84	132
7/2/2002	4139391342		0636303	3649284	WGS 84	168
1/23/2005	4144070345		0637193	3647504	WGS 84	189
7/1/2002	4145642509		0636113	3650457	WGS 84	144
1/23/2005	4145682471		0637193	3647504	WGS 84	145
1/23/2005	4146140119		0637270	3647380	WGS 84	121
2/2/2003	4146223932		0636319	3649472	WGS 84	140
1/23/2005	4146253511		0637324	3647288	WGS 84	167
4/10/2005	4146253511	R	0637270	3647380	WGS 84	164
1/23/2005	4146337678		0637270	3647380	WGS 84	127
9/15/2002	5048764733		0637048	3647496	WGS 84	122
7/1/2002	41364f7635		0636113	3650457	WGS 84	129
4/13/2002	413825713b		0636343	3649560	WGS 84	130
4/13/2002	413855113f		0636343	3649560	WGS 84	135
4/13/2002	4138636c54		0636343	3649560	WGS 84	115
4/25/2002	41386b6d20		0637315	3647284	WGS 84	101
3/21/2002	41386C3F6C		0637960	3646848	WGS 84	126
9/15/2002	41386d6f7f		0637048	3647496	WGS 84	115
3/21/2002	41386F4C50		0636152	3650182	WGS 84	158
4/13/2002	413870082b		0636343	3649560	WGS 84	103
4/13/2002	4138751d74		0636343	3649560	WGS 84	152
3/21/2002	4138776472		0637960	3646848	WGS 84	155

Tag Date	PIT Code	PIT (R for recapture)	GPS Coordinates (UTM)		GPS Coordinate System	Length (mm TL)
6/13/2003	41387b016a		0636054	3650687	WGS 84	123
6/13/2003	413907525f		0636054	3650687	WGS 84	146
2/2/2003	413907671b		0636319	3649472	WGS 84	111
2/5/2004	413907671b	R	0636319	3649472	WGS 84	120
5/25/2003	41390B5C6C		0637222	3647459	WGS 84	129
9/15/2002	41390d3f6a		0637048	3647496	WGS 84	120
7/2/2002	413912176b		0636303	3649284	WGS 84	135
6/13/2003	4139122f50		0636054	3650687	WGS 84	120
2/2/2003	4139151d66		0636319	3649472	WGS 84	127
7/1/2004	4139175962		0637270	3647380	WGS 84	165
1/8/2005	4139175962	R	0637270	3647380	WGS 84	168
5/25/2003	4139195C67		0637222	3647459	WGS 84	119
7/1/2002	41391a6d4a		0636113	3650457	WGS 84	164
5/25/2003	41391C0D5B		0637222	3647459	WGS 84	136
7/1/2004	41391C0D5B	R	0637270	3647380	WGS 84	153
1/23/2005	41391C0D5B	R	0637193	3647504	WGS 84	155
4/25/2002	41391e0f08		0637315	3647284	WGS 84	123
4/13/2002	41391e3c62		0636343	3649560	WGS 84	120
9/16/2002	41391f7267		0636963	3647870	WGS 84	135
7/2/2002	413921263d		0636303	3649284	WGS 84	118
6/13/2003	4139221f56		0636054	3650687	WGS 84	136
9/16/2002	4139227c3b		0636963	3647870	WGS 84	165
5/25/2003	413923412D		0637222	3647459	WGS 84	106
2/23/2003	4139234d71		0637145	3647521	WGS 84	132
3/21/2002	4139235367		0637960	3646848	WGS 84	134
3/21/2002	4139241361		0637960	3646848	WGS 84	121
4/13/2002	4139251d1d		0636343	3649560	WGS 84	137
2/2/2003	4139253f2a		0636319	3649472	WGS 84	147
7/2/2002	413925522f		0636303	3649284	WGS 84	124
4/25/2002	413927453b		0637315	3647284	WGS 84	115
4/13/2002	413927583a		0636343	3649560	WGS 84	124
3/21/2002	4139276C5D		0636152	3650182	WGS 84	145
2/2/2003	4139292f0f		0636319	3649472	WGS 84	129
3/27/2005	4139292F0F	R	0636322	3649497	WGS 84	153
9/15/2002	41392a2c6b		0637048	3647496	WGS 84	139
7/2/2002	41392a2e5f		0636303	3649284	WGS 84	137
7/2/2002	41392a7113		0636303	3649284	WGS 84	122
9/15/2002	41392c372f		0637048	3647496	WGS 84	168
9/15/2002	41392c6e4d		0637048	3647496	WGS 84	130
4/25/2002	41392e220f		0637315	3647284	WGS 84	118
6/13/2003	41392e295e		0636054	3650687	WGS 84	113
7/2/2002	41392e5d40		0636303	3649284	WGS 84	126
3/21/2002	4139316E6E		0637960	3646848	WGS 84	143
3/21/2002	4139326241		0637960	3646848	WGS 84	163
4/25/2002	413933530b		0637315	3647284	WGS 84	103
4/10/2005	413933530B	R	0637270	3647380	WGS 84	145

Tag Date	PIT Code	PIT (R for recapture)	GPS Coordinates (UTM)		GPS Coordinate System	Length (mm TL)
3/21/2002	413936615A		0636152	3650182	WGS 84	109
4/13/2002	413938011c		0636343	3649560	WGS 84	148
9/16/2002	41393b4d4a		0636963	3647870	WGS 84	135
3/21/2002	41393C2B61		0636152	3650182	WGS 84	97
3/21/2002	41393C5951		0636152	3650182	WGS 84	111
4/25/2002	41393c773a		0637315	3647284	WGS 84	119
4/13/2002	41393d1e31		0636343	3649560	WGS 84	143
9/16/2002	41393d4670		0636963	3647870	WGS 84	128
7/2/2002	41393e0b05		0636303	3649284	WGS 84	125
4/13/2002	41393e2566		0636343	3649560	WGS 84	153
4/25/2002	41393e3510		0637315	3647284	WGS 84	170
3/6/2005	4143730E63		0636253	3649630	WGS 84	152
3/27/2005	41437E574E		0636322	3649497	WGS 84	145
1/8/2005	4144070345		0637270	3647380	WGS 84	194
3/27/2005	41440C396E		0636322	3649497	WGS 84	152
1/8/2005	41440E1641		0637270	3647380	WGS 84	232
1/8/2005	4145584C36		0637270	3647380	WGS 84	184
1/23/2005	41455D712C		0637270	3647380	WGS 84	151
2/5/2004	41455e1125		0636319	3649472	WGS 84	131
2/5/2004	41455e7c20		0636319	3649472	WGS 84	124
1/23/2005	4145616F1A		0637270	3647380	WGS 84	116
1/8/2005	414563642C		0637324	3647288	WGS 84	131
1/8/2005	4145682471		0637270	3647380	WGS 84	144
2/27/2005	41456A2B5D		0636310	3649390	WGS 84	161
1/23/2005	41456C2649		0637270	3647380	WGS 84	178
3/27/2005	4145715806		0636322	3649497	WGS 84	132
1/19/2005	414574026B		0636160	3650158	WGS 84	204
1/23/2005	4145745C04		0637270	3647380	WGS 84	148
1/23/2005	41457A5C38		0637270	3647380	WGS 84	135
3/27/2005	41457D3061		0636322	3649497	WGS 84	150
3/27/2005	4146074751		0636322	3649497	WGS 84	151
1/23/2005	4146087C55		0637270	3647380	WGS 84	110
1/23/2005	41460A3300		0637324	3647288	WGS 84	134
1/23/2005	41460A7E06		0637193	3647504	WGS 84	152
1/19/2005	41460C307C		0636160	3650158	WGS 84	132
2/5/2004	41460E0B37		0636319	3649472	WGS 84	136
1/23/2005	41460E422B		0637270	3647380	WGS 84	118
3/27/2005	41460F277C		0636322	3649497	WGS 84	155
3/27/2005	4146115728		0636322	3649497	WGS 84	130
3/6/2005	4146115C79		0636253	3649630	WGS 84	145
3/27/2005	4146120817		0636322	3649497	WGS 84	143
1/8/2005	4146122B03		0637270	3647380	WGS 84	190
2/27/2005	4146126E43		0636310	3649390	WGS 84	167
2/27/2005	4146130803		0636310	3649390	WGS 84	140
1/23/2005	414615356A		0637193	3647504	WGS 84	164
3/27/2005	4146161C7F		0636322	3649497	WGS 84	154

Tag Date	PIT Code	PIT (R for recapture)	GPS Coordinates (UTM)		GPS Coordinate System	Length (mm TL)
1/23/2005	4146173E5C		0637324	3647288	WGS 84	168
1/19/2005	4146184E70		0636160	3650158	WGS 84	170
3/27/2005	4146196D70		0636322	3649497	WGS 84	165
3/27/2005	41461A0D79		0636322	3649497	WGS 84	146
3/27/2005	41461B2132		0636322	3649497	WGS 84	136
1/23/2005	41461C3529		0637270	3647380	WGS 84	171
1/23/2005	41461C4C48		0637270	3647380	WGS 84	146
1/23/2005	41461C637A		0637193	3647504	WGS 84	162
1/8/2005	41461C7069		0637270	3647380	WGS 84	212
1/23/2005	41461C7069	R	0637193	3647504	WGS 84	211
3/27/2005	41461E1074		0636322	3649497	WGS 84	132
3/27/2005	41461E3A05		0636322	3649497	WGS 84	142
5/23/2005	41461E3A05	R	0636846	3648273	WGS 84	136
5/21/2004	4146202A3C		0637501	3647200	WGS 84	124
3/27/2005	4146225219		0636322	3649497	WGS 84	166
1/8/2005	414623166E		0637358	3647189	WGS 84	139
1/23/2005	4146233E2B		0637270	3647380	WGS 84	161
1/23/2005	4146241C63		0637324	3647288	WGS 84	170
1/23/2005	4146257F1A		0637193	3647504	WGS 84	142
1/8/2005	4146263178		0637324	3647288	WGS 84	126
2/5/2004	414627435c		0636319	3649472	WGS 84	139
1/23/2005	414628355E		0637324	3647288	WGS 84	159
3/27/2005	4146297B5B		0636322	3649497	WGS 84	148
1/8/2005	41462C2136		0637358	3647189	WGS 84	122
3/27/2005	41462D7963		0636322	3649497	WGS 84	171
1/8/2005	4146302A50		0637324	3647288	WGS 84	140
1/23/2005	4146311C17		0637270	3647380	WGS 84	137
2/27/2005	4146316B1E		0636310	3649390	WGS 84	139
3/6/2005	4146320C1A		0636253	3649630	WGS 84	138
3/27/2005	4146320C1A	R	0636322	3649497	WGS 84	136
1/23/2005	414C60C4D07		0637193	3647504	WGS 84	152
4/13/2002	504c133008		0636343	3649560	WGS 84	125

Appendix A.2. Identification and location information for PIT-tagged Gila chub *Gila intermedia* from Cienega Creek, Arizona (Pima County).

Tag Date	PIT Code	PIT (R for recapture)	GPS Coordinates (UTM)		GPS Coordinate System	Length (mmTL)
3/29/2002	4066391536		0540355	3524550	WGS 84	125
3/29/2002	4139243241		0540355	3524550	WGS 84	113
6/26/2002	4139370972		0538714	3519281	NAD 27	136
1/4/2005	4146310950		0540607	3525563	WGS 84	100
4/14/2005	4146354806		0540601	3525571	WGS 84	97
6/26/2002	413862764c		0538714	3519281	NAD 27	121
3/29/2002	4138727d71		0540355	3524550	WGS 84	121
6/27/2002	413873097b		0538714	3519281	NAD 27	94
4/7/2002	41387a6e4f		0538714	3519281	NAD 27	91
6/26/2002	4139136d09		0538714	3519281	NAD 27	116
6/26/2002	41392b0945		0538714	3519281	NAD 27	106
3/10/2002	41392B2100		0540557	3525571	WGS 84	132
4/7/2002	41392b572a		0538714	3519281	NAD 27	142
3/29/2002	4139300b39		0540355	3524550	WGS 84	112
6/7/2003	413939221C		0538743	3519492	NAD 27	97
1/4/2005	41436E2A59		0540607	3525563	WGS 84	113
1/4/2005	41437F333A		0540308	3524964	WGS 84	159
6/3/2004	414566694B		0540607	3525563	WGS 84	118
7/16/2004	414566694B	R	0540601	3525571	WGS 84	115
6/3/2004	4145666A5C		0540687	3525646	WGS 84	119
8/17/2004	41456D554E		0540332	3524743	WGS 84	108
2/2/2005	4145790856		0538957	3516978	NAD 27	112
1/4/2005	41460B7543		0540607	3525563	WGS 84	130
2/21/2005	41460B7543	R	0540607	3525563	WGS 84	136
5/8/2005	41460B7543	R	0540607	3525563	WGS 84	127
1/16/2005	41460F7658		0541718	3527387	WGS 84	156
8/17/2004	4146123949		0540332	3524743	WGS 84	105
7/16/2004	4146134E54		0540601	3525571	WGS 84	118
2/2/2005	414615021A		0538988	3516967	NAD 27	109
8/17/2004	4146196621		0540332	3524743	WGS 84	107
6/3/2004	41461C0908		0540687	3525646	WGS 84	108
6/3/2004	41461E7620		0540687	3525646	WGS 84	116
2/21/2005	41461F013D		0540607	3525563	WGS 84	135
5/8/2005	41461F013D	R	0540607	3525563	WGS 84	128
2/21/2005	41461F1826		0540557	3525571	WGS 84	145
4/14/2005	41461F1826	R	0540601	3525571	WGS 84	144
1/4/2005	4146201956		0540607	3525563	WGS 84	110
6/25/2004	4146225B7C		0540308	3524964	WGS 84	227
1/11/2005	4146226264		0538940	3517244	NAD 27	131
8/17/2004	4146230329		0540332	3524743	WGS 84	102
1/4/2005	414628297C		0540607	3525563	WGS 84	109
1/11/2005	41462D5D7A		0538940	3517244	NAD 27	115

Tag Date	PIT Code	PIT (R for recapture)	GPS Coordinates (UTM)		GPS Coordinate System	Length (mmTL)
2/2/2005	41462F0065		0538957	3516978	NAD 27	132
8/17/2004	414630072C		0540332	3524743	WGS 84	114
5/26/2005	414630072C	R	0540332	3524743	WGS 84	127
2/2/2005	414633756F		0538957	3516978	NAD 27	122

APPENDIX B

Spawning and hatching of Gila chub

SPAWNING AND HATCHING OF GILA CHUB

Andrew A. Schultz

Arizona Cooperative Fish and Wildlife Research Unit

School of Natural Resources

104 Biological Sciences East

University of Arizona

Tucson, AZ 85721

ABSTRACT

Information on reproductive characteristics of the endangered Gila chub *Gila intermedia* is largely limited and qualitative, and culture techniques and requirements are virtually unknown. Here I provide the first published data on spawning and selected reproductive and developmental characteristics of Gila chub. Fish were brought to the laboratory in March 2003 from Sabino Creek, Arizona (12.3°C). Fish were then warmed slowly and spawned at 14.93°C, 10 d following collection. Following this initial spawning, Gila chub spawned consistently in the laboratory without hormonal, chemical, photoperiod, temperature, and substrate manipulation, during all times of the year. Spawns were noted at temperatures ranging from about 15 to 26°C; however, I noted that Gila chub spawned less frequently at temperatures above 24°C. Multiple spawning attempts per year per individual are likely. There was a strong, inverse relationship between time to hatch and incubation temperature. Hatch rate of eggs was high (mean = 99.43%) and larval Gila chub accepted a variety of natural and formulated diets at first feeding. The future of Gila chub may someday depend in part on hatchery propagation to provide specimens for restocking formerly occupied habitats and establishing refuge populations. Information from my study can aid future efforts to successfully spawn and rear Gila chub and related species.

INTRODUCTION

The requirements necessary to culture the Southwest's threatened native fishes for recovery efforts are unknown for certain species, yet may prove critical for conservation. Gila chub *Gila intermedia* are one of seven chub species of the genus *Gila* inhabiting the Colorado River Basin. All are threatened by non-native species, habitat loss, and other factors within the basin. Roundtail chub *Gila robusta* remains the only species not listed or proposed for listing as endangered. Published accounts of captive spawning/culture efforts for these chubs are few. Hamman (1982a; 1982b; 1985) reported on the spawning and reproductive biology of humpback chub *Gila cypha* and bonytail *Gila elegans* in captivity. Muth et al. (1985) did the same for roundtail chub. Current research on spawning/culture techniques and requirements for both headwater chub *Gila nigra* and roundtail chub is ongoing (Scott Bonar and Erica Sontz, personal communication, U.S. Cooperative Fish and Wildlife Research Unit, University of Arizona; and personal observation). Previous observations (Ken Wintin, personal communication, Arizona-Sonora Desert Museum; Jeanette Carpenter, personal communication, U.S. Geological Survey; and personal observation) confirm that Gila chub have the ability to spawn and be maintained in captivity but spawning/culture techniques and requirements are largely unknown. The limited information available on culture techniques and general life-history of Gila chub hampers recovery of this species (Vives 1990). The future of Gila chub may someday depend in part on hatchery propagation to provide specimens for restocking formerly occupied habitats and establishing refuge populations. The objectives of this study were to establish a group of adult Gila chub in the laboratory, identify methods to successfully spawn Gila chub in captivity, and develop Gila chub eggs through post-hatch to the larval phase.

METHODS

In March 2003 I collected Gila chub from Sabino Creek, Arizona to serve as broodstock. Fish were transported to the laboratory at the University of Arizona in aerated containers and then acclimated to laboratory temperatures. Because the temperature of Sabino Creek was 12.3°C, I cooled the laboratory to about 15°C and allowed fish to slowly warm in rectangular glass tanks with water capacities of about 280 and 330 L. After their first spawn (at 14.93°C), I varied temperatures to estimate the range of temperatures at which Gila chub would spawn. Most spawning trials were conducted between 18-24°C with temperatures held relatively static. Approximate length range of adults was 110-175 mm TL and sex ratio was unknown. Groups of 5-9 adult Gila chub were maintained and spawned in rectangular glass tanks filled with dechlorinated municipal water and capacities from about 110-330 L, with a maximum density of about 0.08 chub/L. All spawning/holding and egg-incubation tanks were aerated and fitted with recirculating bio-filters with a combined filtering capacity of about 3784 L/h for spawning tanks and 1135 L/h for egg-incubation tanks. The returned water from the bio-filters created a surface disturbance and slight flow within the spawning tanks. The main diet of adults consisted of thawed natural feeds, mainly chironomid larvae (Hikari Bio-Pure Bloodworms, Hikari, Inc., Hayward, CA). I fed adult Gila chub in slight excess twice during each day at an interval of anywhere from about 6-9 hours. Adult Gila chub were observed at least twice daily and tanks checked for signs of spawning activity. I thoroughly cleaned tanks of all debris at least twice daily using a siphon hose, which resulted in a water exchange of about 5-20% daily. Water quality (i.e., pH, ammonia, nitrite, and temperature) was monitored daily.

I placed 11 x 11-cm glazed, beige-colored ceramic tiles on the bottom of the spawning tanks each time I needed a spawn. A rigid plastic grating (pattern was 15 x 15-mm [open space])

squares, 8 mm high and 2 mm thick) cut to fit the dimensions of the tank sides was raised 2-4 inches off the tile substrate using 4-6 pieces of 1.27-mm diameter PVC pipe glued directly to the underside. Following spawning, tiles were removed from spawning tanks, tiles with eggs were gently rinsed clean of debris by dunking in water from which they originated, and the number of eggs present on the tiles was recorded. Tiles with eggs were then placed vertically in vinyl covered metal dish racks submersed in 57-L aquaria. I counted larval Gila chub following hatch, which usually occurred within 24 h.

I used an ocular micrometer to measure diameter of spawned eggs and total length (to nearest 0.1 mm) of larval Gila chub. I measured wet-weight (to nearest 0.0001 g) of Gila chub larvae using an electronic scale. Particular care was taken to systematically remove excess water from larval Gila chub prior to measurement. Larval Gila chub were euthanized with MS-222 (3-aminobenzoic acid ethyl ester) prior to measurement.

RESULTS

Gila chub taken from Sabino Creek, Arizona in March at a temperature of 12.3°C spawned at 14.9°C within 10 days of initial introduction into the lab. Gila chub consistently spawned in the laboratory thereafter without hormonal, chemical, photoperiod, temperature or substrate manipulation, during all times of the year. Spawns were noted at temperatures ranging from about 15 to 26°C; however, I noted that Gila chub spawned less frequently at temperatures above 24°C. Most trials were conducted between 18-24°C and groups of Gila chub would usually spawn within 14 d of tanks being set up for spawning within this temperature range.

Spawning behavior of Gila chub was observed several times in the laboratory and for those acclimated, behavior appeared little affected by observers. Before spawning, several

presumed males chased what appeared to be a lone female. Presumed males were often noted to have more vivid spawning colors than females. Spawning colors were present to varying degrees near ventral and pectoral fin bases, ventral body areas, opercle, and mouth, with strong, dark-colored horizontal banding noted on the most active fish. Nudging and nipping of the female posteriorly by males was noted. The actual release of gametes was often immediately preceded by a slight upward turn and then a light to violent shudder by the female, especially when against a rough surface or wedged between in-tank structures. Roughly 30 eggs were released during each act. Following the act, nearby fish, perhaps including those involved in the act, immediately began eating available eggs. Such spawning acts were repeated several times by what appeared to be the same female. Video footage taken in the laboratory confirmed my visual observations. Spawning events often lasted over an hour.

Total number of viable eggs counted following a spawn ranged from 106 to 2750 (mean = 1044; SD = 667) and egg counts had no obvious relationship to temperature at time of spawn. Mean percent of non-viable eggs from each spawn was 6.36 % (SD = 8.8). Eggs of Gila chub were demersal, adhesive, ovoid, and translucent with the inner 80-90% of the egg a light yellow cream color and the remainder colorless. Mean diameter of fertilized eggs about 24 h after spawn was 2.16 mm (SD = 0.05). Not including spawns affected by fungal outbreaks, mean hatch rate was 99.43% (SD = 1.39). I found a strong inverse linear relationship ($r^2 = 0.88$; $df = 1, 32$; $P < 0.001$) between mean incubation temperature and time to hatch for the temperature range examined (Figure 1). The regression equation for this relationship was:

$$\text{Time to Hatch (d)} = 21.77 - 0.72 \text{ Mean Incubation Temperature (C}^\circ\text{)}$$

Mean length and weight of larval Gila chub ($n = 20$) within 6 h or less of hatch was 6.55 mm TL (SD = 0.12) and 1.69 mg (SD = 0.29), respectively. Larval Gila chub remained benthic upon emergence. Slight yolk present upon hatch was quickly reduced and swim-up appeared to occur within the first 48 h. Larval Gila chub accepted several types of natural and prepared/commercial feeds upon exogenous feeding.

DISCUSSION

Much life-history information can be learned when spawning and culturing a species in captivity. Often this life-history information is difficult to observe in nature. Life-history information can help identify factors limiting natural and introduced populations. Other culture studies have provided vital information for many federally-listed threatened or endangered species (Johnson and Jensen 1991).

The highly adhesive nature of Gila chub eggs created challenges when first trying to efficiently count, aerate, and rear the eggs, and develop the embryos in a timely, efficient, space-saving fashion. Preliminary efforts to remove the adhesive eggs of Gila chub and subsequently rear them were largely unsuccessful. Rakes et al. (1999) were able to remove adhesive fish eggs and incubate them. Other spawning substrates proved difficult to clean thereby leading to higher losses of eggs due to fungal outbreaks. My described spawning set-up allowed most of the spawned eggs to fall through the grating and adhere to the glazed ceramic tiles. The grating protected the eggs from adult predation and the tiles provided an easily cleaned, efficient system for transfer and counting. Some eggs were cannibalized prior to falling through the grating. Cannibalization of eggs might be reduced by having spawning tanks contain only a single brood pair. It is unknown how such pairing would affect spawning behavior.

Debris was easily rinsed off tiles with eggs and the slick nature of the tile surface may have been a contributing factor. Rakes et al. (1999) used unglazed ceramic tiles to facilitate spawning in species that spawn in crevices or angled spaces behind current. An unglazed or rough tile surface may offer a more natural feel and potential spawning stimulus than glazed tiles, or allow for a stronger attachment point for eggs. However, in situations where contact between adult fish and tiles is unnecessary, the glazed tiles are more easily cleaned, and I found Gila chub eggs strongly adhered to the slick glazed surface. The equipment needed for my spawning set-up was inexpensive and most parts could be found at a typical hardware store and easily modified to fit varying needs. However, construction, maintenance, and monitoring of my spawning system did require considerable labor.

Schultz and Bonar (2006) stated reproduction of Gila chub in Bonita Creek and Cienega Creek, Arizona commenced in February, peaked at the beginning of spring, and dropped off as summer began. Additional spawning activity in the fall was suggested by some of the data. My observations suggest that spawning of Gila chub in captivity is possible year-round. Multiple spawnings per year per individual are likely given my observations. It is unknown what mechanism triggered Gila chub to spawn out of season within the laboratory. I first collected Gila chub broodstock from Sabino Creek, Arizona at 12.3°C and began acclimating them to laboratory conditions. Within ten days of collection these fish had spawned at 14.9°C. Because Gila chub first spawned without much of a temperature increase and readily spawned at a variety of temperatures without inducement afterwards, I cannot say that temperature manipulation is necessary to spawn Gila chub in captivity. However, temperature manipulation was helpful to spawn other similar species in captivity, including Yaqui chub *Gila purpurea* (Kline and Bonar 2009) and Mohave tui chub *Siphateles bicolor mohavensis* (Archdeacon and Bonar, Accepted).

Minckley (1973) noted Gila chub had an extended spawning regime in a relatively constant temperature and water-level spring-fed pond. The goal of maximizing fitness via reproductive effort and success of future progeny is central to evolutionary theory. The cost of reproductive efforts may be lessened over time within stable environments having moderate, steady temperatures, consistent high-quality food resources, consistent access to mates, and/or reduced predator threats.

Gila chub often exhibit brilliant orange/red colors when in a heightened reproductive state. A previous field study described reproductive colors and a subsequent rating system for Gila chub (Schultz and Bonar 2006). I found that spawning color of Gila chub that released gametes when collected in the field ranged from moderate to very strong. The most intensely colored Gila chub (\geq strong spawning colors) were captured where daytime water temperatures ranged from 12-28 °C. Spawning colors for Gila chub were noted throughout the year in the laboratory but often failed to achieve the intensity of colors in the field. Gila chub presumed to be males (due to spawning behavior and slower growth in the laboratory) expressed a greater intensity in spawning coloration than other captive Gila chub. This is supported by field data as males dominated the catch of Gila chub having strong and very strong spawning coloration (Schultz and Bonar 2006). Based on spawning coloration patterns, Nelson (1993) hypothesized Gila chub in Cienega Creek, Arizona greater than 75 mm could spawn. Qualitative observations in the laboratory suggest that Gila chub can mature quickly under intensive conditions. Although spawning coloration is undoubtedly related to the reproductive cycle it is not clear if a definitive relationship exists between intensity of spawning colors and time before spawning.

Chasing behavior attributed to spawning activity of Gila chub in the wild (Bonita Creek, Arizona) was similar to that observed in the laboratory (Schultz and Bonar 2006). Minckley

(1973) described similar behavior for Gila chub in a pond where large presumed females were followed by numerous smaller presumed males.

The total counts of eggs following a spawn in my study should be considered underestimates due to cannibalization of eggs prior to falling through the protection grid, and any loss of eggs from tiles during transfer. In addition, unavoidable disturbance of tanks (e.g., cleaning activity) may have arrested spawning activity, accounting for occasional spawns of low magnitude. The disparity between estimates of fecundity from the enumeration of actual spawns in the laboratory and extrapolation of total ova from ovaries of sacrificed Gila chub in a related field study (Schultz and Bonar 2006) could not be explained by size differences in Gila chub or partial cannibalization in the laboratory. The actual production of viable oocytes (functional fecundity) may differ from true reproductive potential due to incomplete spawning or degeneration and resorption of oocytes (Crim and Glebe 1990). In spite of the strong relationship noted between mean incubation temperature and time to hatch, measurement of time to hatch was likely biased at times as detection of a spawning occurrence or final hatch was dependent on visual observation.

Roundtail chub *Gila robusta*, a closely related but larger species, had a larger mean fertilized egg diameter and length at hatch (Muth et al. 1985) than Gila chub. A formal description of Gila chub larvae was not undertaken as part of my study but given the consistency with which Gila chub will spawn in the laboratory and the proven ability to rear young to the juvenile stage, specimens needed for a larval developmental studies should be possible to obtain.

The ability to domesticate and spawn adult fish of a species without inducement may reduce effort and costs in production, and be deemed advantageous when the synchronicity and timing of cohorts is not a priority. My results provide the first published data on spawning and

selected reproductive characteristics of Gila chub. My observations have shown that given proper care and environmental conditions, Gila chub have the ability to spawn year-round without inducement or natural surroundings, with likely multiple spawning attempts per year per individual possible. In addition, hatch rate of eggs is often high and larval Gila chub accept a variety of natural and formulated feed types at first feeding.

The future of Gila chub may someday depend on culture of the species. The increasing prevalence and importance of culturing imperiled fish species as a conservation and management strategy (Johnson and Jensen 1991; Modde et al. 1995) is a regrettable reality. Nonetheless it can be a powerful tool when needing stock to repatriate extirpated populations or establish refuge populations. Culture techniques can also be used to perpetuate a species during a crisis. Lack of such knowledge has led to the extinction of certain species (Minckley and Deacon 1991).

ACKNOWLEDGMENTS

I thank staff at the Arizona Game and Fish Department (AZGFD) including Robyn Beck, Robert Bettaso, Chris Cantrell, Mike Childs, Dean Foster (former AZGFD), Melissa Kreighbaum, Don Mitchell, John Romero, Jeff Sorensen, David Ward, and many others. I thank Dr. Paul Barrett, Doug Duncan, Roger Hamman, and Manuel Ulibarri of the United States Fish and Wildlife Service (USFWS); Rob Clarkson from the Bureau of Reclamation, Dr. David Propst from the New Mexico Game and Fish Department and all the other professionals and staff associated with the Central Arizona Project (CAP) Transfer Fund. I thank Joshua Taiz of the United States Forest Service (USFS) and the staff associated with the USFS Santa Catalina Ranger District. I thank Craig Ivanyi and Ken Wintin of the Arizona Sonora Desert Museum. I thank Jeff Simms of the Bureau of Land Management. I thank my Ph.D. committee members

Dr. Scott Bonar (major advisor), Dr. Courtney Conway, Dr. Kevin Fitzsimmons, Dr. Peter Reinthal, and Dr. Cecil Schwalbe for all their guidance and continued support. I thank all staff and faculty at the University of Arizona that assisted in any respect during this study, especially Anne Hartley, Linda Lee, Dee Simons, Cecily Westphal, and Carol Yde for administrative support. A special thanks goes to Alison Iles of the Arizona Cooperative Fish and Wildlife Research Unit, and Dr. Patrick Reid and Dr. Malcolm Zwolinski of the University of Arizona's School of Renewable Natural Resources for logistical and administrative support. I thank Dr. Mark Borgstrom and Dr. Robert Steidl for statistical advice, Dr. William Matter for design support, and Andrew Honaman and Gina Schultz for technical support. I thank Daniel Arevalo, Sachiko Aso, Devin Beck, Sheldon Caldwell-Meeks, Brian Chaszar, Stan Culling, Andrea Francis, Jason Kline, Guillermo Ley, Naomi Lupe, Michelle Martin, Shawna Minor, Jake Mundy, Michelle Riley, Gina Schultz, Jasmine Schultz, Skye Schultz, Erica Sontz, Greg Stoehr, Sean Tackley, David Ward, and Sebastian Zeltzer for their assistance in the laboratory and field. I thank Roger Sorensen and Kirk Young of AGFD for their review of the manuscript. This project was funded by the AGFD Heritage Fund, the CAP Transfer Fund, the University of Arizona, and the U.S. Geological Survey Arizona Cooperative Fish and Wildlife Research Unit.

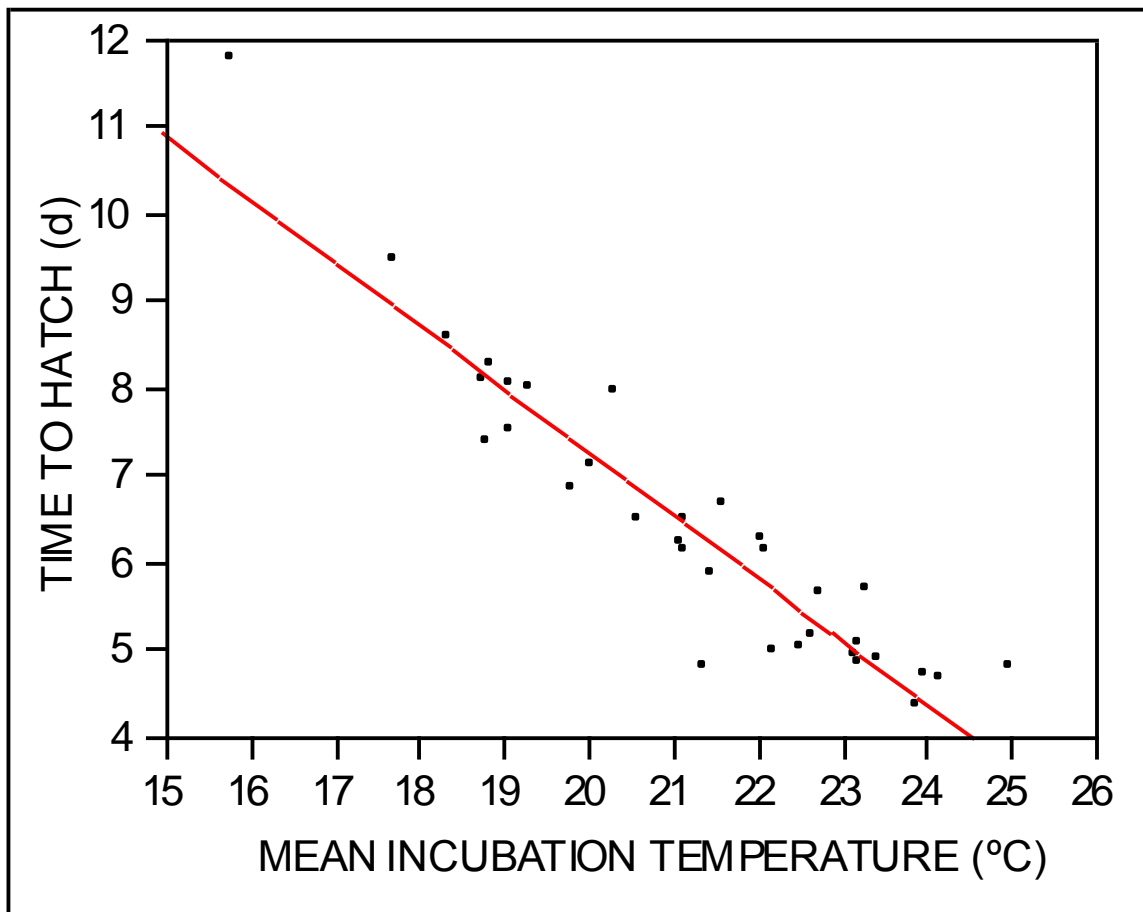
LITERATURE CITED

- Archdeacon, T. P., and S. A. Bonar. Accepted. Captive breeding of endangered Mohave tui chub. *North American Journal of Aquaculture*.
- Crim, L. W., and B. D. Glebe. 1990. Reproduction. Pages 529-553, in C.B. Schreck and P.B. Moyle, editors. *Methods for fish biology*. American Fisheries Society, Bethesda, Maryland.

- Hamman, R. L. 1982a. Induced Spawning and Culture of Bonytail Chub. *The Progressive Fish-Culturist* 44 (4):201–203.
- Hamman, R. L. 1982b. Spawning and Culture of Humpback Chub. *The Progressive Fish-Culturist* 44 (4):213–216.
- Hamman, R. L. 1985. Induced Spawning of Hatchery-Reared Bonytail. *The Progressive Fish-Culturist* 47 (4):239–241.
- Hendrickson, D. A., and W. L. Minckley. 1984. Cienegas-vanishing climax communities of the American Southwest. *Desert Plants* 6:141-175.
- Johnson, J. E., and B. L. Jensen. 1991. Hatcheries for endangered freshwater fishes. Pages 199-217 *in* W. L. Minckley and J. E. Deacon, editors. *Battle against extinction: native fish management in the American West*. University of Arizona Press, Tucson, Arizona.
- Kline, S. J., and S. A. Bonar. 2009. Captive breeding of Yaqui topminnow and Yaqui chub for recovery purposes. *North American Journal of Aquaculture* 71:73-78.
- Minckley, W. L. 1973. *Fishes of Arizona*. Sims Printing Company, Inc., Phoenix, Arizona.
- Minckley, W. L., and J. E. Deacon, editors. 1991. *Battle against extinction: native fish management in the American West*. University of Arizona Press, Tucson, Arizona.
- Modde, T., A. T. Scholz, J.H. Williamson, G. B. Haines, B. D. Burdick, and F. K. Pfeifer. 1995. Augmentation plan for razorback sucker in the Upper Colorado River Basin. *American Fisheries Society Symposium* 15:102-111.

- Muth, R. T., C. M. Haynes, and C. C. Carlson. 1985. Culture of roundtail chub *Gila robusta robusta* (Cyprinidae) through the larval period. *Southwestern Naturalist* 30:152-154.
- Nelson, B. 1993. Spawning characteristics of Gila chub (*Gila intermedia*) in Cienega creek, Pima County, Arizona. Report for USDI Bureau of Land Management, Tucson Resource Area, Arizona.
- Rakes, R. L., J. R. Shute, and P. W. Shute. 1999. Reproductive behavior, captive breeding, and restoration ecology of endangered fishes. *Environmental Biology of Fishes* 55:31-42.
- Schultz, A. A. and S. A. Bonar. 2006. Selected aspects of the natural history of Gila chub. Arizona Cooperative Fish and Wildlife Research Unit, Fisheries Research Report 02-06, Tucson, Arizona.
- United States Fish and Wildlife Service. 2005. Endangered and threatened wildlife and plants; listing the Gila chub as endangered with critical habitat; final rule. *Federal Register* 70:66664-66721.
- Vives, S. P. 1990. Element stewardship abstract for *Gila intermedia*. The Nature Conservancy, Arizona Field Office, Tucson, Arizona.

FIGURE 1.—Time to hatch plotted against mean incubation temperature (with linear regression fit) for larval Gila chub *Gila intermedia*.



APPENDIX C

Effect of feed type, water temperature, and rearing density on growth and survival of larval and juvenile Gila chub

ABSTRACT

Culture techniques and requirements are virtually unknown for Gila chub *Gila intermedia*, a species federally listed as endangered. I investigated the effect of different feed types, water temperatures, and rearing densities on growth, survival, and overt health/appearance of larval and juvenile Gila chub. Larval Gila chub fed a commercial diet grew the same or slightly better than those fed thawed *Artemia* sp. nauplii, and significantly better than those fed chicken *Gallus domesticus* egg-yolk powder, but survived significantly better when fed *Artemia*. Despite the latter, observations suggest *Artemia* nauplii may be difficult for first-feeding larval Gila chub to handle. Thawed chironomid sp. larvae clearly outperformed prepared commercial feeds for small and large juvenile Gila chub with respect to growth; however, survival was 100% for all feed treatments. Overt health/appearance of larval and juvenile Gila chub remained largely unchanged during all experiments. My results have shown first-feeding larval Gila chub may be reared on a natural or prepared diet but I recommend larval Gila chub be fed a natural feed if survival is paramount to objectives. Based on diets tested I recommend juvenile Gila chub be fed a natural diet if faster growth is paramount to objectives. Further work is suggested to define the nutritive requirements and identify the most efficient feeding regimen for Gila chub.

Growth of larval Gila chub was highest at 28°C and lowest at 32°C, while survival of larval Gila chub was highest at 24°C and lowest at 20°C. Spinal deformities were common for larval Gila chub reared at 32°C but generally uncommon for those reared at lower temperatures. Although growth of small (32-49 mm TL) and large (52-72 mm TL) juvenile Gila chub generally increased with temperature, differences were not statistically significant. Survival was 100% and no external abnormalities were noted in any experiment testing small and large juveniles. Water

temperatures from 20-28°C appear suitable for rearing larval Gila chub, with temperatures from 24-28°C optimal. Water temperatures from 20-29°C appear suitable for rearing juvenile Gila chub.

Mean length and weight gain was inversely related to rearing density for larval and large juvenile Gila chub. Survival of larval Gila chub was significantly greater when reared at low densities. Survival for juvenile Gila chub approached 100% for all density treatments. Few oddities in overt fish health and appearance were noted during the experiments and physical development for larval Gila chub largely followed growth rates. My data strongly support increasing rearing density has a negative effect on growth and survival of larval Gila chub. My results may assist in forming preliminary guidelines for rearing Gila chub, with possible relevance to other similar species.

INTRODUCTION

Gila chub have been maintained and grown in captivity (Ken Wintin, personal communication, Arizona-Sonora Desert Museum; Jeanette Carpenter, personal communication, U.S. Geological Survey; and personal observation) but the environmental requirements necessary to efficiently culture the endangered Gila chub *Gila intermedia* are unknown at this time. Gila chub appear to eat a variety of artificial and natural feeds in captivity (personal observation). It has been demonstrated that feed characteristics (Bardi et al. 1998; Mohler et al. 2000; Barrows and Hardy 2001; Mischke et al. 2001), water temperature (Harrelson et al. 1988; Abdel et al. 2005; Fitzsimmons and Perutz 2006), and rearing density (Irwin et al. 1999; Alvarez-Gonzalez et al. 2001; Anderson et al. 2002; Jodun et al. 2002; Sahoo et al. 2004; Rahman et al. 2005) have significant impacts on growth, survival, and health of fishes in captivity. The purpose of my study was to identify the effects of different water temperatures, feed types, and rearing densities, on growth, survival, and overt health/appearance of Gila chub larvae and juveniles under laboratory conditions.

METHODS

Feed Type

I randomly assigned three size classes of Gila chub to each treatment group (feed type) and replicate tank (39-L recirculating aquarium tanks). Feed treatments for first-feeding larval Gila chub (6.1-7.7 mm TL) included an enriched natural feed (thawed *Artemia* sp. nauplii, Hikari Bio-Pure Baby Brine Shrimp, Hikari, Inc., Hayward, CA), a prepared feed (chicken *Gallus domesticus* egg-yolk powder, John Oleksy, Inc., Schaumburg, IL), and a commercial larval fish diet (Hikari First-Bites, Hikari, Inc.) fed to excess four times daily (Table 1). I defined “feeding

to excess” to mean that there was feed left in the tanks 15 min following a feeding. Feed treatments for small (22-29 mm TL) and large (44-68 mm TL) juvenile Gila chub included an enriched natural feed (thawed chironomid sp. larvae, Hikari Bio-Pure, Hikari, Inc.) and the following complete commercial feeds (Hikari Micro Pellets, Hikari, Inc.; Wardley Staple Food Flakes [small juveniles only] and Wardley Premium Shrimp Pellets Formula [large juveniles only], Hartz Mountain, Co., Secaucus, NJ; Golden Pearls Weaning and Juvenile Diet, Brine Shrimp Direct, Inc., Ogden, UT; Silver Cup, Nelson and Sons, Inc., Murray, UT), respectively, fed to excess three times daily (Table 1). Feedings were spaced by 2-3 hours between about 6AM and 8PM. Initial biomass of Gila chub per tank was 0.008 g/L or less for larval chub, 0.083 g/L or less for small juveniles, and 0.396 g/L or less for large juveniles. Tanks varied with laboratory temperature, which rarely deviated from 20-22°C. Experiments ran for 14 d for Gila chub larvae and 21 d for Gila chub juveniles.

I used an ocular micrometer to measure initial length (to nearest 0.1 mm) of larval Gila chub and calipers to measure final length (to nearest 0.1 mm) of larval Gila chub. I measured length (to nearest 1 mm) of juveniles using a measuring board. I measured wet-weight (to nearest 0.0001 g) of all Gila chub using an electronic scale. Particular care was taken to systematically remove excess water from all larval Gila chub prior to measurement. Larval Gila chub were euthanized with MS-222 (3-aminobenzoic acid ethyl ester) prior to measurement. Initial larval length and weight measurements were derived from a random subsample ($n = 20$) acquired within 24-h of hatching. Final larval length and weight measurements were derived from a random subsample ($n = 10$) of survivors from each treatment group. For large juvenile fish, I measured lengths and weights of all individual fish. For small juveniles I measured lengths of all individuals but compared mean weight of all individuals per tank for the analysis.

I used one-way analysis of variance (ANOVA) to test for significant differences in mean weight and length gain, and percent survival, of larval and juvenile Gila chub among feed types. If a statistically significant ($P \leq 0.05$) difference was detected in ANOVA tests, I used a Tukey-Kramer HSD Multiple Comparison Procedure to identify which means differed.

Temperature

I randomly assigned Gila chub to each of four different treatment levels (test temperatures) with three replications (tanks) per treatment level for each size class tested. Each 38-L rectangular glass tank was fitted with a recirculating filter system with a stocking density of 40 larval chub (6.0-7.5 mm TL), 7 small juveniles (32-49 mm TL), or 5 large juveniles (52-72 mm TL) for a mean initial biomass of 0.004 g/L, 0.19 g/L, and 0.49 g/L, respectively. Gila chub were acclimated by increasing water temperature in equally divided intervals over a five-day period until the desired test temperature was reached. Larval Gila chub were tested at 20, 24, 28, and 32°C. Juvenile Gila chub were tested at 20, 23, 26, and 29°C. Test temperatures were monitored daily for accuracy and adjusted when necessary. Experiments ran for 29-30 days.

Larval Gila chub were euthanized with MS-222 (3-aminobenzoic acid ethyl ester) prior to measurement. Initial larval measurements were derived from a random subsample ($n = 18$) of all fish acquired within 24-h of hatching. Final larval measurements were derived from a random subsample ($n = 10$) of survivors from each treatment group. I measured wet-weight (to nearest 0.0001 g) of all Gila chub using an electronic scale. Particular care was taken to systematically remove excess water from all larval Gila chub prior to measurement. I used an ocular micrometer to measure initial length (to nearest 0.1 mm) of larval Gila chub and calipers to

measure final length (to nearest 0.1 mm) of larval Gila chub. I measured length (to nearest 1 mm) of juveniles using a measuring board.

Each replicate group of larval Gila chub was fed to excess four times daily using a combination of thawed *Artemia* sp. nauplii (Hikari Bio-Pure, Hikari, Inc., Hayward, CA) and Hikari First-bites (Hikari, Inc.). Each replicate group of juvenile chub was fed to excess three times daily using a combination of unfrozen chironomid larvae and Hikari Micro-pellets (Hikari, Inc.) for small juveniles or Silver Cup (Nelson and Sons, Inc., Murray, UT) for large juveniles.

I used one-way analysis of variance (ANOVA) or Welch's ANOVA test (when group variances were significantly different, $P \leq 0.05$) to test for significant differences in mean weight and length gain, and percent survival of larval and juvenile Gila chub among test temperatures. If a statistically significant ($P \leq 0.05$) difference was detected in ANOVA tests I used a Tukey-Kramer HSD Multiple Comparison Procedure to identify which means differed. I used Pearson's chi-squared test to determine if the incidence of spinal deformity of larval Gila chub differed among test temperatures.

Density

I randomly assigned Gila chub to each of three different treatment densities and four replications (tanks) per treatment density. Mean initial density (low, moderate, and high, respectively) of Gila chub was 0.065 g/L (38.9 fish/L), 0.540 g/L (319.5 fish/L), and 1.343 g/L (795 fish/L) for larval chub (6.3-6.8 mm TL); 3.618 g/L (4.0 fish/L), 16.986 g/L (20.1 fish/L), and 60.145 g/L (68.3 fish/L) for small juveniles (36-47 mm TL); and 1.681 g/L (0.4 fish/L), 14.346 g/L (2.7 fish/L), and 53.942 g/L (8.4 fish/L) for large juveniles (57-95 mm TL). All experiments were conducted within closed recirculating systems. Larval Gila chub were tested

in 11 x 11 cm cylindrical, acrylic, floating pods set to contain about 0.25 L of water.

Experimental pods were set within a 340-L rectangular glass tank which gravity fed water to a smaller 189-L rectangular glass tank in which water was then pumped back to the larger tank. The smaller tank was fitted with 2 recirculating bio-filters with a maximum combined filtering capacity of 3784 L/h. Pod bottoms consisted of stainless steel mesh (0.25-mm open-space). A drip system allowed each pod to receive a flow of at least 2.4 mL/s. Small juvenile Gila chub were tested in floating hard plastic pods (9.6 x 9.6 x 9.6 cm) set to contain 0.25 L water. Pods were contained within 38-L aquarium tanks. Large juvenile Gila chub were tested in 4.75-L (8.5 x 22 x 25.4 cm) sections of standard 38-L aquarium tanks. All juvenile tanks were fitted with a recirculating bio-filter with a filtering capacity of 1135 L/h. Tanks for all experiments were maintained near 24°C. Experiments ran for 33 d for Gila chub larvae, 48 d for small juveniles, and 45 d for large juveniles.

Larval Gila chub were euthanized with MS-222 (3-aminobenzoic acid ethyl ester) prior to measurement. Initial larval measurements were derived from a random subsample ($n = 20$) of all fish acquired within 24-hr of hatching. Final larval measurements were derived from a random subsample ($n = 10$) of survivors from each treatment group. I measured wet-weight (to nearest 0.0001 g) of all Gila chub using an electronic scale. Particular care was taken to systematically remove excess water from all larval Gila chub by blotting and air drying fish prior to measurement. I used an ocular micrometer to measure initial total length (to nearest 0.1 mm) of larval Gila chub and calipers to measure final total length (to nearest 0.1 mm) of larval Gila chub. I measured total length (to nearest 1 mm) of juveniles using a measuring board.

Each replicate group of larval Gila chub was fed to excess four times daily using a combination of thawed *Artemia* sp. nauplii (Hikari Bio-Pure, Hikari, Inc., Hayward, CA) and

Hikari First-Bites (Hikari, Inc.). Each replicate group of juvenile chub was fed to excess three times daily using a combination of thawed chironomid larvae and Hikari Micro Pellets (Hikari, Inc.) for small juveniles or Silver Cup (Nelson and Sons, Inc., Murray, UT) for large juveniles.

I used one-way analysis of variance (ANOVA) to test for significant differences in mean weight and length gain, and percent survival, of larval and juvenile Gila chub among test temperatures. If a statistically significant ($P \leq 0.05$) difference was detected in ANOVA tests, I used a Tukey-Kramer HSD Multiple Comparison Procedure to identify which means differed.

RESULTS

Feed Type

Mean length gain of larval Gila chub was significantly different ($F = 6.649$; $df = 2, 13$; $P = 0.010$) among feed types with the commercial feed outperforming the others (Table 2). Mean weight gain showed a similar pattern with respect to feed types but the difference was not statistically significant ($F = 1.208$; $df = 2, 13$; $P = 0.330$) (Table 2). Mean percent survival of larval Gila chub was significantly different ($F = 6.087$ $df = 2, 13$; $P = 0.013$) among feed types with a consistently higher survival for those groups fed *Artemia* sp. nauplii (Table 2). Few oddities in overt fish health/appearance were noted during the experiment, and physical development largely followed growth rates.

Mean length gain of small juvenile Gila chub differed ($F = 9.096$; $df = 4, 5$; $P = 0.016$) among feed types with chironomid larvae strongly outperforming the remaining commercial feeds (Table 2). As in the larval experiments, mean weight gain for small juveniles showed a similar pattern with respect to feed types but the difference was not statistically significant ($F = 3.011$; $df = 4, 5$; $P = 0.128$) (Table 2).

Mean length and weight gain of large juvenile Gila chub was significantly different ($F = 7.076$ and 11.725 ; $df = 4, 5$; $P = 0.027$ and 0.009 , respectively) among feed types with chironomid larvae strongly outperforming the remaining commercial feeds (Table 2). Outside of two escapees for both small and large juvenile experiments, survival was 100% for all replicate tanks and no oddities in overt fish health or appearance were noted during either experiment.

Temperature

Mean weight and length gains of larval Gila chub were significantly different ($F = 6.87$ and 11.05 ; $df = 3, 8$; $P = 0.05$ and 0.03 , respectively) among test temperatures. Growth of larval chub was greatest at 28°C but decreased markedly at 32°C (Table 3). Mean weight gain of larval Gila chub was significantly greater at 28°C than 20°C and 32°C . Mean weight and length gain of small ($F = 0.17$ and 1.80 ; $df = 3, 8$; $P = 0.91$ and 0.22 , respectively) or large ($F = 0.47$ and 0.67 ; $df = 3, 8$; $P = 0.70$ and 0.59 , respectively) juvenile Gila chub did not differ significantly among test temperatures (Table 3).

Mean percent survival appeared highest for larval chub reared at 24°C but there was no statistical evidence ($F = 2.76$; $df = 3, 8$; $P = 0.11$) of a difference in survival among test temperatures (Table 3). Mortalities were all but non-existent (one accidental) for either juvenile size-class. There was strong evidence (Chi-square = 31.11 ; $P < 0.001$) that spinal deformities of larval Gila chub differed among test temperatures. Spinal deformities were present in almost half (47%) of the larval chub reared at 32°C , less common (23%) for those reared at 24°C , and non-existent for those reared at 20°C and 28°C . No other overt abnormalities were noted for larval Gila chub. All juvenile Gila chub tested appeared overtly healthy throughout the experiment.

Density

There was convincing evidence that mean length and weight gain of larval Gila chub differed ($F = 66.201$ and 15.637 ; $df = 2, 9$; $P < 0.001$ and 0.001 , respectively) among rearing densities. Mean length and weight gain decreased as rearing density increased (Table 4). There was also convincing evidence that mean percent survival of larval Gila chub differed ($F = 25.258$; $df = 2, 9$; $P < 0.001$) among rearing densities with consistently higher survival for those groups reared at a low density (Table 4). Few oddities in overt fish health or appearance were noted during the experiment and physical development largely followed growth rates. Mean length gain of small juvenile Gila chub differed ($F = 5.025$; $df = 2, 9$; $P = 0.034$) among rearing densities and appeared least for those reared at a high density. However, the multiple comparisons procedure used was unable to identify which treatments differed statistically (Table 4).

Mean weight gain of small juvenile Gila chub differed ($F = 7.418$; $df = 2, 9$; $P = 0.012$) among rearing densities, and was greatest for those reared at a moderate density (Table 4). Survival was 100% for all density treatments with small juvenile Gila chub and no oddities in overt fish health or appearance were noted. Mean length and weight gain of large juvenile Gila chub differed ($F = 22.241$ and 88.155 ; $df = 2, 9$; $P < 0.001$, respectively) among rearing densities. Mean length and weight gain decreased as rearing density increased (Table 4). For large juvenile Gila chub, survival and lack of oddities in fish health/appearance was at or approached 100% for all density treatments. Evidence of reproductive activity (eggs) was noted in one moderate and one high density treatment tank.

DISCUSSION

Although maximizing production is likely not the main goal in the culture of many imperiled native fishes at this time, there are distinct benefits to an efficient grow-out phase when producing fish for stocking and other efforts. Faster grow-out to a certain size allows stocking for a greater part of the year, may lower feed and labor costs, and may increase available rearing space. Where piscivores are present, stocking of large individuals may be necessary to lower their loss due to predation (Marsh and Brooks 1989).

Feed Type

Natural feeds often outperform prepared/commercial feeds with respect to growth (Barrows and Hardy 2001). Larval stages of many species of fishes grow and survive better on natural feed (Mischke et al. 2001; Mohler et al. 2000; Bardi et al. 1998). While survival of larval Gila chub fed a natural feed was greater, growth of those fed the commercial diet was equal or slightly better. Mischke et al. (2001) had similar results for larval bluegill *Lepomis macrochirus*. It is possible that some *Artemia* nauplii are too large for first-feeding larval Gila chub to handle, which may account for this feed not outperforming the commercial diet with respect to growth. I observed several unsuccessful feeding attempts of larval Gila chub before they found an *Artemia* they could ingest. Alternative feeds that are smaller or co-feeding (i.e., feeding more than one feed type/size, Rosenlund et al. 1997) may prove necessary to optimize growth and survival of first-feeding larval Gila chub.

Although differences in growth of juvenile Gila chub among natural and commercial diets were obvious, I did not identify a commercial feed that consistently outperformed other

commercial feeds. A more lengthy experiment may be needed to reveal differences among prepared commercial feed types.

Prior to my feeding experiments I discovered larval Gila chub would consume thawed *Artemia* nauplii with similar enthusiasm to live *Artemia* nauplii. It is unknown if live or thawed *Artemia* affect growth of Gila chub differently. Mohler et al. (2000) found Atlantic sturgeon *Acipenser oxyrinchus oxyrinchus* fed thawed *Artemia* nauplii grew slower than, but had similar survival to, those fed live *Artemia*. I noted that thawed *Artemia* drifted similarly to live *Artemia* when a slight flow was present in tanks. The use of frozen natural feeds produced off site meant that *Artemia* was readily available, and I did not have to culture *Artemia* on site, which is labor intensive. While an economic evaluation was not included in my study, it is likely commercially available frozen natural feeds are more costly per nutritive value than most prepared/commercial feeds. Maximum survival and health of larval cohorts is often valued over short-term cost disadvantages and this value may be even more pronounced for imperiled species such as Gila chub. While growth was equal or slightly less for larval Gila chub fed *Artemia* than a commercial larval fish diet, survival was significantly higher for larval chub fed the natural diet. Both growth and survival of larval Gila chub may have been increased if a smaller natural feed had been given for the first few days of exogenous feeding or if a co-feeding strategy where both live and inert feed was given was employed. Rosenlund et al. (1997) found combining live feed and manufactured diets improved growth and survival of marine fish larvae compared to the use of live feed only. Co-feeding was found to serve two purposes by improving and stabilizing the nutritional condition of the larvae and pre-conditioning larvae to accept the manufactured diet when live feed is withdrawn, resulting in a shorter weaning period.

I did not compare growth in Gila chub with respect to nutritive differences among feed types (e.g., protein). My study provides initial guidelines for the feeding of larval and juvenile Gila chub and further studies will be needed to identify proximate compositions of diet that will optimize the growth, survival, and health of Gila chub.

In summary, my investigation demonstrated that larval Gila chub survived significantly better, but grew comparably to slightly less, when fed a natural diet (i.e., *Artemia nauplii*) versus a commercial larval fish diet and chicken egg-yolk powder. However, further investigation of the efficiency of smaller natural feeds for larval Gila chub is warranted given observations made. It appears prepared or commercial feeds can be used to rear larval Gila chub but longer-term growth, survival, and health was not studied. Juvenile Gila chub clearly grew better when fed a natural diet (i.e., chironomid larvae) versus any of the commercial diets I tested. However, survival and overt health or appearance was similar for both commercial and natural diets. Based on feeds tested, I recommend larval Gila chub be fed a natural diet if survival is paramount to objectives. Based on feeds tested, I recommend juvenile Gila chub be fed a natural diet if faster growth is paramount to objectives. Further work is suggested to define the nutritive requirements and identify the most efficient feeding regimen for Gila chub.

Temperature

Of the temperatures I tested, optimal temperature for growth of larval Gila chub was 28°C and the growth rate markedly decreased somewhere between 28-32°C. The survival and health of larval Gila chub appeared better at 24°C than at other temperatures tested. Although a positive trend with increasing temperatures was sometimes apparent and juvenile Gila chub seemed to grow best between 26–29°C, statistical differences in growth among rearing temperatures were

not found. A statistically significant difference in growth among test temperatures for juveniles may have been revealed by employing a more lengthy experiment, a wider range of test temperatures, or more replicates for a more powerful test.

The temperature at which highest growth rate occurs is probably optimal for most physiological processes (Harrelson et al. 1988). However, further insight as to the relationship between optimal growth and factors independent of growth can shape criteria when determining optimal culture temperature. Disease susceptibility can vary with temperature (Harrelson et al. 1988) and is always a concern. In addition, rearing temperature can contribute to development of deformations (Abdel et al. 2005; Fitzsimmons and Perutz 2006). In general a higher incidence of malformations has been found in cultured rather than wild fishes (Komada 1980 and citations therein) and such malformations are considered an important problem in intensive aquaculture (Aritaki et al. 1996; Fraser et al. 2004). While I found the incidence of spinal deformities for larval Gila chub was much higher at 32°C, any trend in occurrence of spinal deformities was unclear at the other temperatures tested. It is generally considered prudent for culturists to produce fishes that are similar in morphological, physiological, behavioral, and biochemical characteristics to their wild counterparts. I recorded overt signs of deformation, but investigation into unseen affects of various culture conditions upon Gila chub may be warranted. Matsouka (2003) found reared fishes with abnormalities often showed no obvious external signs of deformation.

My tests were conducted under relatively well-controlled laboratory conditions. Study of growth and other factors under more variable conditions, such as outdoor ponds, is needed for Gila chub. Growth rates can be greater in a cyclic rather than a static temperature regime (Harrelson et al. 1988).

Based on the parameters and results of my study water temperatures from 20-28°C appear suitable for rearing larval Gila chub, with temperatures from 24-28°C recommended for faster growth. Water temperatures from 20-29°C appear suitable for rearing juvenile Gila chub.

Density

My data strongly supported that rearing density affected growth of larval and large juvenile Gila chub. The relationship of density to small juvenile growth was less clear. Mean length gain of small juvenile Gila chub decreased as density increased; however, I cannot explain why weight did not show the same relationship. An inverse relationship between rearing density and growth of larvae and juveniles has been noted for other species of fishes as well (Rahman et al. 2005; Sahoo et al. 2004; Anderson et al. 2002; Jodun et al. 2002; Irwin et al. 1999).

Similar to other fishes (Sahoo et al. 2004; Alvarez-Gonzalez et al. 2001), larval Gila chub survived better at low rearing densities. I found little effect of the rearing densities I tested on survival of either small or large juvenile Gila chub during my experiment. Anderson et al. (2002) found no effect of rearing density (up to 667 fish/m³; mean fish weight = 1.76 g) on the survival of juvenile bluegill *Lepomis macrochirus* in a longer study. The few mortalities of juvenile Gila chub I noted took place in high-density treatments. In addition, high density treatments for large juveniles resulted in weight loss over a 45-d period. Thus, high density treatments may have eventually led to a significant increase in mortality rates during a longer experiment.

Irwin et al. (1999) stated relationships between density and growth may not always be linear, and that a threshold level may exist for certain species. My study was conducted at three broadly separated rearing densities and it is unknown how growth and survival of Gila chub

between these ranges would be influenced and what type of relationships exist therein. It is unknown by what mechanism(s) rearing density affects the growth and survival of larval and juvenile Gila chub as observations of social interactions, individual behaviors, and physiological measurements were not conducted, or were limited, during my study.

As referred to prior, the effect of rearing density upon Gila chub is undoubtedly influenced by surrounding factors. The effect of density upon Gila chub in more natural conditions such as outdoor ponds will likely vary from my results. The probable interactive effects between density and vital factors such as feeding regime, temperature, and water quality, warrants study. Furthermore, my results are for closed recirculating systems, and in other types of systems, rearing density may affect growth patterns differently. Given the increasing limitations on space, water use, and funding often encountered by hatchery managers, recirculating systems may become more prevalent in the future.

My results provide the first published data on the effects of specific rearing densities upon growth and survival of Gila chub. These results may assist in developing guidelines for initial rearing densities for Gila chub in recirculating systems, with possible relevance to other similar species. Recommended initial rearing densities for Gila chub are dependent upon management objectives and the culture system used. Based on my tests, I recommend initial stocking densities near 39 fish/L if growth and/or survival of larval Gila chub in aquaria are primary considerations. For juvenile Gila chub all densities I tested gave acceptable survival, at least in the short term. If maximizing growth rate of juvenile Gila chub is important, I recommend fish be raised at approximately 16.986 g/L for small juveniles and approximately 1.681 g/L for large juveniles. Further research is needed to further define the relationship(s) and any thresholds between rearing density and growth and survival for early life stages of Gila chub.

I recommend further research for closed recirculating systems concentrate on testing densities within the range of the low to moderate treatment levels I employed.

The increasing prevalence and importance of culturing imperiled fish species as a conservation and management strategy (Johnson and Jensen 1991; Modde et al. 1995) is a regrettable reality. Nonetheless, captive breeding and rearing can be a powerful tool when needing stock to repatriate extirpated populations or establish refuge populations. Culture techniques can also be used to perpetuate a species during a crisis. Lack of such knowledge has led to the extinction of certain species (Minckley and Deacon 1991).

ACKNOWLEDGMENTS

This project was funded and supported by the Arizona Game and Fish Department (AGFD) Heritage Fund, the Central Arizona Project Transfer Fund, the University of Arizona, and the Arizona Cooperative Fish and Wildlife Research Unit and its cooperators. I thank all the staff and professionals at the AGFD, especially those at the Region V office. I thank my Ph.D. committee members Scott Bonar (major professor), Courtney Conway, Kevin Fitzsimmons, Peter Reinthal, and Cecil Schwalbe, and William Matter for design support. I thank Paul Barrett, Roger Hamman, and Manuel Ulibarri of the United States Fish and Wildlife Service for design and logistical support. I thank Joshua Taiz and associated staff of the United States Forest Service Santa Catalina Ranger District. I thank all staff and faculty at the University of Arizona that assisted in any respect during this study, especially Anne Hartley, Linda Lee, Dee Simons, Cecily Westphal, and Carol Yde. Special thanks go to Mark Borgstrom and Robert Steidl for statistical advice. I thank Craig Ivanyi and Ken Wintin of the Arizona Sonora Desert Museum. Thanks to Andrew Honaman and Gina Schultz for technical support. Special thanks go to Alison

Iles of the Arizona Cooperative Fish and Wildlife Research Unit, Dr. Mitch McClaran, Patrick Reid and Malcolm Zwolinski of the University of Arizona's School of Natural Resources.

Thanks to Yashica Anderson, Daniel Arevalo, Sachiko Aso, Brian Chaszar, Sheldon Caldwell-Meeks, Stan Culling, Teresa Drury, Andrea Francis, Shawna Hehr, Jason Kline, Guillermo Ley, Michelle Martin, Jake Mundy, Michelle Riley, Gina Schultz, and Sebastian Zeltzer for their assistance in the laboratory. Thanks to all the other individuals, government agencies, non-governmental groups, and businesses not mentioned here that assisted with the project.

LITERATURE CITED

- Abdel, I., E. Abellan, O. Lopez-Albors, P. Valdes, M. J. Nortes, and A. Garcia-Alcazar. 2005. Abnormalities in the juvenile stage of sea bass (*Dicentrarchus labrax*) reared at different temperatures: types, prevalence and effect on growth. *Aquaculture International* 12:523-538.
- Alvarez-Gonzales, C. A., J. L. Ortiz-Galindo, S. Dumas, S. F. Martinez-Diaz, D. E. Hernandez-Ceballos, T. Grayeb-Del Alamo, M. Moreno-Legorreta, and R. Pena-Martinez. 2001. Effect of stocking density on the growth and survival of spotted sand bass *Paralabrax maculatofasciatus* larvae in a closed recirculating system. *Journal of the World Aquaculture Society* 32:130-137.
- Anderson, D., I. P. Saoud, and D. A. Davis. 2002. The effects of stocking density on survival, growth, condition, and feed efficiency of bluegill juveniles. *North American Journal of Aquaculture* 64:297-300.

- Aritaki, M., T. Seikai, and M. Kobayasi. 1996. Reduction of morphological abnormalities in brown sole by larval rearing with higher temperature and early feeding of *Artemia* nauplii. *Nippon Suisan Gakkaishi* 62:857-864.
- Bardi, R. W., Jr., F. A. Chapman, and F. T. Barrows. 1998. Feeding trials with hatchery produced Gulf of Mexico sturgeon larvae. *Progressive Fish Culturist* 60:25-31.
- Barrows, F. T., and R. W. Hardy. 2001. Nutrition and feeding. Pages 483-558 in G. A. Wedemeyer, editor. *Fish hatchery management*, second edition. American Fisheries Society, Bethesda, Maryland.
- Fitzsimmons, S. D., and M. Perutz. 2006. Effects of egg incubation temperature on survival, prevalence and types of malformations in vertebral column of Atlantic cod (*Gadus morhua*) larvae. *Bulletin of the European Association of Fish Pathologists* 26:80-86.
- Fraser, M. R., T. A. Anderson, and R. de Nys. 2004. Ontogenic development of the spine and spinal deformities in larval barramundi (*Lates calcarifer*) culture. *Aquaculture* 242:697-711.
- Harrelson, M. E., J. Hudson, and J. B. Cravens. 1988. Thermal effects. *Journal of Water Pollution Control Federation* 60:978-983.
- Irwin, S., J. O'Halloran, and R. D. FitzGerald. 1999. Stocking density, growth and growth variation in juvenile turbot, *Scophthalmus maximus* (Rafinesque). *Aquaculture* 178:77-88.
- Jodun, W. A., M. J. Millard, and J. Mohler. 2002. The effect of rearing density on growth, survival, and feed conversion of juvenile Atlantic salmon. *North American Journal of Aquaculture* 64:10-15.

- Johnson, J. E., and B. L. Jensen. 1991. Hatcheries for endangered freshwater fishes. Pages 199-217 in W. L. Minckley and J. E. Deacon, editors. Battle against extinction: native fish management in the American West. University of Arizona Press, Tucson, Arizona.
- Komada, N. 1980. Incidence of gross malformations and vertebral anomalies of natural and hatchery *Plecoglossus altivelis*. Copeia 1980:29-35.
- Marsh, P. C., and J. E. Brooks. 1989. Predation by ictalurid catfishes as a deterrent to re-establishment of hatchery-reared razorback suckers. Southwestern Naturalist 34:188-195.
- Matsouka, M. 2003. Comparison of meristic variations and bone abnormalities between wild and laboratory-reared red sea bream. Japan Agricultural Research Quarterly 37:21-30.
- Minckley, W. L., and J. E. Deacon, editors. 1991. Battle against extinction: native fish management in the American West. University of Arizona Press, Tucson, Arizona.
- Mischke, C. C., G. D. Dvorak, and J. E. Morris. 2001. Growth and survival of hybrid sunfish larvae in the laboratory under different feeding and temperature regimes. North American Journal of Aquaculture 63:265-271.
- Modde, T., A. T. Scholz, J.H. Williamson, G. B. Haines, B. D. Burdick, and F. K. Pfeifer. 1995. Augmentation plan for razorback sucker in the Upper Colorado River Basin. American Fisheries Society Symposium 15:102-111.

- Mohler, J. W., M. K. Kim, and P. R. Farrell. 2000. Growth and survival of first feeding and fingerling Atlantic sturgeon under culture conditions. *North American Journal of Aquaculture* 62:174-183.
- Rahman, M. A., M. A. Mazid, M. R. Rahman, M. N. Kahn, M. A. Hossain, and M.G. Hussain. 2005. Effect of stocking density on survival and growth of critically endangered mahseer, *Tor putitora* (Hamilton), in nursery ponds. *Aquaculture* 249:257-284.
- Rosenlund, G., J. Stoss, and C. Talbot. 1997. Co-feeding marine fish larvae with inert and live diets. *Aquaculture* 155:183-191.
- Sahoo, S. K., S. S. Giri, and A. K. Sahu. 2004. Effect of stocking density on growth and survival of *Clarias batrachus* (Linn.) larvae and fry during hatchery rearing. *Journal of Applied Ichthyology* 20:302-305.

TABLE 1.—Nutrient analysis (percent, by weight; from data supplied by feed manufacturers) of 2 natural diets (enriched and processed by manufacturers; frozen *Artemia* sp. nauplii and frozen chironomid larvae, Hikari Bio-Pure, Hikari, Inc.) and 7 prepared/commercial diets (chicken egg-yolk powder, John Oleksy, Inc.; Hikari First-Bites and Hikari Micro Pellets, Hikari, Inc.; Wardley Staple Food Flakes and Wardley Premium Shrimp Pellets Formula, Hartz Mountain, Co; Golden Pearls Weaning and Juvenile Diet, Brine Shrimp Direct, Inc.; Silver Cup, Nelson and Sons, Inc.) fed to three size classes of Gila chub *Gila intermedia*. Values for protein and fat represent minimum guarantee levels, and fiber, phosphorus, and moisture represent a range of minimum or maximum and typical guaranteed levels. Values in parentheses are for a dried version of the feed type.

Diet	Protein	Fat	Fiber	Ash	Phosphorus	Moisture	Size Class Fed
<i>Artemia</i> sp. nauplii	6.8 (47)	1.5 (5.5)	1.2 (0.5)		(0.1)	86 (6)	Larval
Chironomid larvae	6 (65)	0.5 (5)	0.9 (3.5)		(0.1)	89 (6.5)	Sm. & Lg. Juvenile
Egg-yolk powder	34.25	55.8		3.4	<1	2.95	Larval
Hikari First Bites	48	3	1	15	1.3	10	Larval
Hikari Micro Pellets	42	4	3	12		10	Sm. & Lg. Juvenile
Wardley Staple Flakes	40	4	5			8	Sm. Juvenile
Wardley Shrimp Pellets	30	3	10			10	Lg. Juvenile
Golden Pearls	60	18		15		8	Lg. Juvenile
Silver Cup	48-51	14-16	3-1	12-9		<10	Lg. Juvenile

TABLE 2.—Mean weight and length gains (with standard errors of the means) per feed type for larval, small juvenile and large juvenile Gila chub *Gila intermedida*. Feed types for larval Gila chub include thawed *Artemia* sp. Nauplii (Hikari Bio-Pure Baby Brine Shrimp), chicken *Gallus domesticus* egg-yolk powder, and a commercial larval fish diet (Hikari First-Bites). Feed types for small juvenile and large juvenile Gila chub include thawed chironomid larvae (Hikari Bio-Pure Blood Worms) and four commercial feeds (Golden Pearls Weaning and Juvenile Diet [Feed 1], Hikari Micro Pellets [Feed 2], Wardley Premium Shrimp Pellets [Feed 3], and Silver Cup [Feed 4]). Values with different lowercase letters are significantly different ($P \leq 0.05$).

Feed Type	Mean Weight Gain		Mean Length Gain		Mean %	
	(mg/g) ^a	SE	(mm TL)	SE	Survival	SE
Larval Gila Chub						
Artemia Nauplii	3.55	1.22	2.8 xy	0.7	76%	13
Egg Yolk Powder	2.72	0.78	2.3 x	0.3	47%	16
Commerical Feed	3.78	1.94	3.4 y	0.5	49%	23
Small Juvenile Gila Chub						
Chironomid Larvae	0.231	0.024	6.4 x	0.2		
Feed 1	0.093	0.100	2.9 y	0.5		
Feed 2	0.076	0.014	3.2 y	1.0		
Feed 3	0.135	0.080	2.3 y	0.6		
Feed 4	0.031	0.043	2.4 y	1.2		
Large Juvenile Gila Chub						
Chironomid Larvae	1.465	0.185	6.9 x	1.5		
Feed 1	0.838	0.189	3.6 xy	0.2		
Feed 2	0.567	0.054	3.6 xy	1.1		
Feed 3	0.640	0.188	4.1 xy	0.4		
Feed 4	0.765	0.022	2.4 y	0.6		

^aData for larval Gila chub are reported in milligrams (mg) and juvenile Gila chub in grams (g).

TABLE 3.—Mean weight and length gains (with standard errors of the means) per test temperature for larval, small juvenile and large juvenile Gila chub *Gila intermedia*. Values with different lowercase letters are significantly different ($P \leq 0.05$).

°C	Mean Weight Gain (mg/g) ^a	SE	Mean Length Gain (mm TL)	SE	Mean % Survival	SE
Larval Gila Chub						
20	26 x	3.7	8.3 xy	0.7	70%	8
24	56 xy	10.3	11.4 yz	1.1	89%	7
28	67 y	12.0	12.1 z	0.8	83%	2
32	2 x	1.7	7.6 x	0.1	73%	4
Small Juvenile Gila Chub						
20	0.713	0.121	11.6	0.8		
23	0.767	0.270	13.5	1.6		
26	0.857	0.063	12.8	2.2		
29	0.880	0.222	14.5	0.7		
Large Juvenile Gila Chub						
20	2.122	0.395	9.3	1.5		
23	2.439	0.373	11.9	1.6		
26	3.151	1.101	14.1	0.9		
29	2.437	0.292	13.7	2.1		

^aData for larval Gila chub are reported in milligrams (mg) and juvenile Gila chub in grams (g).

TABLE 4.—Mean weight and length gains (with standard errors of the means) per rearing density for larval, small juvenile and large juvenile Gila chub *Gila intermedia*. Values with different lowercase letters are significantly different ($P \leq 0.05$).

Density	Mean Weight Gain (mg/g) ^a	SE	Mean Length Gain (mm TL)	SE	Mean % Survival	SE
Larval Gila Chub						
Low	33 x	2	9.3 x	0.0	93%	5
Moderate	23 y	2	8.5 y	0.1	49%	3
High	16 y	2	6.9 z	0.2	51%	6
Small Juvenile Gila Chub						
Low	0.920 x	0.104	16.5	0.9		
Moderate	1.505 y	0.154	16.5	1.0		
High	1.079 xy	0.049	13.4	0.5		
Large Juvenile Gila Chub						
Low	3.215 x	0.288	10.0 x	2.0		
Moderate	0.580 y	0.150	1.4 y	0.2		
High	-0.331 z	0.099	-0.1 y	0.1		

^aData for larval Gila chub are reported in milligrams (mg) and juvenile Gila chub in grams (g).