Westslope Warmwater Fisheries

GOCO Funded

Patrick J. Martinez
Principal Investigator

Bruce McCloskey, Director

Job Progress Report

Colorado Division of Wildlife

Fish Research Section

Fort Collins, Colorado

August 2006

STATE OF COLORADO

Bill Owens, Governor

COLORADO DEPARTMENT OF NATURAL RESOURCES

Russell George, Executive Director

COLORADO DIVISION OF WILDLIFE

Bruce McCloskey, Director

WILDLIFE COMMISSION

AQUATIC RESEARCH STAFF

Mark S. Jones, General Professional VI, Aquatic Wildlife Research Leader
Richard Anderson, General Professional IV, F-289 Riverine Fish/Flow Investigations
Arturo Avalos, Technician III, Research Hatchery
Stephen Brinkman, General Professional IV, F-243, Water Pollution Studies
Patrick J. Martinez, General Professional V, F-242, Coldwater Reservoir Ecology & GOCO - Westslope Warmwater
R. Barry Nehring, General Professional V, F-237, Stream Fisheries Investigations
Kevin Rogers, General Professional IV, GOCO - Boreal Toad and Colorado Cutthroat Studies
Phil Schler, Hatchery Technician V, Research Hatchery
George Schisler, General Professional IV, F-394, Salmonid Disease Investigations
Kevin Thompson, General Professional IV, F-427, Whirling Disease Habitat Interactions
Rod Van Velson, General Professional IV, F-161, Stream Habitat Investigations and Assistance
Harry Vermillion, Scientific Programmer/Analyst, F-239, Aquatic Data Analysis
Nicole Vieira, Physical Scientist III, Water Quality Issues
Rosemary Black, Program Assistant I

Paula Nichols, Federal Aid Coordinator
Jackie Boss, Librarian

Prepared by: ____
Patrick J. Martinez, GP V, Aquatic Researcher

Approved by: ____
Mark S. Jones, Aquatic Wildlife Research Leader

Date: ________________________________

The results of the research investigations contained in this report represent work of the authors and may or may not have been implemented as Division of Wildlife policy by the Director or the Wildlife Commission.

TABLE OF CONTENTS

LIST OF TABLES

State: Colorado **Name:** Statewide Fish Research

Title: Westslope Warmwater Fisheries

Period Covered: July 1, 2005 to June 30, 2006

Principal Investigator: Patrick J. Martinez

STUDY OBJECTIVE: To evaluate, monitor and recommend select nonnative fish control strategies that fulfill commitments for recovery efforts for the "big river" endangered fishes and to provide guidance for maximizing angling opportunity for nonnative warmwater sport fishes within the regulatory, cooperative and ecological constraints of protecting the "big river" native fish assemblage in the rivers of western Colorado.

OBJECTIVE 1. WARMWATER FISHERY ENHANCEMENT AND NONNATIVE FISH CONTROL STRATEGIES.
To evaluate, facilitate and/or recommend nonnative fish control practices to foster/secure progress/compliance toward sufficient progress for recovery, for stocking agreements and regulations, and acceptable monitoring protocols necessary to perpetuate, expand or replace warmwater sport fisheries on Colorado's western slope.

Segment Objective 1: Facilitate adoption of stricter, harsher regulations for illicit fish introductions in western Colorado, including increased surveillance, increased incentives for informants, more severe penalties including higher fines, restitution and environmental rehabilitation as warranted (removal of unauthorized fishes).

INTRODUCTION

The impetus for work under this Segment Objective is the widespread practice of illicit fish introduction taking place in western Colorado (Martinez 2004a). Part of the approach for encouraging and facilitating responsive action to combat this illicit activity is to develop tools that may aid in the prosecution of such illegal activity. The hope is that such developments will inspire managers and administrators to undertake recommendations for the development and implementation of far stricter regulations to address this damaging and potentially devastating activity with more severe penalties to fit the scale of these crimes. Illicit fish introductions pose threats to prominent, valuable sport fisheries in both streams and reservoirs. An equally serious, and perhaps more ominous, threat is on a drainage scale. Fishes illicitly introduced into reservoirs may or may not deteriorate the existing sport fishery. However, their escape from these reservoirs could allow their spread via streams or diversions thus expanding the range of their potentially deleterious ecological interactions that may severely frustrate or preclude efforts to conserve native fishes or recover endangered ones.

METHODS

By pursuing forensic tools to track illicit introductions of fish, some of the perceived futility in prosecuting violators that currently exists may be allayed. This strategy was presented at an Angler's Roundtable meeting in Grand Junction on 29 March 2006. Work to identify naturally occurring microchemical markers in riverine and floodplain habitats (Martinez, et al. 2001, Whitledge et al In PRESS), in hatcheries (Gibson-Reinemer et al.. 2006) and in reservoirs (Martinez and Johnson 2006) will greatly improve the likelihood of tracing illicit movements of fish back to their source.

RESULTS and DISCUSSION

Martinez (2004a) provided a summary of known illicit introduction from 1980 to 2003. Anita Martinez, Colorado Division of Wildlife (CDOW) Aquatic Biologist, prepared an updated version of that summary by interviewing fellow CDOW Aquatic Biologists. Table 1 shows that known numbers of illicit introductions in the 1980s and 1990s increased by a few incidences following these interviews. However, the number of known illicit introductions since 2003 doubled from 7 (Martinez 2004a) to 14 (Table 1), supporting the observation that this activity continues unabated and may be occurring at an increasing rate in the current decade (Martinez 2004a) and the assertion that this activity is now rampant (Martinez 2005).

Key to combating illicit fish introduction is angler and public education about the illegal nature of this activity and the threat it pose to both the sport fishery and native fish community resources of the State. Programs to increase public awareness about this growing problem should be heightened and expanded within the agency (Warner 2005). In addition, it is hoped that law enforcement will see the application of microchemical markers in water and fish otoliths as a forensic breakthrough. Ideally, this view would lead to optimism about the prospects of securing prosecutions, thereby fostering support to combat this illegal activity and encouraging new regulations that increase monetary fines and collateral penalties including revocation of angling privileges and the seizure of equipment use in these crimes to establish more forceful deterrents. Further, establishing high monetary surcharges similar to those established for the poaching of trophy big game would not only address the drainage scale threat of illicit introductions, but also begin to establish funds that may supplement restoration of affected habitats.

Incorporating these crimes in Operation Game Thief and providing rewards for informants would also encourage public surveillance and testimony to secure prosecution and convey that the State is serious about preventing this activity and protecting its aquatic resources. Another strategy to consider is the application of must-kill regulations which require angler to keep illicitly stocked or established fishes and not release them back into the water alive. This regulation has been in place for illicitly stocked lake trout *Salvelinus namaycush* in Yellowstone Lake (Koel et al. 2006). Similar regulations have recently been adopted for illicitly stocked burbot *Lota lota* in Utah (Roger Schneidervin, UT Div. of Wildlife Res., pers. comm.) and Wyoming, and for illicitly stocked walleye *Sander vitreus* in Wyoming (Kevin Gelwicks, WY Dept, Game & Fish, pers. comm.)

Otolith microchemistry may also facilitate identifying reservoirs that are leaking fish that may reach critical habitat for endangered fishes in western Colorado. The recent expansion of smallmouth bass *Micropterus dolomieu* in the Colorado River (Martinez 2005) raises questions about fish escapement from reservoirs. Escapement of nonnative piscivores into Colorado's western rivers may pose a threat to both trout fisheries and nonsalmonid native fishes. Appendix A shows how stable isotopes may identify source waters of these fishes. Appendix B, a Scope-of-Work, funded by the Recovery Program for Endangered Fishes in the Upper Colorado River Basin, describes the "fingerprinting" of reservoirs to track escapement of smallmouth bass, largemouth bass *M. salmoides*, northern pike *Esox lucius*, black crappie *Pomoxis nigromaculatus* and walleye.

Segment Objective 2: Participate in/lead inter-agency effort to identify strategies to improve prospects for control of smallmouth bass in upper Colorado River basin rivers via their life history, control of their escapement from reservoirs, removal strategies, or environmental manipulations to reduce their abundance and negative ecological impacts in riverine habitats.

INTRODUCTION

Martinez (2005b) reported the chronology of the rapid expansion of smallmouth bass in the Colorado River in Colorado and their abrupt increase in 2003. This explosion of smallmouth bass in the Colorado River and the dominance and projected predatory impact of smallmouth bass established in the Yampa River (Martinez 2005b) provided the impetus to review and formulate strategies to maximize and expedite control of smallmouth bass in these rivers which contain 65% of the critical habitat for endangered fishes within Colorado (Martinez 2005b). Given this scenario, I was directed to convene the principle investigators conducting field studies and removals specific to smallmouth bass, various agency representatives and other researchers, to review this problem, forecast its magnitude and assess prospects for control via current methodologies. Of particular importance was the need to render specific strategies to enhance or redirect current efforts and to identify alternate or companion actions believed necessary to combat smallmouth bass within critical habitat.

Table 1. Summary of known and presumed illicit introductions of nonnative, nonsalmonid sport fishes into ponds and reservoirs in western Colorado, 1980-2005, updated by A. Martinez (after Martinez 2004a).

Name of Colorado reservoir	**<u>Decade of accidental/illicit introduction</u>** :(e) = established, occasional to common in angler catch; (r) = rare in angler & biologist catch; (c) = chemically removed			**Comments**	**Number of illicit species introductions**	**Biologist & DWM**
	1980's	**1990's**	**2000's**			
Billy Creek Lake (water code 88600)			Goldfish (e)	Billy Creek SWA (Ouray County)	1	D. Hale
Blue Mesa		Yellow perch (e)	Northern pike (r)	perch in creel	2	D Brauch
Casey Pond			Goldfish (r)		1	B Atkinson
Catamount		Northern pike (e)		Northern pike escaped from Stagecoach into Catamount	0	K Rogers
Chipeta Lake (Montrose County)			Goldfish (r)		1	T Mathieson
Connected Lakes		Walleye (r)			1	D Kowalski
Crawford		Black crappie (e) northern pike (e) walleye (r)		Size limit for northern pike	3	D Kowalski
Dike Rd Pond			Goldfish (e)	T&E grow-out	1	A Martinez
Elkhead	Black crappie (e)			Crappie in creel	1	B Atkinson
Granby			Northern pike (r)		1	B Atkinson
Harvey Gap	Northern pike (e) Black crappie (e)	Yellow perch (e) Walleye (r)		Pike, black crappie and perch in creel	4	B Elmblad, B Gray
Highline	Yellow perch (e), black crappie (e)			Smallmouth bass stocked in 2003	2	B Elmblad
Juniata		Walleye (e)	Bluegill (e)	Walleye and bluegill in creel	2	B Elmblad

Table 1. **Continued.** Summary of known or presumed illicit introductions of nonnative, nonsalmonid sport fishes into ponds and reservoirs in western Colorado, 1980-2005, updated by A. Martinez (after Martinez 2004a).

Name of Colorado reservoir	**Decade of accidental/illicit introduction** :(e) = established, occasional to common in angler catch; (r) = rare in angler & biologist catch; (c) = chemically removed			**Comments**	**Number of illicit species introductions**	**Biologist & DWM**
	1980's	**1990's**	**2000's**			
Kenney	Black crappie (e) Bluegill (r) largemouth bass (r) Northern pike (r)		Walleye (r)	Black crappie escape downstream	5	B Elmblad
Lake Christine	Goldfish (e) Sunfish (e)			Common dumping ground of unwanted aquarium fish by Basalt residents	2	A. Czenkusch
McPhee		Northern pike (r) Walleye (r)		Mercury in piscivores	2	M Japhet
Prewitt Reservoir		Alewife (r)			1	P Walker
Ridgway		Yellow perch (e)	Green sunfish (e)		2	D Kowalski, K Crane
Rifle Gap		Black crappie (e) Northern pike (e) Yellow perch (e)	Golden shiner (r)	Bag limit for yellow perch	4	B Elmblad, B Gray
Stagecoach		Northern pike (e)	Walleye (r)	Pike escape	2	K Rogers
Vallecito	Smallmouth bass (e)		Yellow perch (r)	(SMB) - Alleged perpetrator known to CDOW but insufficient evidence for prosecution	2	M Japhet
Vega			Northern pike (r)	Unconfirmed?	1	B Elmblad
West Lake (Grand Junction)			Goldfish (r)	Drained and stocked with roundtail chub	1	A Martinez
Totals	13	17	14		**44**	

METHODS and MATERIALS

An invitation was sent to announce a Smallmouth Bass Summit that was held in Grand Junction on 28-29 November 2005, at the Colorado Division of Wildlife. The invitation was intentionally sent to a small group to facilitate presentation and discussion of data and potential strategies to optimize control options revealed by our current knowledge of the problem in combination with a review of pertinent literature. To assist participants in their preparation for the meeting, a collection of smallmouth bass literature was sent to them in advance of the Summit. Many of these references are cited herein, but this collection of smallmouth bass papers are included in the literature cited section of this report and are denoted by bold font.

The goal of this gathering was to identify and recommend practicable measures (Best Management Practices – BMPs) to expedite the removal/reduction of smallmouth bass (SMB) in the Upper Colorado River Basin (UCRB) to control their proliferation/invasiveness and their negative impacts/impediments to native fish conservation/endangered fish recovery and to recommend public relations (PR) actions to minimize public opposition to implementing/expanding these control measures. The individual objectives addressed during the Smallmouth Bass (SMB) Summit are listed below, but only a synopsis of some of these is summarized below.

OBJECTIVES:

1. Review SMB ecology in conjunction with what is known about SMB in UCRB to establish SMB role in UCRB river food webs.

2. Review geographic implications of SMB origins and invasiveness in UCRB.

3. Rank threat posed by SMB to native fish preservation and endangered fish recovery.

4. Clarify priority/urgency of SMB removal/reduction strategies and recommend PR elements to support this message.

5. Identify SMB "Achilles Heel" and recommend primary methodology for exploiting this aspect of SMB ecology/vulnerability to expedite and maximize SMB removal/reduction, in conjunction with other measures required to facilitate and perpetuate effectiveness of primary methodology.

6. In absence of defensible or practicable "Achilles Heel" for SMB, identify and rank incremental, additive measures to effect removal/reduction for SMB in UCRB.

7. From search for SMB "Achilles Heel" or identification of incremental/additive measures to advance SMB removal/reduction, recommend Best Management Practices (BMPs) based on present understanding of SMB ecology in UCRB, effectiveness of past and present SMB removal/reduction efforts, adequacy of past

and present SMB stocking, escapement, and harvest policies and practices, and insights from literature or expertise and experiences from elsewhere.

8. Identify bio-political challenges or obstacles to implementing BMPs for SMB removal/reduction and recommend strategies to overcome these with minimal public disapproval.

9. Identify, as needed, experts/expertise from outside of UCRB for invitation to review/advise/guide regarding current information and data interpretations relative to goal of maximizing SMB removal/reduction in UCRB.

10. Provide 0.5 hour summary of SMB Summit discussion and recommendations for deliberation at 12-13 December 2005 Nonnative Fish Management Workshop.

11. Prepare written brief summarizing SMB Summit highlights and recommendations for reference, tracking progress toward SMB removal/reduction and comparison to future findings.

RESULTS and DISCUSSION

Only key highlights of the review of smallmouth bass ecology and formulation of recommendations for their control in the Upper Colorado River Basin are presented. Tables 2 and 3 show the expansion of this species in the Colorado and Yampa Rivers.

ECOLOGY: Temperature

The preferred temperature range for smallmouth bass is 20-26^{o}C (Bevelheimer 1996). In their northern range more growth occurs with warmer water temperatures with the key period for growth being from July to September (Coble 1967). While temperature is important for growth, smallmouth bass tend to achieve their highest relative weights where the water does not reach optimum temperatures (McClendon & Rabeni 1987). Temperature also has a profound effect on the activity level and likely catchability of smallmouth bass by anglers. Smallmouth bass often cease feeding in water temperatures < 13^{o}C (McClendon & Rabeni 1987). Below 10^{o}C, only a few SMB do not hibernate, or lie dormant (Oliver et al. 1979). Smallmouth bass become winter quiescent in water < 8^{o}C (Kolok 1991).

ECOLOGY: Habitat

Smallmouth bass prefer clear water to facilitate feeding (Bevelheimer 1996) and tend to occupy rocky substrate composed of cobble and boulders (Bevelheimer 1996, Lyons 1991, McClendon & Rabeni 1987, Newcomb et al. 1995), but the may utilize all forms of submerged cover (Hubert & Lackey 1980). Smallmouth bass have been shown to exhibit homing behavior to stream reaches (VanArnum et al. 2004), and juveniles and adults will occupy same habitats (Newcomb et al. 1995). Adults typically nest in gravel cobble areas at <1-m depth (Neves 1975) with larger substrate being associated with higher production of free-swimming larvae (Lukas & Orth 1995). In contrast to largemouth bass *Micropterus salmoides*, smallmouth bass juveniles are more vulnerable to predation in vegetation than in cobble, and smallmouth bass feed more among cobble substrates (Olson et al. 2003).

Table 2. Chronology of the relative abundance of smallmouth bass in fish collections made in the Colorado (COR) and Gunnison River (GUR) below Redlands Diversion Dam by various agencies in a variety of habitats using an assortment of sampling methods from 1975-2005. USFWS=U.S. Fish and Wildlife Service; CDOW=Colorado Division of Wildlife; CSU=Colorado State University; LFL=Larval Fish Lab; SWCA=Environmental Consultants. MC=main channel; BA=backwater; SH=shoreline. EL=electrofishing, SN=seine, TN=trapnet.

Agency	River	Year	Habitat	Method	No. fish	No. SMB
USFWS	COR	86-92	MC & BA	EL & SN	27,135	1
USFWS	GUR	92-93	MC & BA	EL & SN	100,617	(1)
CDOW	COR	93-95	MC & BA	EL & SN	45,072	(129)
USFWS	COR	93-97	MC & BA	EL & SN	8,251	1
USFWS	COR	96	GP-PD	EL & TN	2,185	2
CSU-LFL	COR	97-98	BA	SN & EL	108,542	0
USFWS	COR	98-00	GP-PD	SN & TN	16,227	15
SWCA	COR	99-01	BA	SN	207,734	0
USFWS	COR	99-01	BA	EL	27,977	27
CDOW	COR	99-01	MC & BA	EL & SN	29,938	47
CDOW	COR	03	MC	EL	7,864	45
USFWS	COR	03	MC & BA	EL & TN	3,929	318
USFWS	COR	04	MC & BA	EL	2,768	1,508
USFWS	COR	05	MC & BA	EL	3,835	1,875

Table 3. Chronology of the relative abundance of smallmouth bass in fish collections made in the Yampa River (YAR) by various agencies in a variety of habitats using an assortment of sampling methods from 1975-2005. CSU=Colorado State University; BLM=Bureau of Land Management; USFWS=U.S. Fish and Wildlife Service; ISMP=Interagency Standardized Monitoring Program; CDOW=Colorado Division of Wildlife; LFL=Larval Fish Lab. MC=main channel; BA=backwater; SH=shoreline. EL=electrofishing, SN=seine, TN=trapnet

Agency	River	Year	Habitat	Method	No. fish	No. SMB
CSU-BLM	YAR	75-77	MC & BA	EL & SN	21,462	0
USFWS	YAR	81	MC & BA	EL,SN,TN	23,890	2
CSU-CDOW	YAR	81-82	MC & BA	EL & SN	25,798	1
CDOW	YAR	87-91	MC & BA	EL & TN	1,652	3
ISMP	YAR	86-91	MC & BA	EL	204	22
ISMP	YAR	92-97	MC & BA	EL	662	237
CDOW	YAR	95-00	MC	EL	1,757	66
CDOW	YAR	98-99	MC	EL	11,880	1,815
CDOW	YAR	00-01	MC & BA	EL	12,298	1,943
CSU-LFL	YAR	03	MC & BA	EL	2,314	1,135
CDOW	YAR	03-04	MC	EL	9,977	4,020
CSU-LFL	YAR	04	SH & BA	SN & EL	3,937	1,535
CSU-LFL	YAR	04	MS & BA	EL	6,251	5,231
CSU-LFL	YAR	05	MS & BA	EL	6,740	5,240

ECOLOGY: Reproduction

Differences in age at maturation between populations of smallmouth bass are due to differences in growth rate (Dunlop et al. 2005). Smallmouth bass males provide paternal care and extended nest guarding in spring & early summer (Dunlop et al. 2005). Males are solitary nesters and the nesting period may span 7 weeks (Knotek & Orth 1998). Larger, older males spawn earlier in the breeding season than smaller, younger males (Baylis et al. 1993). Larger males also receive more egg in their nests from similar sized females, maintain larger broods and contribute a larger proportion of the total number of juveniles reared (Knotek & Orth 1998). Earlier spawning is often unsuccessful due to slow hatching, increased exposure to predation and flow fluctuation, but larger males succeed by repeatedly re-nesting after failures, plus egg development being accelerated at higher temps (Lukas & Orth 1995). Males caught by anglers and played to exhaustion resulted in nests being exposed to more predation risk (Kieffer et al. 1995).

ECOLOGY: Feeding

Smallmouth bass are more active predators than largemouth bass or other ambush predators and tend to prefer comparatively deeper colder waters where they can search for and pursue their prey (Furimsky 2003, Zorn & Seelbach 1995). While smallmouth bass may prey heavily on crayfish (Roell & Orth 1993, Weidel et al. 2000), their diet often includes both small fish and crayfish (Bevelheimer 1996, Fritts & Pearsons 2004). Smallmouth bass tend to grow faster if they eat more fish vs. invertebrates (Hanson & Curry 2005). Crayfish are generally unavailable (too big) as prey for smallmouth bass young-of-year (DeAngelis et al. 1991), but crayfish <14 mm carapace length are also unavailable as prey for smallmouth bass (Roell & Orth 1993). Smallmouth bass < 80 mm total length do not prey on crayfish (Robertson & Winemiller 2001). Smallmouth bass prefer medium-sized crayfish, 32-40 mm TL, which are more vulnerable and provide best return on energy expenditures in searching, attacking and handling (Probst et al. 1984). However, in some instances, smallmouth bass will prey most heavily on fish even if crayfish are abundant (Fayram & Sibley 1997). However, the relative weights of smallmouth bass tend to be higher where crayfish densities are higher (McClendon & Rabeni 1987). On a caloric basis, importance of the prey of smallmouth bass is ranked as crayfish > fish > invertebrates (Probst et al. 1984). Smallmouth bass as small as 13-mm total length begin to prey on fish; their elongate body shape adapts them for fast powerful swimming and is ideal for a piscivorous predator that tend to feed on the largest items that can be ingested (George & Hadley 1979). Many young-of-year smallmouth bass consume fish and smallmouth bass 52-62 mm in total length (Robertson & Winemiller 2001). The predacious nature of smallmouth bass may affect the behavior and habitat selection of their prey (Schlosser 1988).

ECOLOGY: Age and Growth

Longer age-0 smallmouth bass survive winter better than shorter ones (Oliver et al. 1979). Young-of-year must reach sufficient size to escape winter starvation

(DeAngelis et al. 1991). Low food availability, intraspecific competition for prey and lack of large prey contributes to slow growth, increased reproductive investment and higher mortality following reproduction (Dunlop et al. 2005). Despite these constraints, waters such as Flaming Gorge Reservoir, which has among the shortest growing seasons of any self-sustaining smallmouth bass populations and slowest growth rate of any lentic population (Mullner & Hubert 1993), maintains a fishery of interest to anglers. Smallmouth bass are long-lived, perhaps reaching 18 years old, but the maximum age attained by the species appears to be about 15 years (Coble 1975). Smallmouth bass that grow more slowly tend to live longer than those in their southern range or at high temperatures, which tend to die sooner (Mullner & Hubert 1993). Population compensation by smallmouth bass in growth or mortality appears unlikely in populations with low productivity (Beamesderfer & North 1995).

THREAT: Smallmouth Bass

Smallmouth bass are known to pose serious threat to native fish faunas (Iguchi et al. 2004). They impact native fishes by competition for food resources and predation (Weidel et al. 2000) which reduce the abundance of small-bodied fish (MacRae & Jackson 2000). Smallmouth bass have also been shown to prey on salmonids (Fritts & Pearsons 2004, Weidel et al. 2000). Smallmouth bass are ecologically similar to Sacremento pikeminnow *Ptycocheilus grandi* and they have replaced other native fishes in California streams (Gard 2004). There is concern that resource actions such as habitat alterations, flow maipulations, or temperature modifications, even if intended to enhance salmonid abundance, could indirectly diminish salmonid abundance by enhancing the functional or numerical response of smallmouth bass functioning in streams as predators (Fritts & Pearsons). Factors favoring invasive smallmouth bass include their small size at the onset of piscivory, juvenile use of cover and low overlap with other predators (Gard 2004).

Nesler and Hawkins (1991) surveyed fishery biologists and provided a preliminary ranking of the ecological threat posed to endangered fishes of the Colorado River Basin by nonnative fishes. At that time, smallmouth bass was the last species ranking at the tenth level of this survey. This historic perspective was discussed in relation to the current knowledge and demonstrated invasiveness of smallmouth bass in the Upper Colorado River Basin. This discussion led to the participants at the Summit to recommend a number one ranking for smallmouth bass due to the explosive nature of their numbers and the ongoing expansion of their range.

CONTROL: "Achilles Heel"

Effort to identify an approach to maximize the control of invasive smallmouth bass in rivers of the Upper Colorado River Basin was deemed the search for an "Achilles Heel". It was noted in the literature that nesting male smallmouth bass caught by anglers and played to exhaustion exposed nests to more predation risk (Kieffer et al. 1995). In one study, all cases of male removal from nests resulted in predation by other fishes, which destroyed eggs & fry, and it was noted that the larger males produced more fry

(Neves 1975). Further, a significant positive relationship between male mating success and male size has been demonstrated. Nest abandonment is > 50% when return times of males to nest is > 5 minutes. The nests of both smallmouth and largemouth bass are often visible to anglers, the males of both species are aggressive during nesting and both are highly vulnerable to angling during spawning season. Male smallmouth bass are more vulnerable to angling than male largemouth bass and their nests are more easily detected because they tend to be closer to shore, located on exposed gravel (Phillip et al. 1997). Female smallmouth bass choose to spawn with the largest males and males mated to larger, more fertile females produce more fry. Females also prefer to nest with males having nests composed of rock and placed closer to shore (Wiegmann et al. 1992). Large smallmouth bass males with the largest broods defend those broods most aggressively, have the greatest mating success and are the most vulnerable to angling, and as a consequence are the fish most important to reproductive success of the population (Philipp et al. 1997). Pre-season catch and release of male smallmouth bass, and especially catch and harvest, of nesting males, would have negative effect on production of smallmouth bass at the population level (Philipp et al. 1997). These observations suggested that targeting nesting smallmouth bass in optimum, rocky habitats and removing the largest fish would likely contribute to population reductions.

CONTROL: Incremental Strategies

An important means of conserving native biodiversity is to prevent invasion of alien species. Once established, alien species are generally impossible to eliminate completely. Early detection may allow eradication (Iguchi et al. 2004), but minimizing the access of invasive species into habitats and populations where they can become problematic is highly advisable. Possible methods of reducing smallmouth bass include direct removal by electrofishing or trapping, bounty programs, regulation changes, decreasing water temperatures (via irrigation system & reservoir releases) and disruption of spawning by manipulating flows (Fritts & Pearsons 2004). Further, high flows which produce increased water velocities have been shown to be responsible for smallmouth bass nesting failures (Lukas & Orth 1995). Abrupt water level drops also lower nesting success (Neves 1975). Discharge during the spawning/rearing period had greater effect on adult density and fishing yield than did spawning/rearing temperature or winter discharge (Peterson & Kwak 1999). Small stream populations of smallmouth bass are depletable in 3-5 runs of tandem electrofishing boats (Odenkirk & Smith 2005). Based on these observations in the literature, control efforts for smallmouth bass in the Upper Colorado River Basin should include limiting the number of reservoirs containing this species, controlling their escapement from reservoirs into rivers, experimenting, where feasible, with flow manipulations to thwart their nesting success, and testing the use of tandem electrofishing with multiple boats and passes in key habitats.

CONTROL: Biopolitical Challenges

Smallmouth bass and largemouth bass become top predators, but they also become economically important sport fish in lakes and rivers in U.S. (Olson & Young 2003), which can lead to opposition of their removal and control (Martinez 2005b).

Efforts to relocate smallmouth bass from rivers to ponds or reservoirs to offset perceived reductions in fishing opportunity must consider that smallmouth bass are highly susceptible to hypoxia (more sensitive than largemouth bass) and extra care is required for handling during translocation (Furimsky et al. 2003). In addition, smallmouth bass are more susceptible to decompression than largemouth bass due to their tendency to occupy deeper water (>5m), and this can aggravate their susceptibility to hypoxia (Morrissey et al. 2005). Handling of smallmouth bass at low (12^{o} C) or high water (20^{o} C) temperatures is more detrimental than handling at 16^{o} C (Schreer et al. 2001). Obviously, excessive mortality of smallmouth bass during translocation is a potential risk that could become a public relations problem.

The Yampa River channel appears to be suffering from widening due to land use (Anderson 2005). It is speculated that expanded water depletions could result in a warmer thermograph, a condition exacerbated by channel widening, thus further favoring warmwater nonnative species including smallmouth bass, northern pike, channel catfish *Ictalurus punctatus* and verile crayfish *Orconectes virilis*. The projected capacity of smallmouth bass predation to exceed the standing crop plus the annual production by small-bodied fishes in some reaches (discussed later in this report) could result in replacement of native fishes by smallmouth bass in the Yampa River.

SUMMARY: Recommendations

A PowerPoint summary of the Smallmouth Bass Summit was presented at the Nonnative Fish Control Workshop held in Grand Junction, 12-13 December 2006. Appendix C contains this presentation which provides the recommendations produced at the Summit. In terms of priority, the participants indicated that policy changes were most necessary to facilitate control of smallmouth bass in the Upper Colorado River Basin.

OBJECTIVE 2. TROPHIC AND BIOENERGETICS INVESTIGATIONS FOR WARMWATER FISH MANAGEMENT
To improve/identify methods to evaluate/pin-point sources, species, life-stages of nonnative fishes that are most problematic to facilitate efficient control of nonnative fish, to protect/recover native fish and to facilitate continued or expanded stocking/translocation/management of warmwater sport fish on Colorado’s western slope.

Segment Objective 1: Facilitate and participate in Recovery Program Scope-of-Work: Stable Isotope Analysis of Centrarchid Concentration Areas.

INTRODUCTION, METHODS and DISCUSSION

Martinez (2004b) provided the background and impetus for this Segment Objective, which was funded primarily by the Recovery Program for the endangered fishes in the Upper Colorado River Basin. The basic framework of this investigation was to determine if the nonnative centrarchids, largemouth bass, black crappie, bluegill and green sunfish, that occur in backwaters of the upper Colorado River within the Grand Valley originate primarily from off-stem sources such as floodplain ponds, or if these centrarchids are the result of in-river reproduction and recruitment. This distinction in the origins of these nonnative fishes was to be determined by the use of otolith microchemistry (Martinez 2005).

The annual report submitted to the Recovery Program (Appendix D) contains a summary of the projects finding and resulting recommendations. A draft final report was submitted to the Recovery Program on 12 April 2006. The research from this project produced two manuscripts for submission to peer-review outlets. These include Whitledge et al. 2006 and G. W. Whitledge, B. M. Johnson, P. J. Martinez and A. Martinez IN REVIEW. Provenance of non-native fishes in the upper Colorado River revealed by stable isotope and microchemical analyses of otoliths. Transactions of the American Fisheries Society.

Segment Objective 2: Prepare draft manuscript for bioenergetics evaluation (diet composition, age structure - otoliths) of smallmouth bass in the Yampa River, including subsampling for stable isotope analyses.

INTRODUCTION, METHODS and DISCUSSION

Based on collaborative work with Colorado State University (CSU), Martinez (2003b, 2004b and 2005b) has reported on the progress toward identifying and quantifying the food web role of smallmouth bass in the Yampa River. This data set, incorporating demographics, age and growth, gut contents, and stable isotope analyses for smallmouth bass has been assembled into the draft manuscript entitled: Smallmouth bass the primary predatory threat to recovery of the native fish assemblage of the Yampa River, Colorado. The draft, co-authored with Dr.'s Brett Johnson and Kevin Bestgen, and John Hawkins of CSU, is nearing completion, and submission to a peer-reviewed outlet is expected by summer 2006. Appendix E contains a portion of the information that will be included in this manuscript.

Segment Objective 3: Explore origins of nonnative smallmouth bass in the Colorado River in the Grand Valley via laser ablation, trace element and stable isotope techniques using otoliths.

INTRODUCTION

Given the rapid expansion in both abundance and range of smallmouth bass in the Colorado River, microchemical techniques for water and otoliths have been explored to attempt to identify the potential source(s) of smallmouth bass that abruptly populated the river. While the rapid expansion of smallmouth bass and the presence of young-of-year fish indicate reproduction in the river, the presence of larger fish in collections suggests that older fish appeared in the river prior to the population being noticed at its present density. This suggests that escapement or emigration from an established population may have contributed to a "critical mass" of adult fish capable of reproducing en masse and accounting for the seemingly sudden explosion of smallmouth bass in the Colorado River (Martinez 2005b).

METHODS and MATERIALS

Martinez (2005b) described the collection of water and otolith samples gathered to commence this study, including samples from Lake Powell, UT, Rifle Gap Reservoir and the Colorado and Yampa Rivers. Appendix C describes how these samples were processed and analyzed. As single smallmouth bass was collected from Rifle Gap in 2004 by angling since the reservoir's water level was too low to allow access with an electrofishing boat. Electrofishing was performed on 19 April 2005 in Rifle Gap to obtain additional smallmouth bass otoliths. A total of 22 smallmouth bass ranging from 112 to 379 mm in total length were collected and dissected to remove their otoliths. These otoliths were sectioned in my office and 15 of them were subjected to microchemical analysis by Dan Gibson-Reinemer, M.S. Candidate, Colorado State University.

RESULTS and DISCUSSION

Appendix E provides a summary of some otolith microchemistry analyses performed to assess the potential origins of smallmouth bass in the Colorado River in Colorado. At this point, the analysis excludes Lake Powell as the source of any of the fish analyzed from the Colorado River. Appendix F contains more recent work incorporating smallmouth bass captured in Rifle Gap Reservoir in April 2005. Preliminarily, the results suggest that Rifle Gap fish can be distinguished microchemically from the other sites sampled. This analysis will receive more in depth attention as part of the reservoir fingerprinting research funded by the Recovery Program (Appendix B).

Segment Objective 4: Continue to establish data set needed for bioenergetics evaluation of piscivory by smallmouth bass and channel catfish within critical habitat for endangered fishes in the Colorado River.

INTRODUCTION

The projected piscivory by channel catfish, northern pike and smallmouth bass in the Yampa River (Martinez 2005b) provided insight and impetus to estimate the piscivory and food web role of channel catfish and smallmouth bass in the Colorado River. The work on the Yampa suggested that channel catfish ate comparatively few fish, but their piscivory could be substantial in river reaches where they are abundant. Northern pike, rare in the Colorado River, are virtually obligate piscivores, and while their comparatively low density may lessen their potential predation demand, their large size allows them to consume adult native fish, including endangered Colorado pikeminnow. Smallmouth bass, due to their abundance, can exert heavy predation demand in riverine food webs, and if their diet is dominated by small-bodied fish, this can have profound negative implication for native fishes.

In 2004, the Recovery Program shifted from channel catfish removal to focus primarily on smallmouth bass removal in the Colorado and Yampa Rivers. Martinez (2005b) described the effort to assess the food web roles of channel catfish and smallmouth bass in the Colorado River by cooperatively obtaining samples of these species from the U.S. Fish and Wildlife Service (USFWS – Bob Burdick) during their smallmouth bass removal efforts, and funding Dr. Brett Johnson's lab to process stomach samples from these fish.

METHODS and MATERIALS

All centrarchids, 3,754, collected by the USFWS from July to August in 2005 were provided to me, including 1,875 smallmouth bass (Table 2). Some fish were processed when we received them, but most were frozen in a walk-in freezer that was purchased to accommodate storage of nonnative fish samples from various nonnative fish control projects so that these fish can be subsampled in the future for otoliths, stomachs or muscle tissue for stable isotope analyses, and date or location of capture.

In August 2005, we accompanied the USFWS during their smallmouth bass removal and collected 105 channel catfish which were measured for total length to the nearest mm and weighed to the nearest 5g, prior to being frozen. These fish were later thawed and dissected to remove otoliths, stomachs and muscle tissue for stable isotope analyses. Otoliths were sectioned and aged in my office. Stomachs and muscle samples were sent to CSU for processing and analyses.

RESULTS and DISCUSSION

Some age and growth and diet data has been presented for smallmouth bass from the Colorado River (Martinez 2005b, Appendix C, E and G). The data from 2005 will be incorporated into these preliminary analyses and finalized along with data 2006, in the 2007 report. Preliminary diet analysis results for channel catfish from the Yampa River are shown in Appendix G. The aging of otoliths for 2004 and 2005 has been completed, but it will be summarized with the 2006 samples in the 2007 report.

Segment Objective 5: Confirm age and growth of channel catfish and northern pike in the Yampa River via otoliths/cleithra.

INTRODUCTION, METHODS and DISCUSSION

Martinez (2005b) reported bioenergetics projections of predation demand in the Yampa River for channel catfish, northern pike and smallmouth bass. The age and growth for smallmouth bass was determined from otoliths from my current research, but historic age and growth data used relied upon fin spines for channel catfish (Tyus and Nikirk 1990) and scales for northern pike (Martinez 1994). It now appears most suitable to use otoliths to determine ages of channel catfish (Buckmeier et al. 2002) and while scales can be relied upon to provide accurate age for young northern pike, cleithra are more accurate for fish older than age 10 (Laine et al.1991).

I obtained about 40 specimens each of both channel catfish and northern pike from the sampling efforts of Lori Martin (CDOW), John Hawkins (CSU) and Sam Finney (USFWS). These samples have been processed, but the data sets will be incorporated with the results of samples taken and processed in 2006. This updated data set for 2005-2006 will be summarized in the 2007 report.

Segment Objective 6: Begin monitoring of verile crayfish *Orconectes virilis* in the Yampa River, estimating their size structure and density.

INTRODUCTION

Martinez (2003b) discussed the possibility that the larger smallmouth bass in the Yampa River may begin to lose weight given the severely depleted small-bodied fish component in the river that coincided with the increase in smallmouth bass around 1999-2000. However, despite this reduction in this prey source, smallmouth bass in the Yampa, particularly the larger ones, appear to have maintained high relative weights. An apparent explosion of nonnative verile crayfish *Orconectes virilis* appears to have coincided with the onset of drought conditions in 2001. Crayfish were not reported in a survey of macro-invertebrates in the Yampa River in 1975-1976 (Carlson et al. 1979). While crayfish densities had not been previously quantified in the Yampa, most fishery workers on the River during late 1990s-2000’s anecdotally reported the same perception about a massive increase in crayfish abundance.

Given the propensity for smallmouth bass to exploit crayfish as a primary food item, I wanted to estimate the density of crayfish in the Yampa River to determine if they could be responsible for the maintenance of high relative weights among smallmouth bass. I also wanted to estimate the standing stock and annual production of crayfish for comparison to these parameters in the fish population. Last, updating the diet composition for channel catfish and northern pike would reveal if crayfish were contributing more to the diet of these species compared to past data-sets.

METHODS and MATERIALS

Appendix G outlines the development and use of the 1-m^2 quadrat method to estimate crayfish density in the Yampa River at three stations in late summer. Crayfish carapace length (CL) was measured to the nearest mm and weights were measured to the nearest gram to facilitate estimating the biomass of the standing crop. Appendix F also illustrates the method used to expand the crayfish densities derived by weighting their abundance by the proportion of habitat types and the subsequent calculation of their density and biomass per square meter and for the population in a selected portion of the river.

RESULTS and DISCUSSION

Appendix F shows an estimate of 6.7 verile crayfish/ m^2 averaging 17.2 mmCL and representing about 9g/m^2 in the middle Yampa River from rivermile 45-125. These estimates translate into an annual biomass combining standing crop and production estimates for crayfish (Huryn and Wallace 1987) that rival or exceed those for the fish population (Appendix G). Based on bioenergetics response of verile crayfish to temperature (Whitledge and Rabeni 2003), it is theorized that the apparent explosion of this species in the Yampa River may have been accelerated and exacerbated by warmer water temperatures during the recent drought (Martinez 2005).

Comparisons of historic diet data for channel catfish (Tyus and Nikirk 1990) and northern pike (Nesler 1995) indicate an increased utilization of crayfish by these species in 2005, especially for channel catfish (Appendix G). The diet of smallmouth bass in the Yampa River is dominated by verile crayfish and food web mapping using stable isotopes verifies this observation (Appendix G). While there is considerable concern about the smallmouth bass predation demand in the Yampa River food web, the density of verile crayfish may also be of concern. Carpenter (2005) demonstrated the potential for competition of verile crayfish with suckers and chubs native to the Colorado River Basin. Due to this and other evidence indicating that nonnative crayfish can have severe negative effects on native fishes, it is recommended that policies be undertaken to restrict introduction and intentional or illicit transplants of crayfish. It is also recommended that crayfish continue to be studied in the Yampa River to see if their population responds to cooler water temperature resulting from a normal to above-normal level of runoff.

LITERATURE CITED

Anderson, R. A. 2005. Riverine fish flow investigations. Federal Aid in Fish and Wildlife Restoration Project F-288-R8, Progress Report. Colorado Division of Wildlife, Fort Collins.

Baylis, J. R., D. D. Wiegmann and M. H. Hoff. 1993. Alternating life histories of smallmouth bass. Transactions of the American Fisheries Society 122: 500–510.

Beamesderfer, R. C. P., and J. A. North. 1995. Growth, natural mortality, and predicted response to fishing for largemouth and smallmouth bass populations in North America. North American Journal of Fisheries Management 15:688-704.

Bevelhimer, M. S. 1996. Relative importance of temperature, food, and physical structure to habitat choice by smallmouth bass in laboratory experiments. Transactions of the American Fisheries Society 125:274-283.

Buckmeier, D. L., E. R. Irwin, R. K. Betsill and J. A. Prentice. 2002. Validity of otoliths and pectoral spines for estimating ages of channel catfish. North American Journal of Fisheries Management 22:934-942.

Carlson,C. A., C. G. Prewitt, D. E. Snyder, E. J. Wick, E. L. Ames and D. W Fronk. 1979. Fishes and macroinvertebrates of the White and Yampa Rivers. U.S. Bureau of Land Management, Biological Series 1:1-276.

Carpenter, J. 2005. Competition for food between an introduced crayfish and two fishes endemic to the Colorado River basin. Environmental Biology of Fishes 72:335-342.

Coble, D. W. 1967. Relationship of temperature to total annual growth in adult smallmouth bass. Journal of the Fisheries Research Board of Canada 24:87-99.

Coble, D. W. 1975. Smallmouth bass. Pages 21-33 *in* R.H. Stroud and H. Clepper, editors. Black Bass Biology and Management, Sport Fishing Institute, Washington, D. C.

Cooke, S. J., D. P. Phillipp, J. F. Schreer and S. McKinley. 2000. Locomotory impairment of nesting male largemouth bass following catch-and-release angling. North American Journal of Fisheries Management 20:986-977.

DeAngelis, D.L., Godbout, L. and Shuter, B.J. 1991. An individual-based approach for predicting density-dependent dynamics in smallmouth bass populations. Ecological Modelling. 57:91-115.

Dunlop, E. S., J. A. Orendorff, B. J. Shuter, F. H. Rodd and M. S. Ridgeway. 2005. Diet and divergence of introduced smallmouth bass (*Micropterus dolomieu*) populations. Canadian Journal of Fisheries and Aquatic Sciences 62:1720-1732.

Dunlop, E. S., B. J. Shuter and M. S. Ridgeway. 2005. Isolating the influence of growth rate on maturation patterns in the smallmouth bass (*Micropterus dolomieu*). Canadian Journal of Fisheries and Aquatic Sciences 62:844-853.

Fayram, A. H., and T. H. Sibley. 2000. Impact of predation by smallmouth bass on sockeye salmon in Lake Washington, Washington. North American Journal of Fisheries Management 20:81-89.

Fritts, A. L., and T. N. Pearsons. 2004. Smallmouth bass predation on hatchery and wild salmonids in the Yakima River, Washington. Transactions of the American Fisheries Society 133:880-895.

Furimsky, M., S. J. Cooke, C. D. Suski, Wang, Y. and B. L. Tufts. 2003. Respiratory and circulatory responses to hypoxia in largemouth and smallmouth bass: implications for "live-release" angling tournaments. Transactions of the American Fisheries Society 132:1065-1075.

Gard, F. M. 2004. Interactions between an introduced piscivore and a native piscivore in a California stream. Environmental Biology of Fishes 71:287-295.

Gibson-Reinemer, D., B. Johnson, D. Winkelman and P. Martinez. 2005. Forensic applications of otolith microchemistry: tracking sources of stocked rainbow trout in CO. Whirling Disease Initiative, Montana Water Center, MSU. 26 p.

George, E. L., and W. F. Hadley. 1979. Food and habitat partitioning between rock bass (*Ambloplites rupestris*) and smallmouth bass (*Micropterus dolomieui*) young of the year. Transactions of the American Fisheries Society 108:253-261.

Hanson, S. D., and R. A. Curry. 2005. Effects of size structure on trophic interactions between age-0 smallmouth bass and juvenile anadromous alewives. Transactions of the American Fisheries Society 134:356-368.

Hawkins, J. A., and T. P. Nesler. 1991. Nonnative fishes of the upper Colorado River basin: an issue paper. Final Report. Larval Fish Laboratory, Colorado State University, Fort Collins. 72 p.

Hinch, S. G., and N. C. Collins. 1991. Importance of diurnal and nocturnal nest defense in the energy budget of male smallmouth bass: insights from direct video observations. Transactions of the American Fisheries Society 120:657-663.

Horning, W. B., II, and R. E. Pearson. 1973. Growth temperature requirements and lower lethal temperatures for juvenile smallmouth bass (*Micropterus dolomieui*). Journal of the Fisheries Research Board of Canada 30:1226-1230.

Hubert, W. A. 1988. Altitude as a determinant of distribution of largemouth bass and smallmouth bass in Wyoming. North American Journal of Fisheries Management 8:386-387.

Hubert, W. A., and R. T. Lackey. 1980. Habitat of adult smallmouth bass in a Tennessee River reservoir. Transactions of the American Fisheries Society 109:364-370.

Huryn, A. D., and J. B. Wallace. 1987. Production and litter processing by crayfish in an Appalachain mountain stream. Freshwater Biology 18:277-286.

Iguchi, K., K. Matsura, K. M. McNyset, A. T. Peterson, R. Scachetti-Pereira, K. A. Powers, D. A. Veiglais, E. O. Wiley and T. Yodo. 2004. Predicting invasions of North American basses in Japan using native range data and a genetic algorithm. Transactions of the American Fisheries Society 133:845-854.

Johnson, M. G., J. H. Leach, C. K. Minns and C. H. Oliver. 1977. Limnological characteristics of Ontario lakes in relation to associations of walleye (*Stizostedion vitreum*), northern pike (*Esox lucius*), lake trout, (*Salvelinus namaycush*), and smallmouth bass (*Micropterus dolomieui*). Journal of the Fisheries Research Board of Canada 34:1592-1601.

Kieffer, J. D., M. R. Kubacki, F. J. S. Phelan, D. P. Philipp and B. L. Tufts. 1995. Effects of catch-and-release angling on nesting male smallmouth bass. Transactions of the American Fisheries Society 124:70-76.

Knotek, W. L., D. J. Orth. 1998. Survival for specific life intervals of smallmouth bass, *Micropterus dolomieui*, during parental care. Environmental Biology of Fishes 51:285-296.

Koel, T. M., P. E. Bigelow, P. D. Doepke, B. D. Ertel and D. L. Mahoney. 2005. Nonnative lake trout result in Yellowstone cutthroat trout decline and impacts to bears and anglers. Fisheries 30:10-19.

Kolok, A. S. 1991. Temperature compensation in two centrarchid fishes: do winter-quiescent fish undergo cellular temperature compensation. Transactions of the American Fisheries Society120:52–57

Laine, A. O., W. T. Momot and P. A. Ryan. 1991. Accuracy of using scales and cleithra for aging northern pike from an oligotrophic Ontario lake. Northe American Journal of Fisheries Management 11:220-225.

Long, J. M., and W. L. Fisher. 2005. Distribution and abundance of black bass in Skiatook Lake, Oklahoma, after introduction of smallmouth bass and a liberalized harvest regulation on spotted bass. North American Journal of Fisheries Management 25:49-56.

Lukas, J. A., and D. J. Orth. 1995. Factors affecting the nesting success of smallmouth bass in a regulated Virginia stream. Transactions of the American Fisheries Society 124:726-735.

Lyons, J. 1991. Influence of winter starvation on the distribution of smallmouth bass among Wisconsin streams: a bioenergetics modeling assessment. Transactions of the American Fisheries Society 126:157-162.

MacRae, P. S. D., and D. A. Jackson. The influence of smallmouth bass (Micropterus dolomieu) predation and habitat complexity on the structure of littoral zone fish assemblages. Canadian Journal of Fisheries and Aquatic Sciences 58:342-351.

Martinez, P. J. 2004. Westslope warmwater fisheries. Great Outdoors Colorado, Progress Report. Colorado Division of Wildlife, Fort Collins. 101 pp.

Martinez, P. J. 1994. Coldwater reservoir ecology. Colorado Division of Wildlife, Federal Aid in Fish and Wildlife Restoration Project #F-85, Job Progress Report, Fort Collins. 155 p.

Martinez, P. J. 2003a. Coldwater reservoir ecology. Colorado Division of Wildlife, Federal Aid in Fish and Wildlife Restoration Project #F-242-R10, Progress Report, Fort Collins. 104 p.

Martinez, P. J. 2003b. Westslope warmwater fisheries. Federal Aid in Fish and Wildlife Restoration, Progress Report. Colorado Division of Wildlife, Fort Collins. 106 pp.

Martinez, P. J. 2004a. Coldwater reservoir ecology. Federal Aid in Fish and Wildlife Restoration Project F-242-R11 Progress Report. Colorado Division of Wildlife, Fort Collins. 122 pp.

Martinez, P. J. 2004b. Westslope warmwater fisheries. Great Outdoors Colorado Job Progress Report. Colorado Division of Wildlife, Fort Collins. 101 pp.

Martinez, P. J. 2005a. Coldwater reservoir ecology. Federal Aid in Fish and Wildlife Restoration Project F-242-R12 Progress Report. Colorado Division of Wildlife, Fort Collins. 148 pp.

Martinez, P. J. 2005b. Westslope warmwater fisheries. Great Outdoors Colorado Job Progress Report. Colorado Division of Wildlife, Fort Collins. 121 pp.

Martinez, P. J., and B. M. Johnson. 2006. Chemically fingerprinting nonnative fishes in reservoirs. UCRB Recovery Program Project C18/19 Scope of Work. 12 p.

Martinez, P. J., B. M. Johnson & J. D. Hobgood. 2001. Stable isotope signatures of native and nonnative fishes in upper Colorado River backwaters and ponds. The Southwest Naturalist 46:311-322.

McClendon, D. D. and C. F. Rabeni. 1987. Physical and biological variables useful for predicting population characteristics of smallmouth bass and rock bass in an Ozark stream. North American Journal of Fisheries Management 7:46-56.

Morrissey, M. B., C. D. Suski, K. R. Esseltine and B. L. Tufts. 2005. Incidence and physiological consequences of decompression in smallmouth bass after live-release angling tournaments. Transactions of the American Fisheries Society 134:1038-1047.

Mullner, S. A., and W. A. Hubert. 1993. Growth of smallmouth bass (Micropterus dolomieu) in Flaming Gorge reservoir, Wyoming-Utah. Great Basin Naturalist 53:180-185.

Nesler, T. P. 1995. Interactions between endangered fishes and introduced gamefishes in the Yampa River, Colorado, 1987-1991. Colorado River Recovery Implementation Program Project No. 91-29, Final Report. Federal Aid Project SE-3. Colorado Division of Wildlife, Fort Collins. 184 p.

Neves, R. J. 1975. Factors affecting fry production of smallmouth bass (Micropterus dolomieui) in South Branch Lake, Maine. Transactions of the American Fisheries Society 104:83-87.

Newcomb, T. J., S. A. Perry and W. B. Perry. 1995. Comparison of habitat suitability criteria for smallmouth bass (*Micropterus dolomieu*) from three West Virginia rivers. Rivers 5:170-183.

Odenkirk, J., and S. Smith. 2005. Single- versus multiple-pass boat electrofishing for assessing smallmouth bass populations in Virginia rivers. North American Journal of Fisheries Management 25:717-724.

Oliver, J. D., G. F. Holeton and K. E. Chua. 1979. Overwinter mortality of fingerling smallmouth bass in relation to size, relative energy stores, and environmental temperature. Transactions of the American Fisheries Society 108:130-136.

Olson, M. H., and B. P. Young. 2003. Patterns of diet and growth in co-occurring populations of largemouth and smallmouth bass. Transactions of the American Fisheries Society 132:1207-1213.

Olson, M. H., B. P. Young and K. D. Blinkoff. 2003. Mechanisms underlying habitat use of juvenile largemouth bass and smallmouth bass. Transactions of the American Fisheries Society 132:398-405.

Patton, T. M., and W. A. Hubert. 1996. Water temperature affects smallmouth bass and channel catfish in a tailwater stream on the Great Plains. North American Journal of Fisheries Management 16:124-131.

Petersen, J. T., and T. J. Kwak. Modeling the effects of land use and climate change on riverine smallmouth bass. Ecological Applications 9:1391-1404.

Pflieger, W .L. 1966. Reproduction of smallmouth bass (*Micropterus dolomieui*) in a small Ozark stream. American Midland Naturalist 76:410-418.

Philipp, D. P., C. A. Toline, M. F. Kubacki and D. B. F. Philipp. 1997. The impact of catch-and-release angling on the reproductive success of smallmouth bass and largemouth bass. North American Journal of Fisheries Management 17:557-567.

Probst, W. E., C. F. Rabeni, W. G. Covington and R. E. Marteney. 1984. Resource use by rock bass and smallmouth bass. Transactions of the American Fisheries Society. 113:283-294.

Raffetto, N. S., J. R. Baylis. And S. L. Serns. 1990. Complete estimates of reproductive success in a closed population of smallmouth bass (Micropterus dolomieui). Ecology 71:1523-1535.

Rankin, E. T. 1986. Habitat selection by smallmouth bass in response to physical characteristics in a natural stream. Transactions of the American Fisheries Society 115:322-334.

Reed, M. S., M. B. Bain and K. C. Weathers. 1991. A quality smallmouth bass fishery: trophies and voluntary catch and release. Pages 151-157 in D. C. Jackson, editor, First International Smallmouth Bass Symposium. Mississippi Agriculture and Forestry Experiment Station, Mississippi State University, Mississippi State.

Ridgeway, M. S. 1988. Developmental stage of offspring and brood defense in smallmouth bass (*Micropterus dolomieui*).

Ridgway, M. S., T. G. Friesen. 1992. Annual variation in parental care in smallmouth bass, *Micropterus dolomieui*. Environmental Biology of Fishes 35:243-255.

Ridgway, M. S., and B. J. Shuter. 1997. Predicting the effects of angling for nesting male smallmouth bass on production of age-0 fish with an individual-based model. North American Journal of Fisheries Management 17:568-580.

Rieman, B. E., R. C. Beamesderfer, S. Vigg and T. P. Poe. 1991. Estimated loss of juvenile salmonids to predation by northern squawfish, walleyes, and smallmouth bass in John Day Reservoir, Columbia River. Transactions of the American Fisheries Society 120:448-458.

Robertson, M. S., and K. O. Winemiller. 2001. Diet and growth of smallmouth bass in the Devils River, Texas. The Southwestern Naturalist 46:216-221.

Roell, M. J., and D. J. Orth. 1993. Trophic basis of production of stream-dwelling smallmouth bass, rock bass, and flathead catfish in relation to invertebrate bait harvest. Transactions of the American Fisheries Society 122:46-62.

Schlosser, I. J. 1988. Predation rates and the behavioral response of adult brassy minnows (*Hybognathus hankinsoni*) to creek chub and smallmouth bass predators. Copeia 1988:691-697.

Schramm, H. L., P. E. McKeown and D. M. Green. 1995. Managing black bass in northern waters: summary of workshop. North American Journal of Fisheries Management 15:671-679.

Schreer, J. F., S. J. Cooke and R. S. McKinley. 2001. Cardiac response to variable forced exercise at different temperatures: and angling simulation for smallmouth bass. Transactions of the American Fisheries Society 130:783-795.

Serns, S. L. 1982. Relation of temperature and population density to first-year recruitment and growth of smallmouth bass in a Wisconsin lake. Transactions of the American Fisheries Society 111:570-574.

Slipke, J. W., M. J. Maceina, V. H. Travnichek and K. C. Weathers. Effects of a minimum length limit on the population characteristics and sport fishery of smallmouth bass in the shoals reach of the Tennessee River, Alabama. North American Journal of Fisheries Management 18:76-84.

Simonson, T. D., and W. A. Swenson. 1990. Critical stream velocities for young-ofyear smallmouth bass in relation to habitat use. Transactions of the American Fisheries Society 119:902-909.

Shuter, B. J., J. A. MacLean. F. E. J. Fry and H. A. Regier. 1980. Stochastic simulation of temperature effects on first-year survival of smallmouth bass. Transactions of the American Fisheries Society 109:1-34.

Shuter, B. J., D. A. Wismer, H. A. Regier and J. E. Matuszek. 1985. An application of ecological modeling: impact of thermal effluent on a smallmouth bass population. Transactions of the American Fisheries Society 114:631-651.

Smith, S. M., J. S. Odenkirk and S. J. Reeser. 2005. Smallmouth bass recruitment variability and its relation to stream discharge in three Virginia rivers. North American Journal of Fisheries Management 25:1112-1121.

Sowa, S. P., and C. F. Rabeni. 1995. Regional evaluation of the relation to habitat to distribution and abundance of smallmouth bass and largemouth bass in Missouri streams. Transactions of the American Fisheries Society 124:240-251.

Suski, C. D., J. H. Svec, J. B. Ludden, F. J. S. Phelan and D. P Philipp. 2003. The effect of catch-and –release angling on the parental care behavior of male smallmouth bass. Transaction of the American Fisheries Society 132:210-218.

Sweka, J. A., and K. J. Hartman. 2003. Reduction of reactive distance and foraging success in smallmouth bass, *Micropterus dolomieu,* exposed to elevated turbidity levels. Environmental Biology of Fishes 67:341-347.

Tyus, H. M., and N. J. Nikirk. 1990. Abundance, growth, and diet of channel catfish, *Ictalurus punctatus*, in the Green and Yampa Rivers, Colorado and Utah. The Southwesterm Naturalist 35:188-198.

VanArnum, C. J. G., G. L Buynak and J. R. Ross. 2004. Movement of smallmouth bass in Elkhorn Creek, Kentucky. North American Journal of Fisheries Management 24:311-315.

Vander Zanden, M. J., J. D. Olden, J. H. Thorne and N. E. Mandrak. 2004. Predicting occurrences and impacts of smallmouth bass introductions in north temperate lakes. Ecological Applications 14:132-148.

Ward, D. L., and M. P. Zimmerman. 1999. Response of smallmouth bass to sustained removals of northern pikeminnow in the lower Columbia and Snake Rivers. Transactions of the American Fisheries Society 128:1020-1035.

Warner, K. 2005. Smallmouth bass introductions in Maine: history and management implications. Fisheries 30:20-26.

Weidel, B., D. Josephson, and C. Krueger. 2000. Diet and Prey Selection of Naturalized Smallmouth Bass in an Oligotrophic Adirondack Lake. Journal of Freshwater Ecology 15:411-420.

Weiss-Glanz, L. S., and J. G. Stanley. 1984. Population structure indicies of largemouth bass and smallmouth bass determined from angler catches. North American Journal of Fisheries Management 4:89-98.

Whitledge. G. W., B. M. Johnson and P. J. Martinez. 2006. Stable hydrogen isotopic composition of fishes reflects that of their environment. Canadian Journal of Fisheries and Aquatic Sciences IN PRESS.

Whitledge, G. W., R. S. Hayward, R. D. Zweifel and C. F. Rabeni. 2003. Development and laboratory evaluation of a bioenergetics model of subadult and adult smallmouth bass. Transactions of the American Fisheries Society 132:316-325.

Whitledge, G. W., and C. F. Rabeni. 2003. Maximum daily consumption and respiration rates at four temperatures for five species of crayfish from Missouri, U.S.A. (Decapoda, *Orconectes* spp.). Crustaceana 75:1119-1132.

Wiegmann, D. D., J. R. Baylis and M. H. Hoff. 1992. Sexual selection and fitness variation in a population of smallmouth bass, *Micropterus dolomieu* (Pisces: Centrarchidae). Evolution 46: 1740–1753.

Wrenn, W. B. 1980. Effects of elevated temperature on growth and survival of smallmouth bass. Transactions of the American Fisheries Society 109:617-625.

Zimmerman, M. P. 1999. Food habits of smallmouth bass, walleyes, and northern pikeminnow in the lower Columbia River Basin during outmigration of juvenile anadromous salmonids. Transactions of the American Fisheries Society 128:1036-1054.

Zorn, T. G., and P. W. Seelbach. 1995. The relation between habitat availability and the short-term carrying capacity of a stream reach for smallmouth bass. North American Journal of Fisheries Management 15:773-783.

Zweifel, R. D., R. S. Hayward and C. F. Rabeni. 1999. Bioenergetics insight into black bass distribution shifts in Ozark border region streams. North American Journal of Fisheries Management 19:192-197.

APPENDIX A

POWERPOINT:
NONNATIVE FISH STOCKING REGULATION REVIEW,
ISOTOPIC ANALYSES OF ILLICIT FISH INTRODUCTIONS AND
STRATEGIES TO ENSURE COMPLIANCE WITH STOCKING REGULATIONS

Designating Conservation Areas to Prioritize, Publicize, Popularize and Optimize Native Fish Protection and Preservation in Colorado

Patrick J. Martinez, Aquatic Researcher
Colorado Division of Wildlife, Grand Junction

Native Fish Conservation Areas in CO

Objectives:

1. Impetus & rationale
2. Identify initial candidate conservation area
3. Administrative guidance & framework
4. Analogous conservation designations
5. Components of designation
6. Expected benefits of designation
7. Recommendations

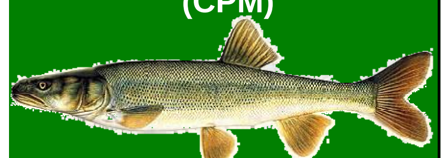
1. Impetus & rationale

- public aware of endangered fish (EDF) recovery efforts
- less aware of policies to protect native fish (NTF)
- EDF recovery often implies single species
- public unaware of importance of NTF communities
- some opposition to recovery of EDF exists
- non-sport NTF protection not seen as priority
- EDF & NTF views persist despite shift of resources
- nonnative fish (NNF) threat to NTF expanding
- anglers often oppose NNF control to benefit NTF
- non-salmonid NNF problematic in W. CO "big rivers"

Humpback chub (HBC)

Bonytail (BTC)

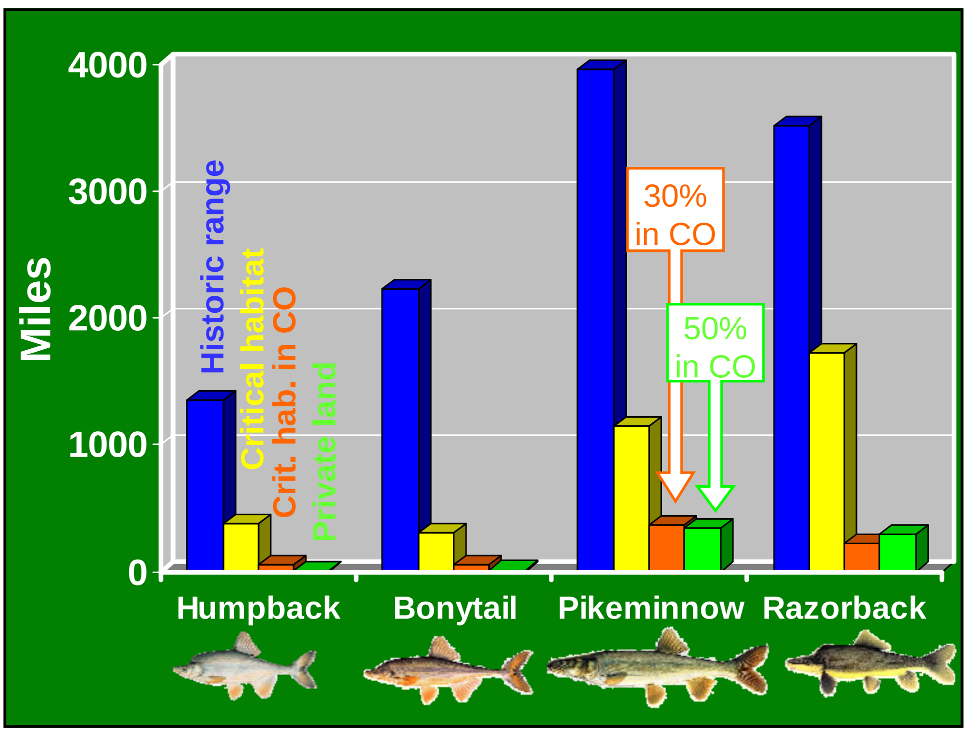
Colorado pikeminnow (CPM)

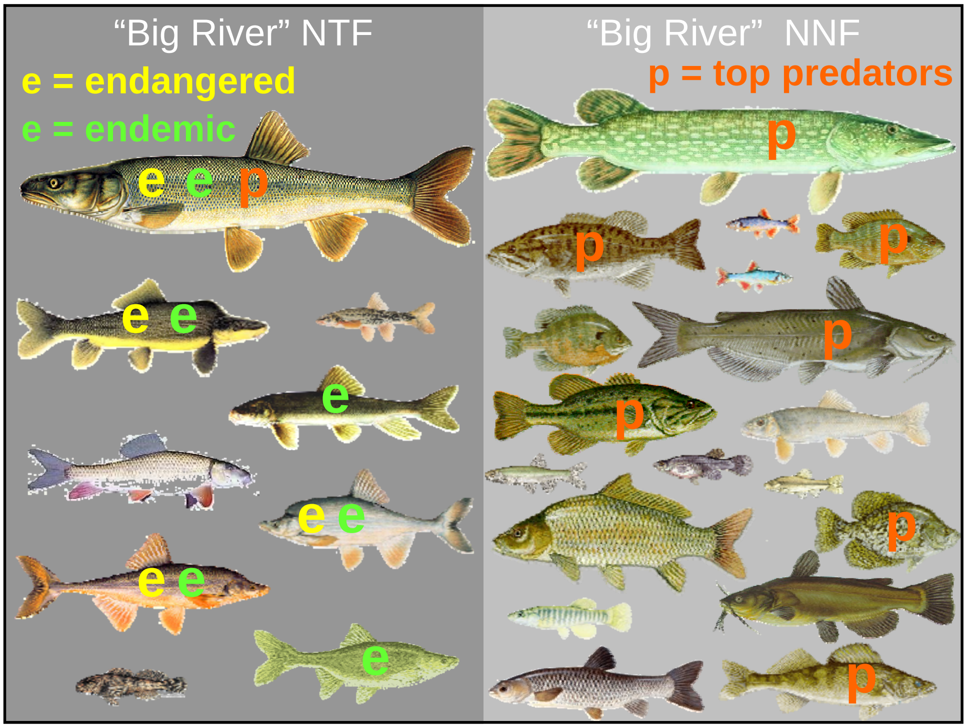


Razorback sucker (RZB)

Colorado River Basin Endangered Fish Critical Habitat

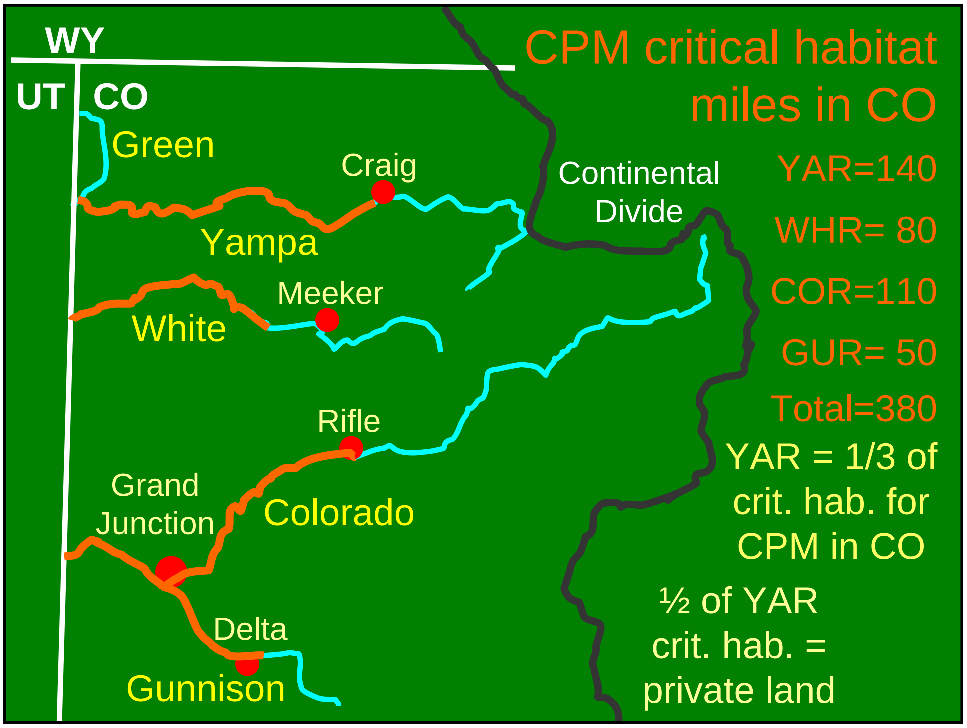
State	HBC	BTC	CPM	RZB
AZ	X	X		X
CA		X		
NM			X	X
NV		X		X
UT	X	X	X	X
CO	X	X	X	X
River				
YAR	X	X	X	X
WHR			X	
COR	X	X	X	X
GUR			X	X





1. Impetus & rationale (con't.)

- maximum sport fish (SPF) & NTF mutually exclusive
- social balance for NTF & SPF in rivers = lose : lose
- maximization of NTF needed for NTF communities
- some SPF far more explosive than formerly believed
- increasingly difficult to serve angler special interests
- mired in tradition of providing SPF in all public waters
- risk replacement, extirpation or extinction of NTF
- if "big river" NTF community not in UCRB, then where?
- optimize chance to preserve "big river" NTF, ASAP
- need NTF refuge/sanctuary in concept & reality



2. Identify initial candidate conservation area

- top predator CPM benefits from intact NTF community
- YAR contains most miles of CPM habitat in CO
- YAR historic stronghold of NTF
- YAR NTF severely suppressed by NNF & SPF (SMB)
- documented predation on NTF & CPM by SPF (NOP)
- Apparent decline in YAR CPM in recent years
- YAR considered most important tributary of Green R.
- peak flows maintain spawning/nursery habitats & cues
- CPM, HBC & RZB spawn in YAR
- YAR HBC one of five remaining populations
- YAR lies entirely within Colorado

3. Administative guidance & framework

CDOW Strategic Plan 2002-2007:

- Recover endangered species
- Prevent further listings
- ***Perpetuate native wildlife***

CDOW Admin. Dir. W-6: Fish Management & Stocking (1999):

- ***Non-salmonid NTF Recovery/Conservation Water***
- Aid recovery or conservation of T&E or NTF
- Stocking NNF & recreation may be restricted

3. Administative guidance & framework (con't.)

Aquatic Wildlife Management Plan Yampa River Basin, CO (CDOW 1998):

- divides YAR drainage & main-stem into 3 segments
- ***all specify control of nonsalmonid NNF & SPF***
- middle segment (55 RM) stresses NTF mgmt.
- lower segment (134 RM) stresses EDF & NTF mgmt.
- *lower YAR = "Nonsalmonid NTF Conservation Water"*
- YAR CPM critical habitat = 140 RM

3. Administative guidance & framework (con't.)

CPM Recovery Goals (USFWS 2002):

- *provide & legally protect habitat* (flow & environment)
- provide passage over barriers (range expansion)
- minimize entrainment in diversion canals
- protect from overutilization
- protect from diseases & parasites
- regulate NNF stocking & escapement
- ***control problematic NNF***
- minimize risk of hazardous spills
- remediate water quality problems
- ***long-term*** *mgmt. & protection of CPM & their habitat*

3. Administrative guidance & framework (con't.)

Mgmt. Plan for EDF in YAR Basin-EA (USFWS 2004)

UPPER COLORADO RIVER ENDANGERED FISH RECOVERY PROGRAM

- offsets EDF impacts due to YAR flow depletions
- addresses EDF Recovery Goals (return BTC to YAR)
- specifies SPF control for CCF, NOP & SMB
- screen reservoirs to control NNF escapement
- implement NNF stocking regulations
- remove NNF from YAR & relocate to off-stem sites
- some NNF will be lethally removed
- some SPF will be relocated to ponds & reservoirs

NNF control carries threat of closure of private land!

4. Analogous designations

WLC Policy D-6 (1992) & Admin. Dir. W-6 (1999)

- **Gold Medal Waters** – CDOW – quality trout >14”

- **Wild Trout Water** – CDOW – naturally sustained trout

- **Native Cutthroat Water** - CDOW, USFS, BLM, NPS

-promote protection/enhancement of aquatic/terrestrial habitat

-loss/degradation of designation due to man requires mitigation

5. Components of designation

- must emphasize & publicize priority of NTF over SPF
- sportfishing allowed but not enhanced or promoted
- SPF bag, possession & size limits removed (done!)
- inform anglers that only residual SPF may remain
- any take of NTF prohibited
- fines & penalties for harming NTF increased 10x
- innovations to control NNF access & abundance
- fund easements for access to manage NTF & NNF
- incentives to protect riparian & riverine habitat
- concept widely applicable for NTF across state

YAMPA RIVER BASIN PARTNERSHIP

"Balancing natural resource and social issues through communication, education, and coordination."

Yampa Valley Land Trust

Northwest Colorado Working Landscape Trust

YAMPA VALLEY PARTNERS

Community Information

Popularize EDF & NTF with private landowners & conservationists!

Yampa River System Legacy Project

Endangered Species Program
Fiscal Year 2005 Private Stewardship Grant Program
Notice of Availability of Federal Assistance

6. Potential & expected benefits of designation

- awareness of NTF communities & conservation needs
- understanding of urgency for actions to benefit NTF
- acceptance of controversial mgmt. (SPF removal)
- instilling concept of refuge/sanctuary for NTF
- off-setting economic impacts by attracting non-anglers
- agreements for long-term access & management
- partnerships to protect riparian & riverine habitat
- shared goal of optimizing NTF communities
- expedite recovery, protection & perpetuation of NTF

7. Recommendations

- formalize "*Non-salmonid NTF Recovery/Conservation Water*" on YAR per Basin Mgmt. Plan & administrative framework, to instill concept of NTF refuge/sanctuary

- apply all "components of designation," especially long-term access to private YAR reaches for NTF mgmt. & NNF control via conservation easements & habitat partnerships stressing importance of NTF communities

- aggressively enforce all existing & necessary regs. & policies to control NNF abundance, proliferation, stocking, illicit introductions & escapement to maximize capacity for NTF recovery, rebound & perpetuation

- apply designation & approach elsewhere to prioritize, publicize, popularize & optimize NTF preservation

APPENDIX B

SCOPE-OF-WORK:
COLORADO RIVER RECOVERY PROGRAM, PROJECT NO.: C18/19
FY-2007 -2009 PROPOSED SCOPE OF WORK FOR:
CHEMICALLY FINGERPRINTING NONNATIVE FISHES IN RESERVOIRS

COLORADO RIVER RECOVERY PROGRAM Project No.: C18/19
FY-2007 -2009 PROPOSED SCOPE OF WORK for:
Chemically Fingerprinting Nonnative Fishes in Reservoirs

Lead Agency: Colorado Division of Wildlife
Submitted by:
Project Leader: Patrick Martinez
Principal Investigators:

Patrick J. Martinez	Brett M. Johnson
Colo. Div. of Wildlife	Dept. Fish & Wildlife Bio.
711 Independent Ave.	Colorado State University
Grand Junction, CO 81505	Fort Collins, CO 80523
Phone: 970-255-6143	970-491-5002
FAX: 970-255-6111	970-491-5091
pat.martinez@state.co.us	brett@warnercnr.colostate.edu

Date: January 11, 2006
Revised: February 28, 2006, per Biology Committee

Category:
___ Ongoing Project
_X_ Ongoing-revised project
___ Requested new project
___ Unsolicited proposal

Expected Funding Source:
_X_ Annual funds
___ Capital funds
___ Other (explain)

I. Title of Proposal: Chemically Fingerprinting Nonnative Fishes in Reservoirs

II. Relationship to RIPRAP:

This proposal addresses movement of nonnative fish into river reaches of critical habitat from reservoirs known to support cool- and warmwater species of nonnative fish. These species include northern pike, smallmouth bass, largemouth bass, black crappie, and walleye. These species are believed to pose a significant predatory threat to the young life stages of endangered and other native fishes (Tyus and Saunders 1996; Martinez et al. 2001; Johnson et al. 2005a). However, it is uncertain to what extent the presence of nonnative species in critical habitat is the result of escapement or illicit transfers from reservoirs. Overall, this study is intended to develop chemical fingerprints of nonnative fishes in 11 reservoirs that are potential sources of nonnative fishes to the critical habitat of Upper Colorado River Basin through microchemical analysis of otoliths. Successful development of these fingerprints will provide the means to assess the proportion of nonnative fishes in these rivers that originate from reservoirs and thereby guide management efforts to reduce this influx of nonnative fishes.

III. Study Background/Rationale and Hypotheses:

Background/Rationale:

Nonnative fishes are present throughout the Upper Basin (Martinez 2002, Trammel et al. 2002), and can adversely impact the recovery of endangered fishes through predation or competition at critical life stages or in critical locales. However, the recruitment sources and origins of nonnative fishes are not well known. Immigration of nonnative fishes from nearby reservoirs has been demonstrated in some cases by the recapture of fishes that had been tagged as part of other studies. However, large scale tagging efforts to address the growing concern about escapement of nonnative piscivores from multiple reservoirs throughout the Upper Basin is impractical. This Scope of Work seeks to verify fish escapement from reservoirs as a source of nonnative fish entering critical habitat by applying newly developed techniques for identifying naturally occurring markers via microchemical analysis of otoliths.

Otolith microchemistry provides a means to trace the origins and movements of fishes in marine (Humpreys et al. 2005, Campana et al. 2000; Bath et al. 2000) and freshwater environments (Brazner et al. 2004, Bronte et al. Wells et al. 2003). In freshwater systems differences in underlying geology can result in water chemistry that varies among watersheds. Limnological processes and chemical transformations within reservoirs impart further distinctiveness to water chemistry among lentic and lotic water bodies. Chemical composition of ambient water is imparted to otoliths of resident fish in a highly predictable and temporally referenced manner. Because otoliths are physiologically inert structures their chemical composition does not change after material is accreted. Thus, otoliths record the environmental history of a fish and that information can be used to determine the fish's provenance (origin and movements).

Recent work by Whitledge et al. (in review; in prep.) has demonstrated that otolith microchemistry has excellent potential for tracing the provenance of nonnative fishes in the Upper Colorado River Basin. Further, graduate work by CSU students Ryan Fitzpatrick and Daniel Gibson-Reinemer is showing that many water bodies (ponds, streams, reservoirs) and hatcheries in Colorado possess unique chemical fingerprints, and that these fingerprints are imparted to the otoliths of fish originating from each location. It also appears that transfers of fish can be detected in otoliths as shifts in the chemical composition along laser transects performed with laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS; Johnson et al. 2005b). These findings coupled with the highly heterogeneous nature of the Colorado Plateau's geology suggest that otolith microchemistry is likely to reveal new insights into the movements of nonnative fishes within the Upper Colorado River Basin.

Hypotheses:

We hypothesize that:

a. the chemical composition (fingerprints) of otoliths from nonnative fishes will differ among reservoirs,
b. inter-annual variation in otolith fingerprints will be small relative to inter-reservoir differences,
c. otolith core signatures of fishes that were reared in reservoirs and immigrated to rivers in critical habitat will be distinct from signatures of fishes inhabiting rivers since hatching, and
d. otolith core signatures can be used to identify fishes as having originated from a particular reservoir.

IV. Study Goals, Objectives and End Product:

Study Goals: to determine chemical "fingerprints" of nonnative fishes in reservoirs that are potential sources of nonnative fishes to critical habitat.

Study Objectives:

Primary objectives of the investigation will be to:

1. quantify chemical "fingerprints" of fishes within study reservoirs and evaluate the degree of inter-annual variation in those fingerprints.
2. determine if fish sampled in rivers the vicinity of study reservoir possess otolith core signatures that identify them as having originated from one of the study reservoirs.
3. improve our understanding of the degree to which immigration or transfers from reservoirs contributes to the load of nonnative fishes in critical habitat of the Upper Colorado River basin.
4. provide recommendations to guide management efforts to reduce the influx of nonnative fishes from reservoirs.

End Products:

1. A quantitative tool to determine the proportion of nonnative fishes in critical habitat that originate from reservoirs.
2. A forensic tool to assist conservation officers in prosecuting individuals engaged in the illegal transfer of nonnative fishes from reservoirs.
3. Identification of the origin and contributing sources of target nonnative fishes to critical habitat, to facilitate the fiscal and ecological efficiency of nonnative fish control.

V. Study Area:

The principal area of study for this SOW will be large reservoirs within the Upper Colorado River Basin, including those in northeastern Utah, southwestern Wyoming and western Colorado (Bottle Hollow, Crawford, Flaming Gorge, Harvey Gap, Kenney, McPhee, Paonia, Ridgeway, Rifle Gap, Rio Blanco and Starvation reservoirs).

VI. Study Methods/Approach:

Nonnative fishes will be collected by standard fisheries sampling techniques, collateral to ongoing sampling by state, federal or university efforts. The number of species varies by reservoir and river, but will include northern pike, smallmouth bass, largemouth bass, black crappie and walleye. We will extract sagittal otoliths from up to 20 individuals of each species from each site. Otoliths will be removed from fishes using non-metallic forceps, rinsed with distilled water, and stored dry in polyethylene vials until preparation for analyses. A range of fish sizes/ages will be collected to allow us to examine otolith core (first year of life) signatures across a number of year classes, and thereby assess inter-annual variation in those signatures. We will strive to make two collections per year from seven reservoirs in 2007 and 2008, and one per year in the remaining four. Water samples will be collected for microchemical analysis at the time of fish sampling.

Otoliths will be embedded in Epo-fix® epoxy, sectioned in a transverse plane using an ISOMET low-speed saw, and polished to reveal annuli. Otolith thin sections will be mounted on acid-washed glass slides using double-sided tape, ultrasonically cleaned for 5 min in ultrapure water, and dried for 24 h under a laminar flow hood. We will employ well-established methods for the microchemical analysis using LA-ICP-MS (Campana 1999) in addition to new techniques developed with Recovery Program funding by Whitledge et al. (in review).

Dr. Brett Johnson of the Department of Fishery and Wildlife Biology at CSU will hire and supervise a graduate research associate (M.S.) to identify sampling intensity, conduct and oversee microchemical analyses, evaluate data and provide findings. CDOW will maintain oversight of this project and will assist and coordinate field sample collection in close cooperation with the graduate research associate. Analytical work will be conducted under the guidance of Alan Koenig, U.S.G.S. Research Scientist, using the LA-ICP-MS instrument at the U.S.G.S. Mineral Resources Laboratory in Denver, Colorado.

This study will compliment recent work that estimated the degree of immigration of nonnative fishes to the Colorado River from floodplain ponds and backwaters (Martinez and Martinez 2004, Whitledge et al. in review, Whitledge et al.in prep).

VII. Task Description and Schedule:

FY 2006:

Task 1. Field Collections.

Pat Martinez, CDOW Aquatic Researcher and field technicians will lead field collection efforts in cooperation with the graduate research associate. Preliminary reservoir sampling will be conducted during June-September 2006. This sampling will be coordinated with the respective states and crews operating in the target reservoirs and river reaches.

Task 2. Microchemical Analysis of Otoliths.

Dr. Brett Johnson at CSU will recruit a graduate research associate, and as soon as funding for FY07 is confirmed he will select a graduate research associate to perform analyses and interpretation of otolith samples and assist with field collections.

FY 2007:

Task 1. Field Collections.

Pat Martinez, CDOW Aquatic Researcher and field technicians will lead field collection efforts in cooperation with the graduate research associate. Full scale reservoir and river sampling will be conducted during May through August 2007, with two collections made at seven reservoirs and one in the remaining four. This sampling will be coordinated with the respective states and crews operating in the target reservoirs and river reaches.

Task 2. Microchemical Analysis of Otoliths.

The graduate student will begin in January 2007. Work will involve year round sample and data analysis, the graduate research associate will advise CDOW on field sample collection and ongoing sample preparation for microchemical analyses. The graduate research associate will submit quarterly reports to Pat Martinez, CDOW.

Reporting: An annual report will be submitted to Pat Nelson by December 15, 2006.

FY 2008:

Task 1. Pat Martinez, CDOW Aquatic Researcher and field technicians will lead field collection efforts in cooperation with the graduate research associate. Reservoir and river sampling will be conducted during May through August, with two collections made at seven reservoirs and one in the remaining four.

Task 2. CSU graduate research associate will perform analyses and interpretation of otolith samples. The graduate research associate will submit quarterly reports to Pat Martinez, CDOW.

Reporting: An annual report will be submitted to Pat Nelson by December 15, 2007. Graduate research associate will present preliminary findings at the Upper Basin Researcher's Meeting in January 2008.

<u>FY 2009:</u>

Task 1. No activity unless findings in previous years warrant additional sampling.

Task 2. CSU graduate research associate will perform analyses and interpretation of otolith samples. The graduate research associate will submit quarterly reports to Pat Martinez, CDOW. The graduate research associate will submit M.S. thesis to graduate committee in April 2009.

Reporting: Graduate research associate will present preliminary findings at Upper Basin Researcher's Meeting in January 2009.
Draft final report to Pat Nelson – May 15, 2009.
1st revised draft final report to peer review – June 15, 2009. (peer reviews due to author – July 15, 2009; BC comments due – August 3, 2005).
2nd revised draft final report to Biology Committee – September 3, 2009

Project Timeline:

<table>
<tr><th>Month</th><th>2006</th><th>2007</th><th>2008</th><th>2009</th></tr>
<tr><td>Jan</td><td></td><td>Grad student begins</td><td>Upper Basin presentation</td><td>Upper Basin presentation</td></tr>
<tr><td>Feb</td><td></td><td rowspan="3">Prepare and analyze otoliths (LA-ICP-MS)</td><td rowspan="3">Prepare and analyze otoliths (LA-ICP-MS)</td><td rowspan="2">Data analysis and writing</td></tr>
<tr><td>Mar</td><td></td></tr>
<tr><td>Apr</td><td></td><td>Thesis draft</td></tr>
<tr><td>May</td><td></td><td rowspan="4">Field collections; Prepare and analyze otoliths (LA-ICP-MS)</td><td rowspan="4">Field collections; Prepare and analyze otoliths (LA-ICP-MS)</td><td>Draft final report</td></tr>
<tr><td>Jun</td><td rowspan="4">Field collections; Prepare otoliths for LA-ICP-MS; recruit grad student</td><td>Revised final report, draft 1</td></tr>
<tr><td>Jul</td><td></td></tr>
<tr><td>Aug</td><td></td></tr>
<tr><td>Sep</td><td rowspan="3">Data analysis and report writing; Prepare and analyze otoliths (LA-ICP-MS)</td><td rowspan="3">Data analysis and report writing; Prepare and analyze otoliths (LA-ICP-MS)</td><td>Revised final report, draft 2</td></tr>
<tr><td>Oct</td><td>Recruit grad student</td><td></td></tr>
<tr><td>Nov</td><td>Data analysis and report writing;</td><td></td></tr>
<tr><td>Dec</td><td>Interim report</td><td>Interim report</td><td>Interim report</td><td></td></tr>
</table>

VIII. FY- 2006 through 2009 Work:

FY 2006 Deliverables:

Summary of field collections provided in annual report to Program- December 2006.

FY 2007 Deliverables:

Presentation of preliminary findings at Upper Basin Researcher's Meeting (January 2007). Preliminary findings summarized in annual report to Program- December 2007.

FY 2008 Deliverables:

Presentation of preliminary findings at Upper Basin Researcher's Meeting (January 2008). Findings summarized in annual report to Program- December 2008.

FY 2009 Deliverables:

Draft Final Report distributed for peer-review by 30 May 2009.

Budget

FY 2006 Costs:

Task 1 - Field Collection

Supplies	$500
Travel, vehicle	$4,000
University indirect cost @ 15% (funds passed through existing Larval Lab- or BMR-USBR agreement)	$675
Total	$5175

Task 2 - Otolith Analysis

CSU professor salary, fringe (0.25 months)	$2,392
Student hourly & fringe (800 hours)	$9,984
Travel	$500
Lab supplies	$500
University indirect cost @ 15% (funds passed through existing Larval Lab- or BMR-USBR agreement)	$2006
Total	$15382
TOTAL (FY 2006)	**$20,557**

FY 2007 Costs:

Task 1 - Field Collection

Supplies	500
Travel, vehicle	2,000
University indirect cost @ 15% (funds passed through existing Larval Lab- or BMR-USBR agreement)	375
Total	$2,875

Task 2 - Otolith Analysis

Graduate Research Associate & fringe	12,636
Graduate tuition (1 semester)	1,751
CSU professor salary, fringe (1 month)	9,000
Student hourly & fringe (300 hours)	3,744
Mass spectrometer use fees	5,333
Water analysis fees	1,500
Travel	500
Lab supplies	500
University indirect cost @ 15% (funds passed through existing Larval Lab- or BMR-USBR agreement)	4,982
Total	$39,946
TOTAL (FY 2007)	$42,821

FY 2008 Costs:

Task 1 - Field Collection

Supplies	0
Travel, vehicle	0
University indirect cost @ 15% (funds passed through existing Larval Lab- or BMR-USBR agreement)	0
Total	0

Task 2 - Otolith Analysis

Graduate Research Associate & fringe	$13712
Graduate tuition (1 semester)	$1,839
CSU professor salary, fringe (1.125 months)	11302
Student hourly & fringe (200 hours)	$2,496
Mass spectrometer use fees	$2,800

Water analysis fees	$1,000
Travel	525
Lab supplies	400
University indirect cost @ 15% (funds passed through existing Larval Lab- or BMR-USBR agreement)	4835
Total	$38,909
TOTAL (FY 2008)	$38,909

FY 2009 Costs:

Task 1 - Field Collection

Total	$0

Task 2 - Otolith Analysis

Graduate Research Associate & fringe	7,020
Graduate tuition (1 semester)	1,930
CSU professor salary, fringe (0.56 months)	7,097
Mass spectrometer use fees	0
Water analysis fees	0
Travel	551
Lab supplies	400
Journal page charges	500
University indirect cost @ 15% (funds passed through existing Larval Lab- or BMR-USBR agreement)	2,335
Total	$19,834
TOTAL (FY 2009)	**$19,834**

IX. Budget Summary:

FY-2006	
Field Collection:	$ 5,175
Otolith Analyses:	$15,382
Total	**$20,557**
FY-2007	
Field Collection:	$ 2,875
Otolith Analyses:	$39,946
Total	**$42,821**
FY-2008	
Field Collection:	$ 0
Otolith Analyses:	$38,909
Total	**$38,909**
FY-2009	
Field Collection:	$ 0
Otolith Analyses:	$19,834
Total	**$19,834**
Amount Requested from Recovery Program (FY07 – FY09)	**$122,121**

X. Reviewers: Anita Martinez, Colorado Division of Wildlife

XI. References:

Bath, G. E., S. R. Thorrold, C. M. Jones, S. E. Campana, J. W. McLaren, and J. W. H. Lam. 2000. Strontium and barium uptake in aragonitic otoliths of marine fish. Geochimica et Cosmochimica Acta 64: 1705-1714.

Brazner, J.C., S.E. Campana, D.K. Tanner and S.T. Schram. 2004. Reconstructing habitat use and wetland nursery origin of yellow perch from Lake Superior using otolith elemental analysis. J. Great Lakes Res. 30:492-507.

Bronte, C.R., R.J. Hesselberg, J.A. Shoesmith, and M.H. Hoff. 1996. Discrimination among spawning populations of Lake Superior lake herring based on trace element profiles in sagittae. Transactions of the American Fisheries Society 125: 852-859

Campana, S. E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Mar. Ecol. Prog. Ser. 188:263-297.

Campana SE, Chouinard GA, Hanson JM, Frechet A, Brattey J. 2000. Otolith elemental fingerprints as biological tracers of fish stocks. Fisheries Research 46: 343-357.

Humphreys, R. L., S. E. Campana, E. E. DeMartini. 2005. Otolith elemental fingerprints of juvenile Pacific swordfish *Xiphias gladius*. Journal of Fish Biology 66: 1660-1670.

Irving, D. B., and B. D. Burdick. 1995. Reconnaissance inventory and prioritization of existing and potential bottomlands in the Upper Colorado River Basin: 1993-1994. Recovery Implementation Project for Endangered Fishes in the Upper Colorado River Basin Final Report. U.S. Fish and Wildlife Service, Denver, Colorado.

Johnson, B. M., G. Whitledge, M. Sullivan, and D. Gibson-Reinemer. 2005a. Stable isotopes and statistics. Progress report, Colorado Division of Wildlife, Grand Junction, Colorado, 22 pages.

Johnson, B. M., D. Gibson-Reinemer, D. Winkelman, P. J. Martinez. 2005b. Forensic Applications of Otolith Microchemistry for Tracking Sources of Illegally Stocked Whirling Disease Positive Trout. Final Report, Whirling Disease Initiative, Bozeman, MT, 26 pages.

Johnson, B. M., P. J. Martinez, J. A. Hawkins, and K. T. Bestgen. In prep. Smallmouth bass are the primary predatory threat to the native fish assemblage of the Yampa River, Colorado.

Martinez, A. M. 2002. Nonnative fish control in Colorado, 1997-2001. *in* R. Muth, moderator. Nonnative Fish Control Workshop. Upper Colorado River Endangered Fish Recovery Program, Denver, Colorado.

Martinez, A. M., and P. J. Martinez. 2004. Stable Isotope Analysis of Centrarchid Concentration Areas. Annual Project Report, Project C-18/19, Colorado River Recovery Program, Denver, Colorado.

Martinez, A. M., J. T. Romatzke, and D. R. Powell. 2002. Proposed redirection of the nonnative fish control program in Colorado from pond reclamation/isolation to intensive control of nonnative fish in one area of the Colorado River that is considered a “hot spot” for centrarchids. Report of Colorado Division of Wildlife to Upper Colorado River Endangered Fish Recovery Program, Denver, Colorado.

Martinez, P. J. and B. M. Johnson. 2005. Researchers study ear bones to learn origin and movement patterns of sunfish. Swimming Upstream. Upper Colorado River Recovery Program, U.S. Fish and Wildlife Service, Denver, Colorado.

Martinez, P. J., B. M. Johnson, and J. D. Hobgood. 2001. Stable isotope signatures of native and nonnative fishes in Upper Colorado River backwaters and ponds. The Southwestern Naturalist 46: 311-322.

Trammell, M. A., R. A. Valdez, L. Jonas, and H. Johnstone, 2002. Non-native fish control in backwater habitats in the Colorado River. Final Report to Colorado Division of Wildlife Resources. SWCA, Inc., Environmental Consultants, Flagstaff, AZ.

Tyus, H. M., and J. F. Saunders, III. 1996. Nonnative fishes in natural ecosystems and a strategic plan for control of nonnatives in the Upper Colorado River basin. Recovery Implementation Program DRAFT REPORT. Cooperative Agreement No. 14-48-006-95-923. U.S. Fish and Wildlife Service, Denver, Colorado.

Wells, B.K., B. E. Rieman, J. L. Clayton, D. L. Horan, and C. M. Jones. 2003. Relationships between water, otolith, and scale chemistries of westslope cutthroat trout from the Coeur d'Alene River, Idaho: the potential application of hard-part chemistry to describe movements in freshwater. Transactions of the American Fisheries Society132: 409–424.

Whitledge, G. W., B. M. Johnson and P. J. Martinez. In review. Stable hydrogen isotopic composition of fishes reflects that of their environment. Canadian Journal of Fisheries and Aquatic Sciences.

Whitledge, G. W., B. M. Johnson, P. J. Martinez, and A. Martinez. In prep. Provenance of non-native fishes in the upper Colorado River revealed by stable isotope and microchemical analyses of otoliths. Ecological Applications.

APPENDIX C

POWERPOINT:
SUMMARY OF SMALLMOUTH BASS SUMMIT, HELD 28-29 NOVEMBER 2006, AND PRESENTED AT THE NONNATIVE FISH CONTROL WORKSHOP, HELD 12-13 DECEMBER 2006

Comparison of Smallmouth Bass in the Yampa and Colorado Rivers, Colorado, and Implications for Their Control

Patrick J. Martinez & Kelli J. Rehder
Colorado Division of Wildlife

Mario L. Sullivan & Brett M. Johnson
Colorado State University

SMB life history:

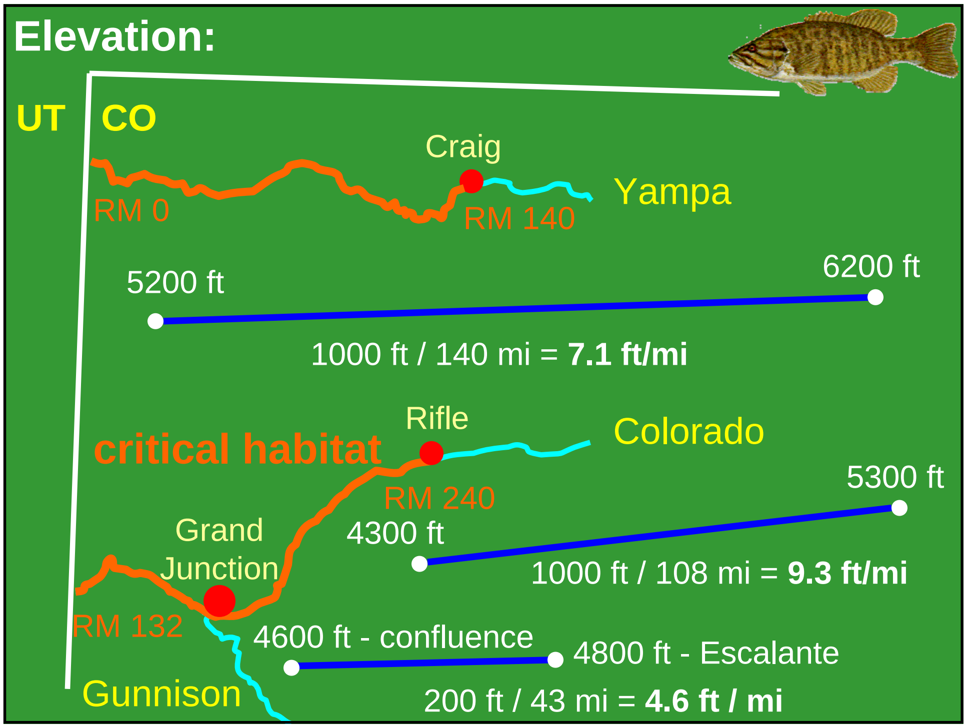
- prefer rocky substrate or structure in streams & lakes
- mature age 2-4 @ 8-12 in., depending on growth rate
- spawn May-June @ 56-64 F
- male builds & tends nest at depths of 2-20 ft.
- females release 2000-3000 eggs/lb of body weight
- several females spawn in same nest
- eggs hatch in 5-8 days, young leave in 5-6 days
- flow & especially temperature affect age 0 survival
- growth variable - temperature & food – best > 70 F
- prey on insects, crayfish & fish
- life span up to 15-18 years

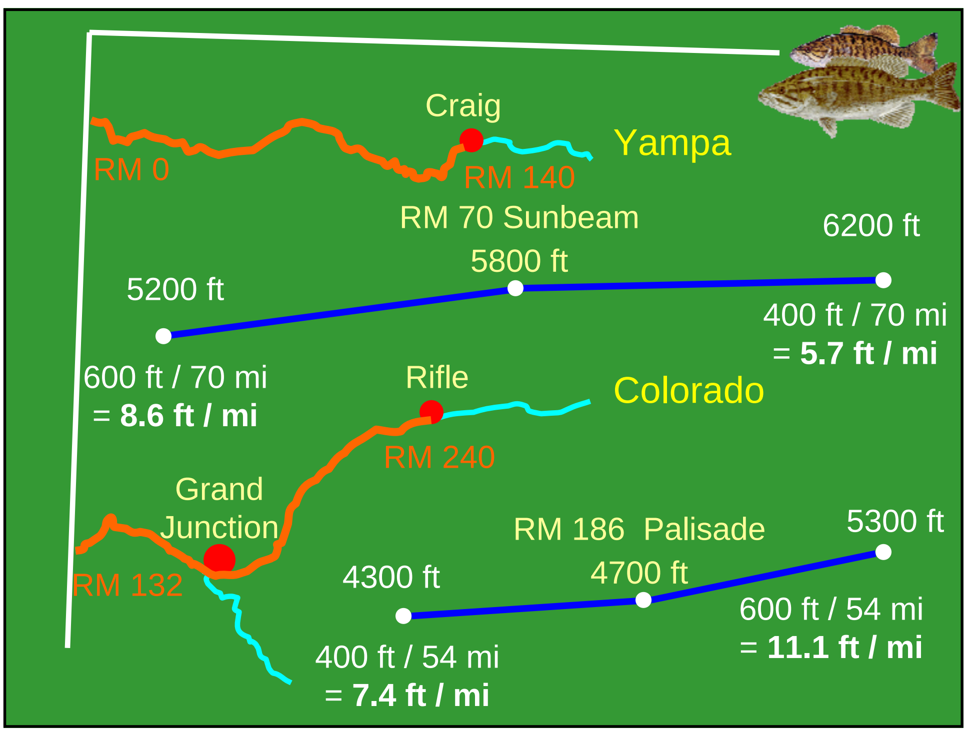
Objectives - to compare:

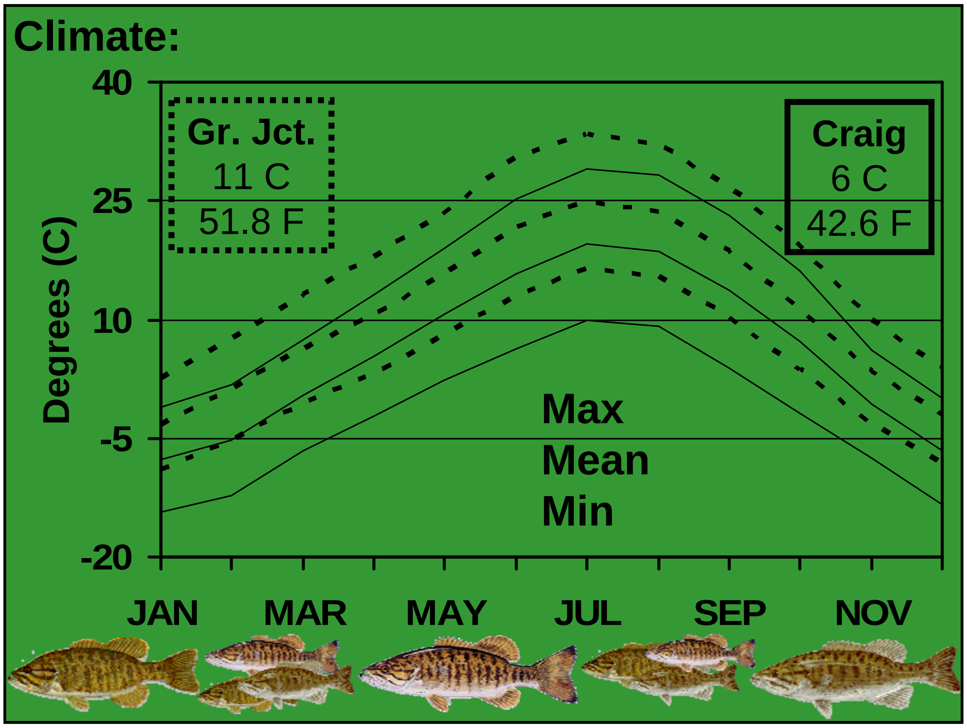
- physical characteristics of YAR & COR as they relate to SMB (elevation, climate, discharge, temperature)
- basic SMB life history parameters in YAR & COR
  - length frequency
  - age & growth
  - relative weight
  - diet
- potential & strategies for control

Methods:

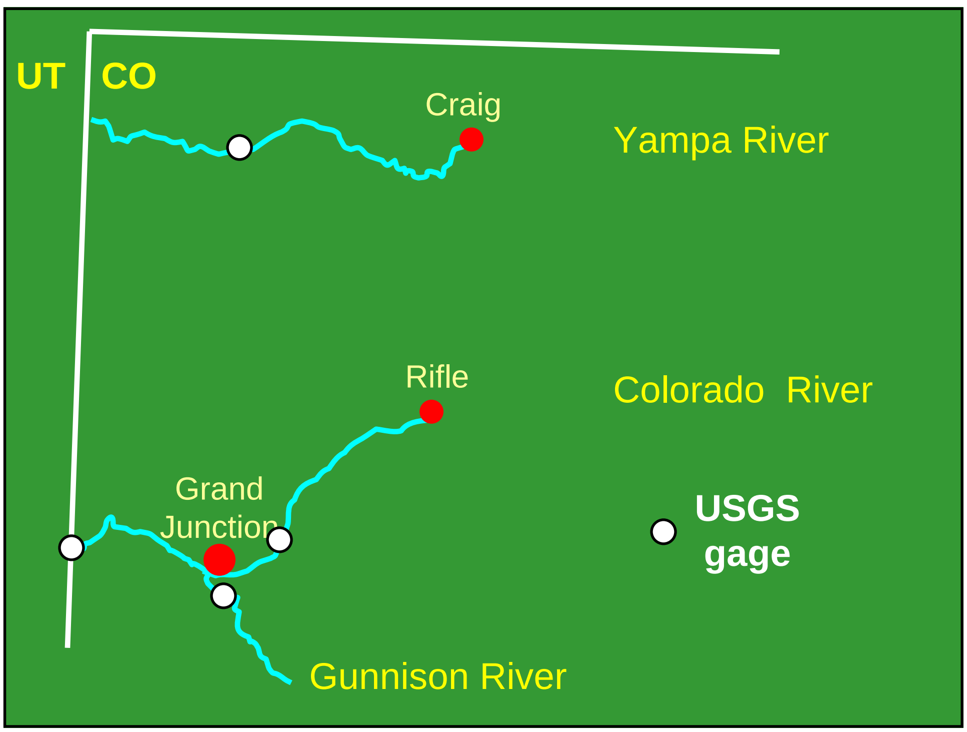
- mean monthly & annual air temp (NOAA)
- mean monthly flow & temperature 1996-2004 (USGS)
- YAR centrarchids 2001-2004, electrofish, RM 50-118 April - September (Hawkins, Anderson, Martin)
- COR centrachids 2004, electrofish, RM 125-188, April - August (Osmundsen, Burdick, A. & P. Martinez)
- SMB age & growth (Rehder, Gross & P. Martinez-CDOW)
- SMB diet (Sullivan, Oplinger & Johnson-CSU)


Elevation:
UT
CO
Craig
Yampa
RM 0
RM 140
5200 ft
6200 ft
1000 ft / 140 mi = **7.1 ft/mi**
Rifle
Colorado
critical habitat
RM 240
5300 ft
Grand Junction
4300 ft
1000 ft / 108 mi = **9.3 ft/mi**
RM 132
4600 ft - confluence
4800 ft - Escalante
Gunnison
200 ft / 43 mi = **4.6 ft / mi**

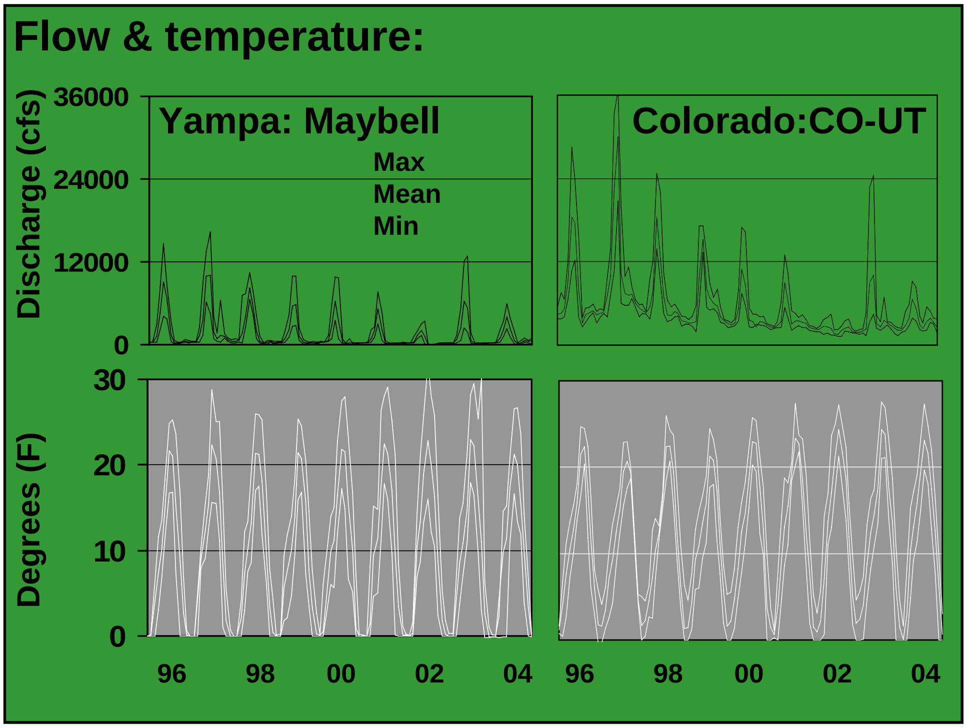

Craig
Yampa
RM 0
RM 140
RM 70 Sunbeam
6200 ft
5800 ft
5200 ft
400 ft / 70 mi
= **5.7 ft / mi**
600 ft / 70 mi
= **8.6 ft / mi**
Rifle
Colorado
RM 240
Grand Junction
RM 186 Palisade
5300 ft
4700 ft
4300 ft
RM 132
600 ft / 54 mi
= **11.1 ft / mi**
400 ft / 54 mi
= **7.4 ft / mi**

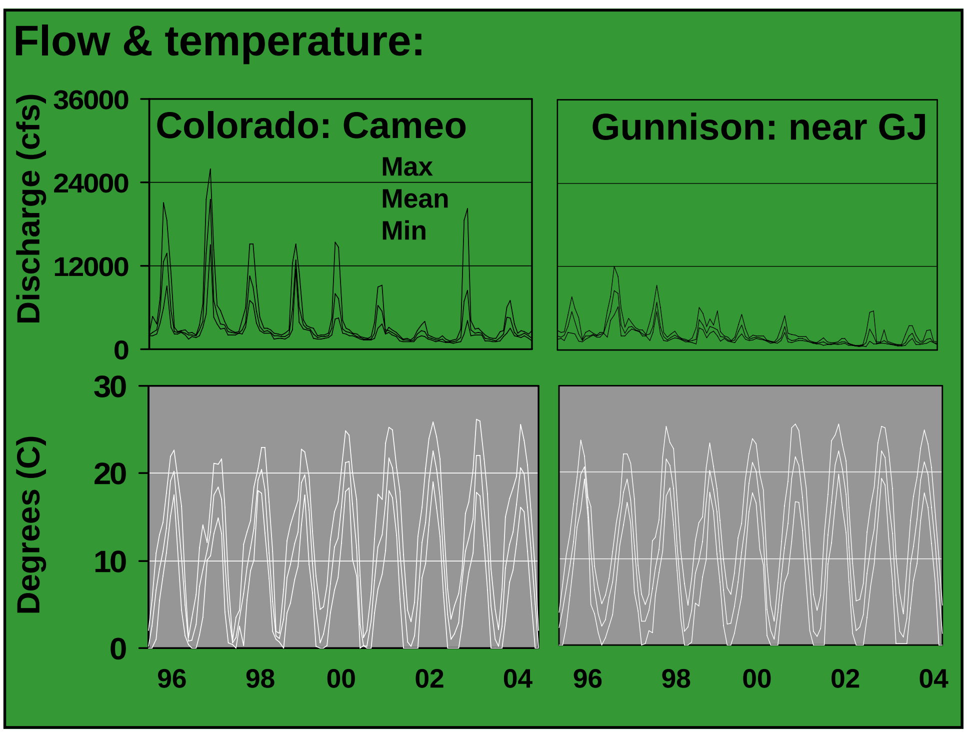


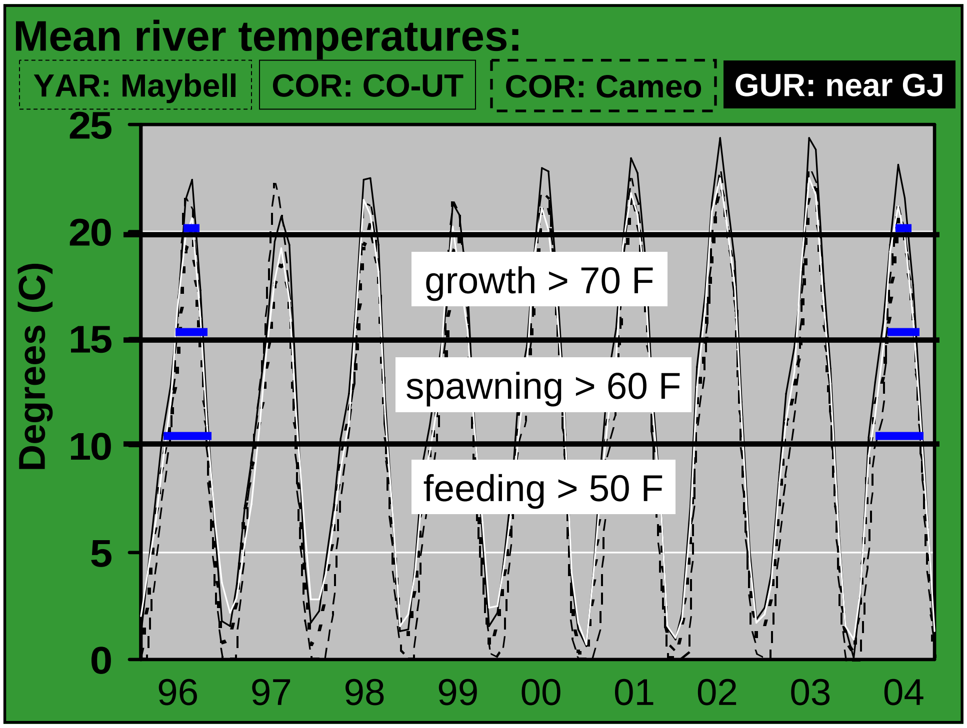
Climate:
40
25
10
-5
-20
Degrees (C)
Gr. Jct.
11 C
51.8 F
Craig
6 C
42.6 F
Max
Mean
Min
JAN
MAR
MAY
JUL
SEP
NOV

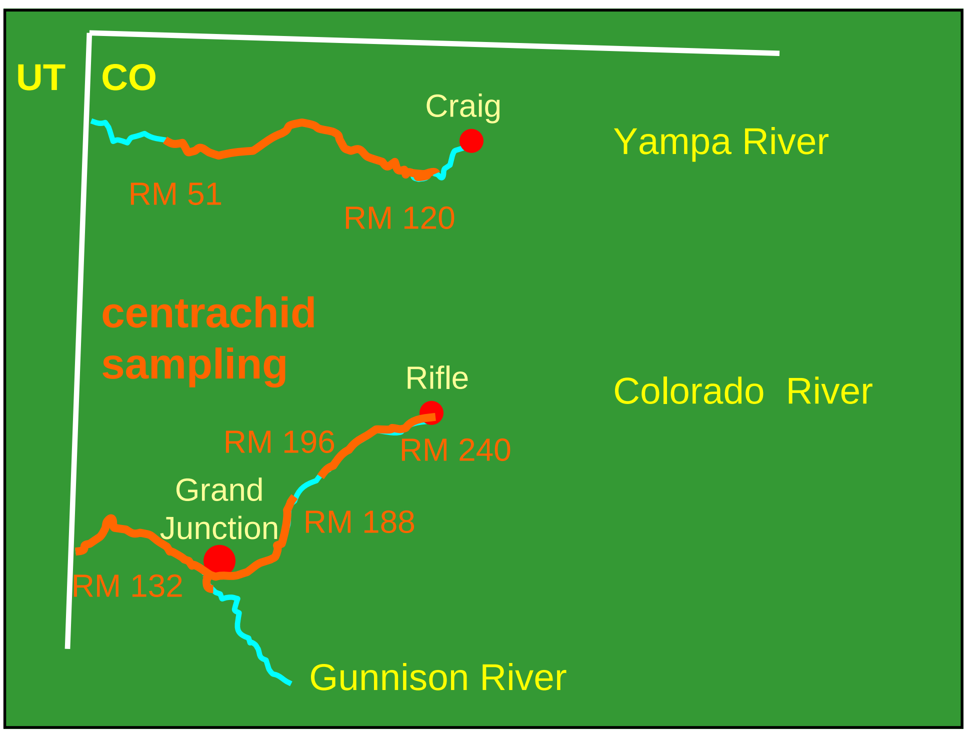


UT
CO
Craig
Yampa River
Rifle
Colorado River
Grand
Junction
USGS
gage
Gunnison River

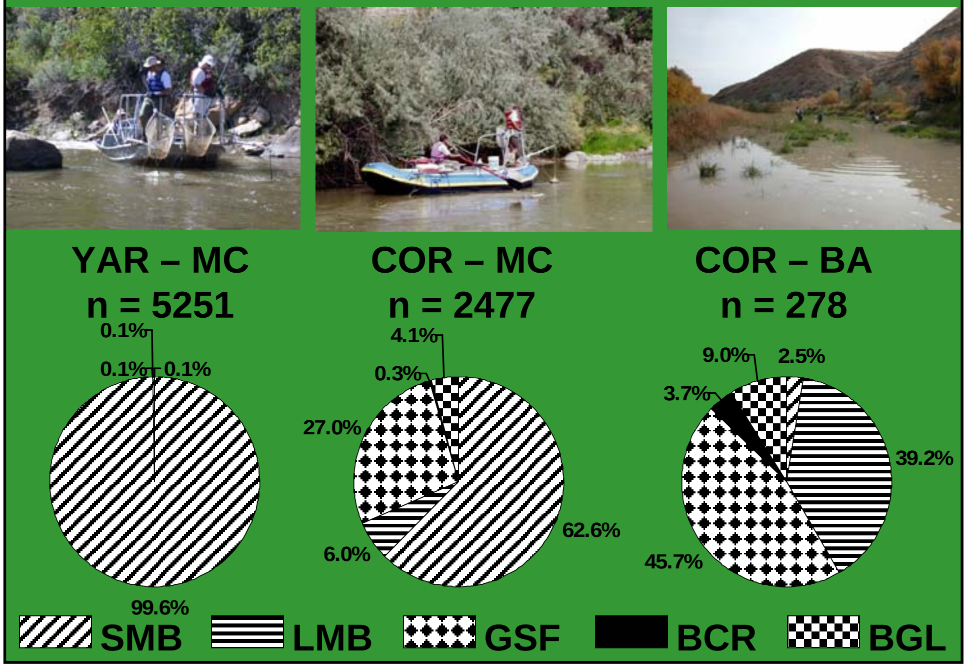

Flow & temperature:
Discharge (cfs)
36000
24000
12000
0
Yampa: Maybell
Max
Mean
Min
Colorado:CO-UT
Degrees (F)
30
20
10
0
96
98
00
02
04
96
98
00
02
04


Flow & temperature:
Discharge (cfs)
36000
24000
12000
0
Colorado: Cameo
Max
Mean
Min
Gunnison: near GJ
Degrees (C)
30
20
10
0
96
98
00
02
04
96
98
00
02
04

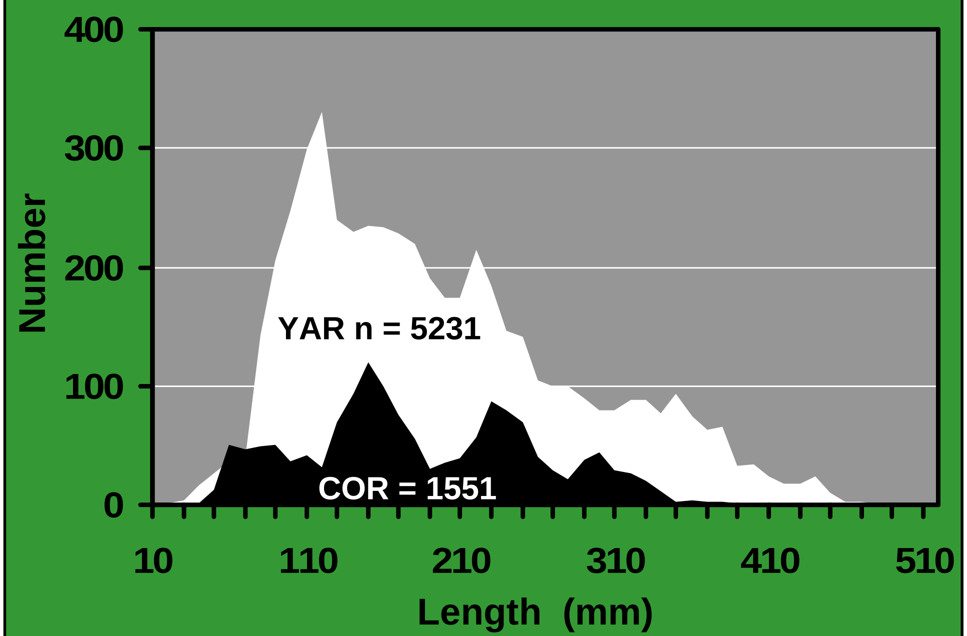

Mean river temperatures:
YAR: Maybell
COR: CO-UT
COR: Cameo
GUR: near GJ
Degrees (C)
25
20
15
10
5
0
growth > 70 F
spawning > 60 F
feeding > 50 F
96
97
98
99
00
01
02
03
04


UT
CO
Craig
Yampa River
RM 51
RM 120
centrachid
sampling
Rifle
Colorado River
RM 196
RM 240
Grand
Junction
RM 188
RM 132
Gunnison River

Centrarchid relative abundance: 2004



SMB length frequency - 2004:



SMB age & growth:

Otoliths - saggita

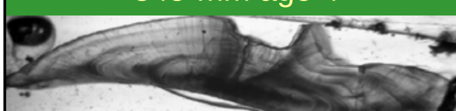
- ➢ taken from sub-sample of SMB across length range
- ➢ handled only with plastic forceps
- ➢ stored in 2-ml micro-centrifuge tubes
- ➢ embedded in Epo-Fix, transverse sectioned with Isomet Saw, polished & lapped
- ➢ digitally enhanced & photographed using Image-Pro Plus
- ➢ 2 readers established age

YAR | **COR**

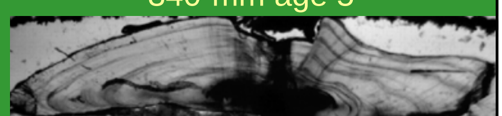
241-mm age 3

211-mm age 3

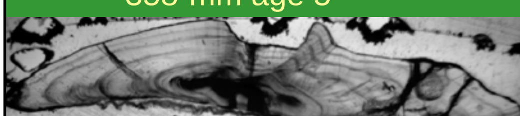
345-mm age 4



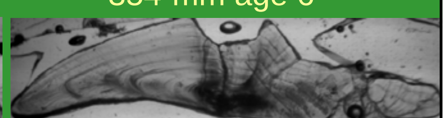
340-mm age 5



358-mm age 5

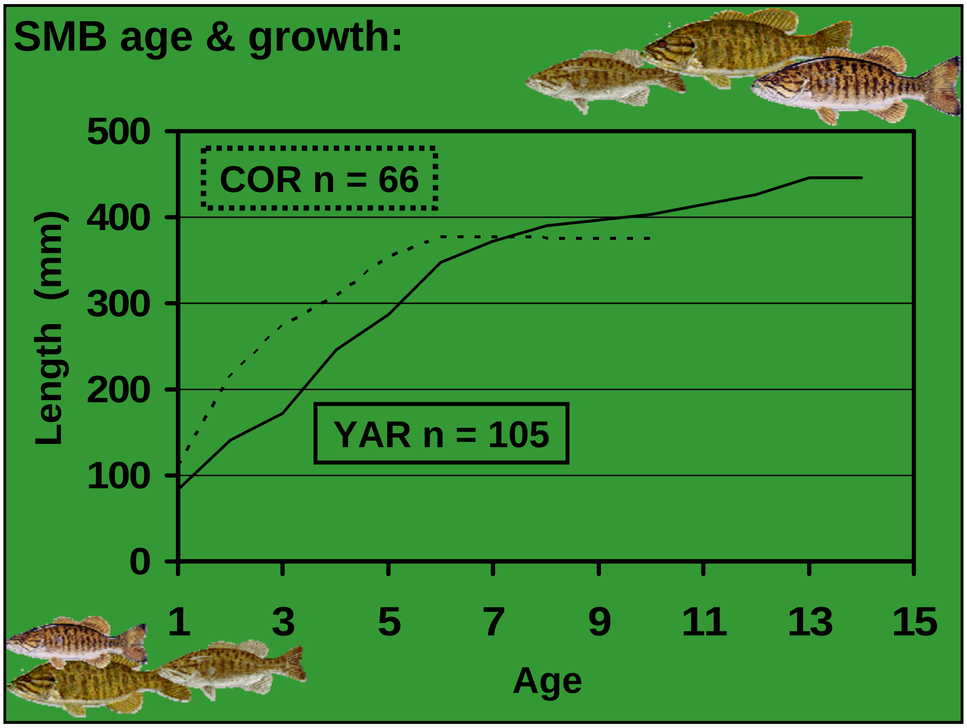


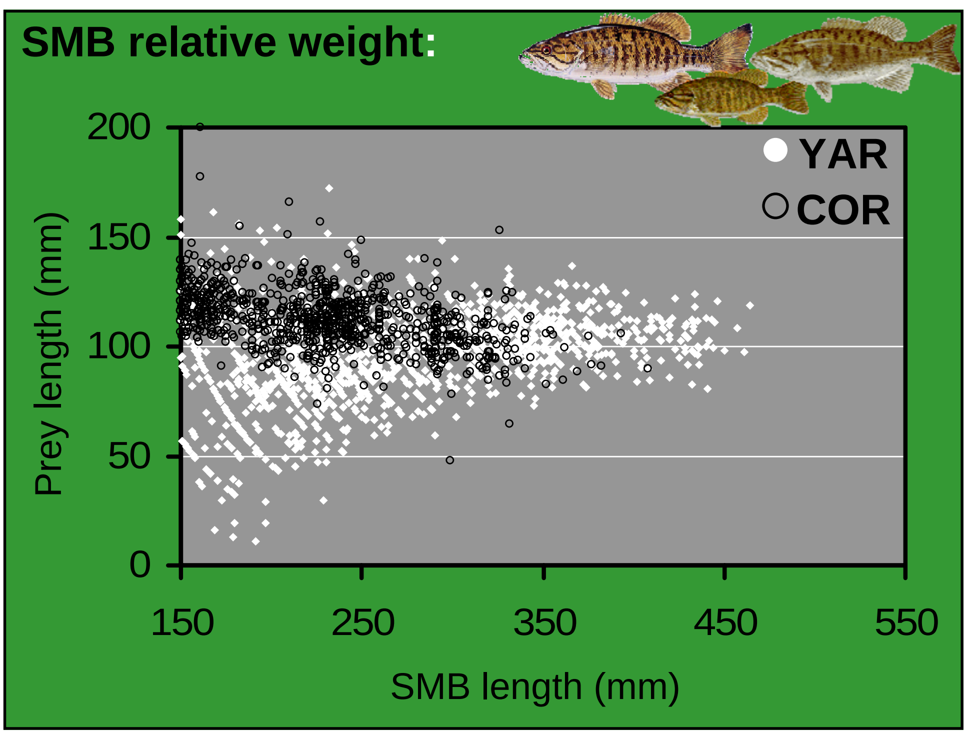
354-mm age 6



446-mm age 14

375-mm age 10

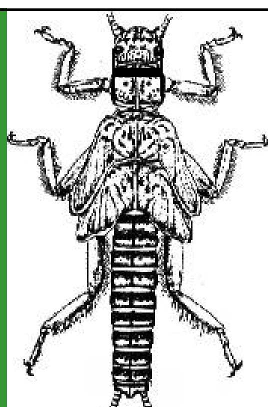

SMB age & growth:
COR n = 66
YAR n = 105
Length (mm)
500
400
300
200
100
0
1
3
5
7
9
11
13
15
Age


SMB relative weight:
YAR
COR
Prey length (mm)
200
150
100
50
0
150
250
350
450
550
SMB length (mm)

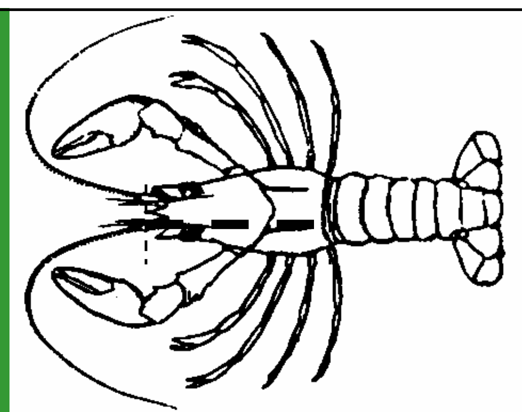
SMB diet:

Stomach & contents

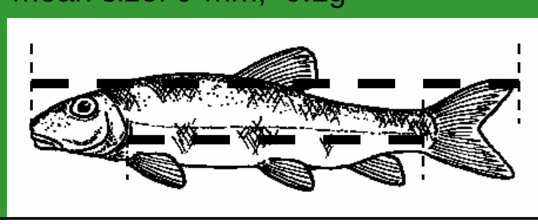
- preserved in formalin
- analyzed at CSU
- invertebrates identified to lowest taxa feasible & measured (northern crayfish = *Orconectes virilis*)
- fish identified to species if feasible & measured
- 3 major prey categories: insects, crayfish & fish
- regression equations used to estimate prey biomass
- insect = 400 cal/kg; fish = 600 cal/kg; crayfish = 800 cal/kg



Head capsule width
mean size: 9-mm, 0.1g



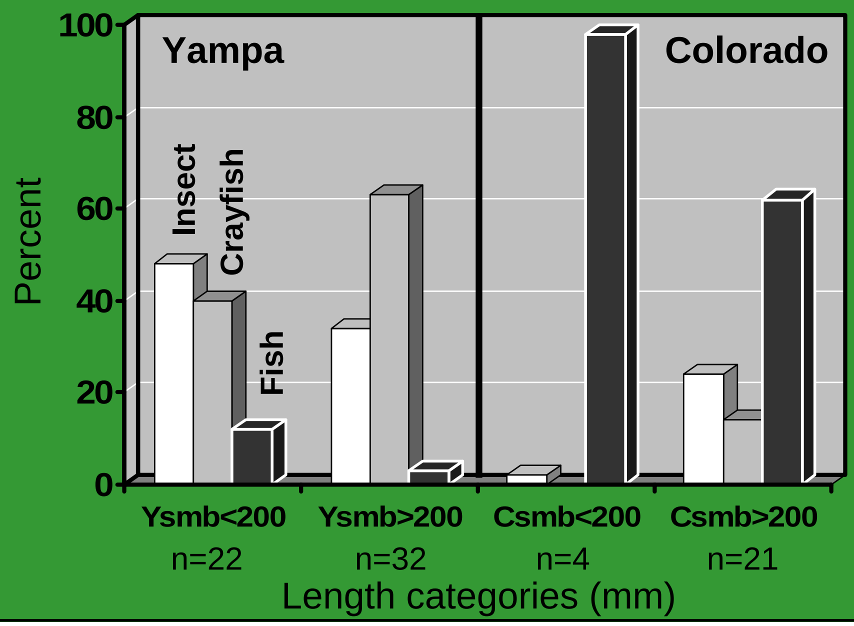
Carapace length
mean size: 40-mm, 6.4g



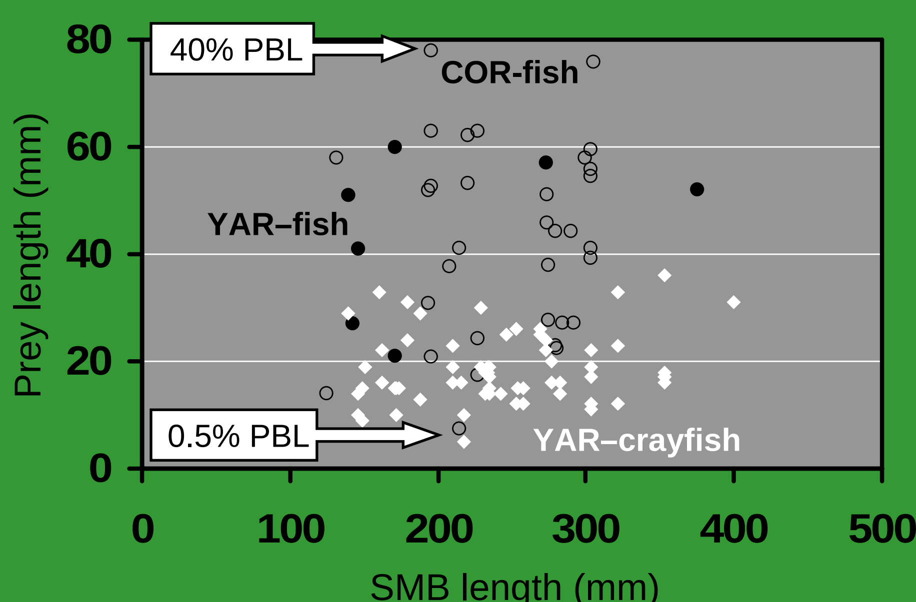
Total length

Backbone length
mean size: 40-mm, 1.2g

SMB diet:



SMB diet:



Characteristic		Population productivity		
		Low	Average	High
ENVIRONMENTAL FACTORS				
Latitude	N^{o}	>45	Y 37-45 C	<37
Elevation	m	Y >1000 C	300-1000	<300
Air temp.	C^{o}	Y <7	7-13 C	>13
POPULATION PARAMETERS				
Age@ 280mm		Y >4.5	3.3-4.5 C	<3.3
Ave. PSD		<30	Y 30-70 C	>70
Nat. mortality		Y >0.6	0.2-0.6 C	<0.2
EXPLOITATION RESPONSE				
Numbers		Y HIGH	MOD C	LOW
Comp. Grow.		Y NO-LOW	MOD C	HIGH
Comp. Mort.		Y NO-LOW	MOD C	HIGH

Conclusions:

- river's water temperature most important factor in SMB recruitment & growth
- subtle increases in water temperature responsible for enhancing SMB abundance
- higher river discharge typically associated with lower river water temperatures
- recent drought conditions reduced flows & generally increased river temperatures
- return to average or above-average flow trends will reduce river temperatures
- reduced river temperatures will reduce SMB recruitment & favor SMB control

Conclusions (con't):

YAR, COR & GUR

- river discharge higher or mean water temperature lower prior to 2000
- river discharge lower or mean water temperature higher since 2000

YAR & COR

- SMB proliferation limited prior to 2000 due lower mean annual water temperatures
- SMB abundance exaggerated since 2004 due more favorable water temperatures
- SMB capable of rapid expansion & high level of predation on SBF & native fish

Conclusions (con't):

YAR

- lower discharge favors warming, but temperature moderated by higher elevation
- SMB abundance increased notably since 1992 escapement/entrainment from Elkhead
- SMB abundance exploded since 2000 due to increased river temperature
- SMB growth limited by cooler river temperatures, despite high body condition
- body condition of larger SMB sustained by abundant northern crayfish
- increase & explosion of SMB associated with decline of SBF & juvenile native fish

Conclusions (con't):

COR – Grand Valley to CO-UT Stateline

- higher discharge favors cooling, but water temperature warmed due to lower elevation
- warmer river = earlier SMB spawning, longer growing season & more recruitment
- rapid increase in SMB abundance/range due to higher temperatures since 2000
- SMB growth comparatively rapid due to temperature & adequate prey
- body condition & growth of largest SMB limited by low availability of northern crayfish
- SMB expansion & predation on SBF will adversely impact native fish community

Conclusions (con't):

COR - above Cameo

- coolest river temperatures, despite comparatively low discharge & elevation
- recent, but limited, expansion of SMB facilitated by higher river temperature

GUR – above Whitewater

- river temperature generally similar to YAR & increased since 2000
- recent & past river conditions would allow SMB establishment

Recommendations:

- evaluations of response by fish community to control of SMB should focus initially on SBF due to prey size
- YAR - lack of compensatory response by SMB to removal suggests focused removal of adult SMB will hasten their decline upon return of average flows due to reduced recruitment, growth & replacement of adults
- COR – moderate compensatory response due to high growth rate of juvenile SMB suggests greater emphasis on removal of YOY & juvenile SMB warranted, including potential off-stem sources
- use caution in “warming” thermographs

Recommendations (con’t):

- flow permitting, consider removal of SMB during spawning late-May through June to disrupt nest success in addition to removal
- document SMB age/size at maturity, track in relation to removal & adapt control effort
- crayfish density should be documented to study response of this nonnative species to SMB expansion or control, or flow
- “maintenance” removal of SMB adults may be adequate to control or reduce SMB in COR above Cameo due to cool flows
- consider gradient in relationship to hot spots or control reaches for SMB

APPENDIX D

COLORADO RIVER RECOVERY PROGRAM, FY 2005 ANNUAL PROJECT REPORT, PROJECT NO. C-18/19, PROJECT TITLE: STABLE ISOTOPE ANALYSIS OF CENTRARCHID CONCENTRATION AREAS

I. Project Title: Stable Isotope Analysis of Centrarchid Concentration Areas

II. Principal Investigators:

Anita Martinez
Colorado Division of Wildlife
711 Independent Ave.
Grand Junction, CO 81505
Phone: 970-255-6143
FAX: 970-255-6111
anita.martinez@state.co.us

Patrick J. Martinez (lead)
Colorado Div. of Wildlife
711 Independent Ave.
Grand Junction, CO 81505
Phone: 970-255-6141
FAX: 970-255-6111
pat.martinez@state.co.us

III. Project Summary:

Non-native centrarchids, including largemouth bass *Micropterus salmoides*, bluegill *Lepomis macrochirus*, green sunfish *L. cyanellus*, and black crappie *Pomoxis nigromaculatus* occur in a variety of aquatic habitats throughout the Grand Valley reach of the Colorado River and represent a significant predatory threat to young life stages of endangered fishes. However, it has been uncertain whether centrarchid presence in critical riverine habitats was primarily the result of escapement from off-channel ponds or resident "in-stream" reproduction. The goal of this project is to identify centrarchid sources to critical riverine habitats and thereby facilitate fiscally and ecologically efficient control of centrarchids through improved knowledge of their sources. The draft final report for this project is due December 15, 2005. The format of the report will be two draft manuscripts prepared for submission to peer-review journals and a section including conclusions and recommendations.

IV. Study schedule:

FY 2003:

Task 1. Post-doctoral scientist Dr. Gregory Whitledge was hired at Colorado State University (CSU) under the supervision of Dr. Bret Johnson, professor in the Department of Fishery and Wildlife Biology. Dr. Whitledge prescribed sample sites and numbers, oversaw or performed isotopic/microchemical analyses of water and otolith samples, evaluated data and provided findings.

An annual report was submitted to Pat Nelson on 26 November 2003. Presentation on project methodology was delivered at Upper Basin Researchers Meeting in Moab, January 2004.

FY 2004:

Task 1. Anita Martinez, CDOW Nonnative Fish Control biologist led field sampling access and collection efforts in cooperation with Pat Martinez, CDOW Aquatic Researcher and field technicians. Consultation with Dr.s Whitledge and Johnson guided all sampling efforts. Cooperation with U.S. Fish and Wildlife Service – Colorado River Fishery Project personnel, under the supervision of Bob Burdick, greatly facilitated sample collection. Sampling site selection and number of samples was based on several factors:

1. the findings of prior isotopic work by Martinez et al. (2001),
2. preliminary floodplain pond/riverine biota isotopic data from 2001-2002 (Martinez 2003),
3. the results of work on centrarchid concentration areas (Martinez 2004),
4. the GIS analysis of fish distributions resulting from the Nonnative Fish Regulation evaluation (Martinez and Nibbelink 2004), and
5. obtaining access from private/municipal landowners.

Task 2. Sampling was conducted approximately two weeks per month, as needed.

Task 3. Dr. Whitledge oversaw or performed analyses and interpretation of isotopic/microchemical data obtained from water and otolith samples. Work involved year round sample and data analysis, including advising CDOW on field sample collection and ongoing sample preparation for isotopic analyses. Dr. Whitledge submitted brief quarterly reports to P. Martinez, CDOW, to maintain coordination and progress for this project. An annual report was submitted to Pat Nelson on 12 November 2004. Preliminary project findings were presented at Upper Basin Researchers Meeting in Grand Junction, January 2005.

FY 2005:

Task 1. A. Martinez maintained records of water and otolith samples sent to Dr. Whitledge for analyses. Final sample preparation and analyses were performed based on results from 2003 and 2004 sampling efforts and analyses results.

Task 2. Minimal field sampling was required in 2005.

Task 3. Dr. Whitledge performed analyses and interpretation of isotopic/microchemical data and submitted brief quarterly reports to P. Martinez to maintain project coordination and progress toward completion. Research and findings will be incorporated into two draft manuscripts for submission to peer-reviewed journals. These manuscripts will constitute the body of the draft final report along with project conclusions and recommendations. This annual report was submitted to Pat Nelson on 16 November 2005.

Reporting: Draft final report due to Pat Nelson – December 15, 2005. Note that this date will likely be delayed due to Dr. Johnson's involvement in a serious accident – if this occurs, subsequent dates may also be adjusted.

1st revised draft final report to peer review – January 15, 2006 (peer reviews due to author – February 15, 2006; Biology Committee comments due – March 3, 2006).

2nd revised draft final report to Biology Committee – April 3, 2006

V. Relationship to RIPRAP:

This project addressed the movement of nonnative fish into river reaches of critical habitat from floodplain habitats known to support large numbers of Centrarchidae fish species. Nonnative fishes, including largemouth bass, bluegill, green sunfish, and black crappie are known to occur in floodplain ponds, backwaters, beaver ponds, washes and irrigation drainage ditches throughout the Grand Valley reach of the Colorado River. In riverine habitats, these fish species are most commonly associated with backwaters or slow-moving side channels. It is in these low-velocity riverine habitats that centrarchids are believed to pose a significant predatory threat to the young life stages of endangered and other native fishes. However, it was uncertain to what extent the presence of centrarchid species in low-velocity riverine habitats is the result of escapement from off-channel ponds or resident "in-stream" reproduction. Overall, this study has identified the sources of nonnative fishes in the Colorado River through isotopic/microchemical analysis of water and otolith samples under the riverine flow and floodplain conditions that existed during the timeframe of this project. These conditions were drier than normal, thus riverine flows were lower and the communication of these flows with floodplain features, including ponds, were likely less than during conditions of average or above average discharge.

<u>General Recovery Program Support Action Plan</u>:

III. Reduce negative impacts of nonnative fishes and sport fish management activities.

III.A.2. Identify and implement viable control measures.

Colorado River Action Plan: Main stem

III. Reduce negative impacts of nonnative fishes and sport fish management activities.

III.A.4.a. Evaluate sources of nonnative fishes and make recommendations.

VI. Accomplishment of FY2005 Tasks and Deliverables, Discussion of Initial Findings and Shortcomings:

A. Martinez coordinated tracking/transfer of water/otolith samples from CDOW, USFWS, and CSU field collections to Dr. Whitledge at CSU. P. Martinez oversaw otolith sectioning and preparation for submission to CSU. Dr. Whitledge coordinated completion of water samples analyses at various labs and performed isotopic/microchemical analyses of otoliths. Dr. Whitledge analyzed data and provided interpretations in conjunction with Dr. Johnson. Dr. Whitledge produced two draft manuscripts, including contributions from co-authors Dr. Johnson, P. Martinez and A, Martinez, summarizing the results and findings of this project.

Field sampling and sample analyses provided data to address the key project components as indicated below:

1. Determine whether the origins and movement patterns (collectively termed provenance) of centrarchids in the Grand Valley reach of the Colorado River can be identified using stable isotope and/or microchemical analyses.

Stable hydrogen isotope ratio ($^2H/^1H$ or D/H, expressed as δD, where ^2H=D=deuterium or heavy hydrogen) represents a naturally occurring environmental marker that has not been applied in any published studies of fish provenance. Results from this project indicate that stable hydrogen isotopic composition in fish otoliths from the Grand Valley reach of the Colorado River can distinguish whether a specific fish spent the bulk of its life in riverine habitats (mainstem, backwater, beaver pond) vs. floodplain ponds. Pond water samples were enriched in ^2H compared to water collected in the three riverine habitats and ranges of pond and riverine water δD values did not overlap. Median water δD was significantly greater for ponds compared to beaver ponds, backwaters, and the river main channel. Water δD was enriched in ^2H in ponds compared to riverine habitats due to greater opportunity for evaporative fractionation to be expressed in ponds as a result of their longer water residence time. A highly significant linear relationship exists between fish otolith values and δD signatures of the waters fish inhabit. Water samples and one otolith from each pair were analyzed for δD using an isotope ratio mass spectrometer. Otoliths < 2.5 mg were analyzed whole while otoliths > 2.5 mg were ground to obtain a 2-2.5 mg core sample centered on the otolith nucleus. The development of this methodology is summarized and discussed in Whitledge, G. W., B. M. Johnson, and P. J. Martinez. IN REVIEW. Stable hydrogen isotopic composition of fishes reflects that of their environment, submitted to Canadian Journal of Fisheries and Aquatic Sciences.

Identifying the origins of nonnative centrarchids based on δD were refined using a natural marker based on strontium (^{88}Sr) and calcium (^{44}Ca) ratios. δD distinguishes pond- from riverine-resident fish, whereas Sr:Ca differentiates between residence in high-salinity habitats (including some ponds) and low-salinity areas. A relationship between otolith Sr:Ca ratio and environmental salinity was developed from known provenance centrarchids from ponds our study area and the highest salinity value recorded in our water samples from riverine habitats (1.2 $^{o}/_{oo}$). The second otolith was embedded in epoxy, sectioned transversely and polished to reveal annuli. These thin sections were then mounted on acid-washed glass slides and ultrasonically cleaned in ultrapure water prior to laser ablating transects from the otolith nucleus to its edge for analyses using an inductively coupled plasma mass spectrometer (LA-ICPMS). Otolith Sr:Ca ratios complemented otolith δD analysis by identifying fish that resided in environments (some ponds, irrigation ditches) whose salinity exceeded that of riverine habitats. A threshold Sr:Ca ratio was used to distinguish periods of residence in high-salinity (salinity exceeding that of riverine habitats, high Sr:Ca) versus low-salinity (salinity not exceeding that of riverine habitats, low Sr:Ca) environments. Age at immigration was determined for individuals that showed evidence of movement from high-salinity to riverine environments by associating locations of abrupt declines in otolith Sr:Ca ratio along laser-ablated transects in relation to annuli.

2. Determine the proportion of centrarchids in backwater and main channel habitats within the study area that originated from out-of-channel ponds versus in-channel habitats.

Of the 368 centrarchids collected in backwater and Colorado River main channel habitats, 82 (22%) possessed an otolith core δD signature characteristic of ponds, 218 (59%) exhibited a signature expected for riverine-resident fish, and 68 (19%) were of uncertain origin. For fishes collected in backwaters, presence or absence of direct inflowing ditches or tributary washes did not have a significant effect on the relative proportions of individuals with pond, uncertain, and riverine otolith core signatures. However, significant differences in relative proportions of centrarchids with pond, uncertain, and riverine otolith core δD signatures were present among species (Figure 1). Approximately 70% of largemouth bass and bluegill collected exhibited an otolith core δD signature expected for riverine-resident fish, with 19% possessing a pond δD signature in the otolith core, and 10-11% being of uncertain provenance. In contrast, 53% of black crappie collected had a pond otolith core δD signature, with 26% having a riverine otolith core δD signature and 21% of uncertain origin. Fifty-three percent of green sunfish examined displayed a riverine otolith core δD signature, with 23% showing evidence of emigration from ponds and 24% of unknown provenance.

The 1-2 mg sample size requirement for δD analysis of otoliths by bulk analysis using isotope-ratio mass spectrometry may limit the resolution of δD as a natural marker of fish's full environmental history. Resolution of the approach used for otolith δD analysis corresponded to approximately the first year of a fish's life based on otolith masses of known age centrarchids collected in our study area. However, a potential shortcoming is that the otoliths of recently hatched fish or the otolith core of a larger fish, representative of the fish's first months of life, may contain too little material for δD analyses. Thus, the possibility exists that individuals that emigrated from ponds very early during age-0 may have been misclassified as being of riverine origin, because material indicative of riverine residence could dominate the otolith core signature under such a scenario despite the fact that the fish originated in a pond. While other advancements in microsampling techniques may overcome this situation with δD, such as the use of an ion microprobe, some verification of otolith core δD signatures can be obtained by elemental ratio analyses (Sr:Ca).

Otolith thin sections from 210 centrarchids collected from Colorado River backwaters were analyzed for Sr:Ca ratio using LA-ICPMS. All 79 individuals with riverine otolith core δD signatures exhibited a riverine otolith core Sr:Ca ratio consistent with that expected for riverine-resident fish. Eight fish whose origins were uncertain based on δD analysis exhibited elevated otolith core Sr:Ca ratios characteristic of residence in high-salinity ponds, resolving uncertainty regarding provenance of these individuals. The 50 centrarchids with pond δD signatures in their otolith cores exhibited a wide range of otolith core Sr:Ca ratios. Median otolith core Sr:Ca ratios were significantly higher for fish with pond and uncertain (59 fish) otolith core δD signatures compared to fish with riverine otolith core δD signatures. Maximum estimated salinity corresponding to otolith core Sr:Ca ratios was highest for black crappie, intermediate for green sunfish and bluegill, and lowest for largemouth bass (Table 1).

The relative abundance of fish with riverine otolith core δD and Sr:Ca signatures indicates that low-velocity backwater and beaver pond habitats are likely the primary source of most centrarchids (recently invading smallmouth bass *Micropterus dolomiei* were not part of this investigation) inhabiting the Colorado River in our study area. All four species analyzed in this study are associated with low-velocity, river margin habitats in rivers and construct nests in these areas. Black crappie was the only species for which the majority of individuals collected showed evidence of having emigrated from ponds, which may be due to their tendency to spawn in or near vegetation. Macrophytes are common in Grand Valley ponds but are rare or absent in backwaters (Martinez et al. 2001).

3. If feasible, pinpoint "hotspots" where centrarchids present in connected backwaters and main channel habitats have originated by narrowing the list of possible sources (e.g. from "off-channel ponds" to specific ponds or groups of ponds).

Relative proportions of fish with pond, uncertain, and riverine otolith core δD signatures were not significantly different above versus within the Grand Valley or among fishes collected in river main channel versus backwater habitats. However, 60 of the 82 fish (73%) with pond δD signatures in their otolith cores were collected below the Gunnison River confluence. Relative proportions of fish with pond, uncertain, and riverine otolith core δD signatures were significantly different above versus below the Gunnison River confluence, with the proportions of pond and uncertain provenance individuals higher below the Gunnison River confluence than above (Figure 2). Twenty-two fish exhibited evidence of emigration from high-salinity habitats to the Colorado River based on changes in otolith Sr:Ca ratios along laser-ablated transects. Seventeen (77%) of these individuals were collected below the Gunnison River confluence. At least four individuals were determined to have immigrated to riverine habitats at each age from 0 to 3 years. Of these, all five fish that showed evidence of immigration to riverine habitats at age 3 were black crappie.

Pinpointing locations within the study area that were contributing large numbers of nonnatives was deemed important for focusing control efforts to problem areas. The greater proportion of fishes with pond otolith core δD signatures collected below in comparison to above the Gunnison River confluence is not likely the result of the Gunnison River contributing substantial numbers of pond-origin fish to the Colorado River, as relatively few ponds are present along the Gunnison River (Martinez and Nibbelink 2004). Rather, the higher incidence of centrarchids emigrating from ponds to the Colorado River below the Gunnison River confluence is likely related to the greater number of ponds and the comparatively high number of irrigation ditches and washes that enter the Colorado River downstream from where the Gunnison River enters (Martinez 2004). Another possible explanation is that the generally larger, more structurally complex backwaters found below the Gunnison River confluence may be more attractive to centrarchids or more conducive to their growth or survival than the generally smaller, and structurally simpler backwaters found above the Gunnison confluence. The centrarchid species collected in this study are typically associated with structure. Lack of a significant association between relative frequencies of individuals with pond, riverine, and uncertain otolith core δ^2H signatures and presence or absence of direct inflowing ditches or washes to backwaters suggests that centrarchids that immigrate to riverine habitats may be selecting the best available habitats rather than simply occupying those closest to their point of entry to the river.

The high proportion (83%) of pond emigrants that left ponds with low δD water values likely reflects a higher probability of immigration to riverine habitats from ponds that are closely associated hydrologically with the Colorado River. Differences in water δ^2D among ponds reflected varying degrees of hydrologic isolation from the Colorado River. Centrarchids with pond δD signatures in their otolith cores exhibited a wide range of otolith core Sr:Ca ratios, reflecting emigration from ponds with differing salinities. Most individuals that exhibited evidence of emigration from high-salinity habitats were collected below the Gunnison River confluence, reflecting the relative abundance of high-salinity ponds and washes in that area. Significantly higher mean otolith core Sr:Ca ratio for black crappie compared to the other three species indicates a greater tendency for black crappie to originate in high-salinity ponds. Although results of δD analyses indicate that any effort to control centrarchid escapement from ponds should be directed primarily toward locations closely associated with the river, our findings do not provide any more specific evidence that particular ponds or groups of ponds are disproportionately contributing to centrarchid abundance in riverine habitats. While largemouth bass displayed predominately pond δD signatures in their otolith cores, they were also associated with the lowest Sr:Ca ratios and salinities. This suggests that if additional control measures were deemed necessary to control movement of this species from ponds, such efforts could be applied on ponds with a salinity < 1.8 $^{o}/_{oo}$, the maximum salinity associated with largemouth bass (Table 1).

No clear pattern with respect to age at immigration was evident from Sr:Ca data, although results indicate that centrarchids have the capacity to move into critical habitat from age-0 to at least age-3. However, the increasing proportion of centrarchids with pond otolith core δD signatures with increasing fish age (Figure 3) and the significantly greater median lengths of individuals with pond otolith core δD signatures compared to those with riverine otolith core δD signatures for three of the four species may be a consequence inter-annual variation in river hydrology and its potential effects on centrarchid reproduction, larval nursery, and immigration to the river. The upper Colorado River basin experienced below average precipitation and mean annual discharge in the upper Colorado River was below average from 2000-2004. During dry years, decreased river-pond connectivity and increased temporal and spatial extent of low-velocity habitat in the river would be expected. Such conditions could be more favorable for centrarchid reproduction and recruitment in riverine habitats due to decreased probability of scouring flows and flushing of larvae from nesting sites while simultaneously limiting access to the river for pond-dwelling fish. Thus, the recent drought may explain why the majority of the smallest, youngest fish carried a riverine δD signature in the otolith core.

The fact that age-4 and older fish had the highest proportion of individuals with pond otolith core signatures and that the largest individuals of three species (particularly largemouth bass and black crappie) almost always carried a pond otolith core δD signature suggests that although the percentage of pond-origin fish in riverine habitats was relatively low at the time of our collections, it may have been higher prior to the current drought and could increase again during years with normal or above average precipitation and river discharge. During wetter years, increased river-pond connectivity and a reduction in temporal and spatial extent of low-velocity habitat in the river would be expected. These conditions would be anticipated to be detrimental to centrarchid reproduction and recruitment in riverine habitats while enhancing access to the river for pond-dwelling fish.

VII. Recommendations:

1. Complete and submit draft manuscript, Whitledge, G. W., B. M. Johnson, P. J. Martinez, and A. Martinez. Provenance of non-native fishes in the upper Colorado River revealed by stable isotope and microchemical analyses of otoliths, for peer-review publication.

2.. Efforts to control abundance of centrarchids (except black crappie and smallmouth bass) in critical habitat for native threatened and endangered fishes should emphasize backwaters and beaver ponds that contain abundant structure irrespective of presence or absence of direct tributaries rather than focusing on those with inflowing washes or ditches.

3. Any efforts to control centrarchid escapement from ponds to the Colorado River should focus on the reach below the Gunnison River confluence, although such actions should be secondary to management activities in riverine habitats given that the majority of centrarchids examined in this study exhibited riverine otolith core δD signatures.

4. If additional control measures were deemed necessary to control movement of largemouth bass from ponds, such efforts could be applied on ponds with a salinity < 1.8 $^{o}/_{oo}$, thus narrowing the number of candidate ponds for treatment.

5. Management of black crappie abundance, in particular, within critical habitat would require an emphasis on restricting escapement from ponds; however, black crappie are the least numerous of the five centrarchids present in our study area.

6. Although results of this project indicate that centrarchid control efforts in the upper Colorado River should focus on riverine habitats when hydrologic conditions are similar to those during this study, reevaluation of relative proportions of riverine-dwelling centrarchids with pond and riverine otolith core signatures is recommended during and immediately following years of above average precipitation and river discharge. Such a follow-up study would be useful for assessing whether management of centrarchid abundance in critical habitat should always be focused within riverine habitats themselves or if

additional emphasis should be placed on controlling centrarchid escapement from ponds to curtail immigration to riverine habitats during high-water years.

VIII. Project Status:

This project should be considered "scheduled for completion". Results address project objectives, provide a basis for management recommendations and funding is sufficient to complete remaining project tasks. A presentation of project findings will be given by P. Martinez or Dr. Johnson at the Upper Basin Researcher's Meeting in Moab, UT in January 2005. A draft final report for this project will be submitted by 15 December 2005, but may be delayed as previously described.

IX. FY2006 Budget Status:

A. Funds Provided: $185,768.00 to Colo. State Univ-CSU. (includes funds "rolled forward" from FY2003)**.** Funds provided to the CDOW for A. Martinez's operations have been expended as outlined in Scope of Work.

B. Funds Expended: $154,774.61 by CSU

C. Difference: $30,993.39 (CSU)

Dr. Whitledge departed in mid-August and is presently an assistant professor in the Fisheries and Illinois Aquaculture Center, Department of Zoology, at Southern Illinois University, in Carbondale, IL. Dr. Johnson has been temporarily sidelined due to injuries sustained in his accident. The remaining funds will be spent for salaries required by Dr.'s Whitledge and Johnson to participate in finalizing manuscripts, conclusions and recommendations which comprise the final report. Some funds are reserved for travel by these professors to attend and participate in Recovery Program workshops and meetings to discuss project findings and management recommendations. Given the implications of low- vs. high-flow scenarios as they relate to the findings of this report, technicians will archive the remaining otoliths samples used in our analyses for potential future comparisons of samples analyzed under normal to high-flow conditions. It is anticipated that up to $3,000 will be consumed in page charges to publish the findings of this research in peer-reviewed journals.

D. Percent of FY2006 Work Completed and Projected Costs to Complete: 90%.

Projected costs to finalize project: $30,993.39 (CSU)

E. Recovery Program Funds Spent for Publication Charges: None to date, anticipate $3,000 for peer-reviewed publication of manuscripts.

X. **Status of Data Submission:** Capture records for fish captured by CDOW in backwaters in 2004 will be submitted to C. McAda by A. Martinez.

XI. **Signed: Patrick J. Martinez, Anita M. Martinez 11-16-05**

LITERATURE CITED

Martinez, A. M. 2004. An evaluation of nonnative fish control treatments in ponds along the Colorado and Gunnison Rivers, 1996-2002. Upper Colorado River Endangered Fish Recovery Program Project No. 18/19, Final Report. Colorado Division of Wildlife, Grand Junction. 60 pp.

Martinez , P. J. 2003. Westslope warmwater fisheries. Federal Aid in Fish and Wildlife Restoration Progress Report. Colorado Division of Wildlife, Grand Junction. 106 pp.

Martinez, P. J., B. M. Johnson, and J. D. Hobgood. 2001. Stable isotope signatures of native and nonnative fishes in upper Colorado River backwaters and ponds. The Southwestern Naturalist 46:311-322.

Martinez, P. J., and N. P. Nibbelink. 2004. Colorado nonnative fish stocking regulation evaluation. Upper Colorado River Endangered Fish Recovery Program Project No. 106, Final Report. Colorado Division of Wildlife, Grand Junction. 84 pp.

Table D-1. Mean (± SE) and maximum otolith core Sr:Ca ratios (mmol/mol) for black crappie (BCR, n=11), bluegill (BGL, n=23), green sunfish (GSF, n=104), and largemouth bass (LMB, n=74). Estimated salinity ($^{o}/_{oo}$) associated with each Sr:Ca ratio is also shown.

Species	Mean Sr:Ca (SE)	Mean salinity	Maximum Sr:Ca	Maximum salinity
BCR	3.11 (0.65)	2.2	7.95	5.0
BGL	1.50 (0.13)	0.8	3.60	3.0
GSF	1.42 (0.06)	0.7	3.70	3.1
LMB	1.28 (0.04)	0.5	2.15	1.8

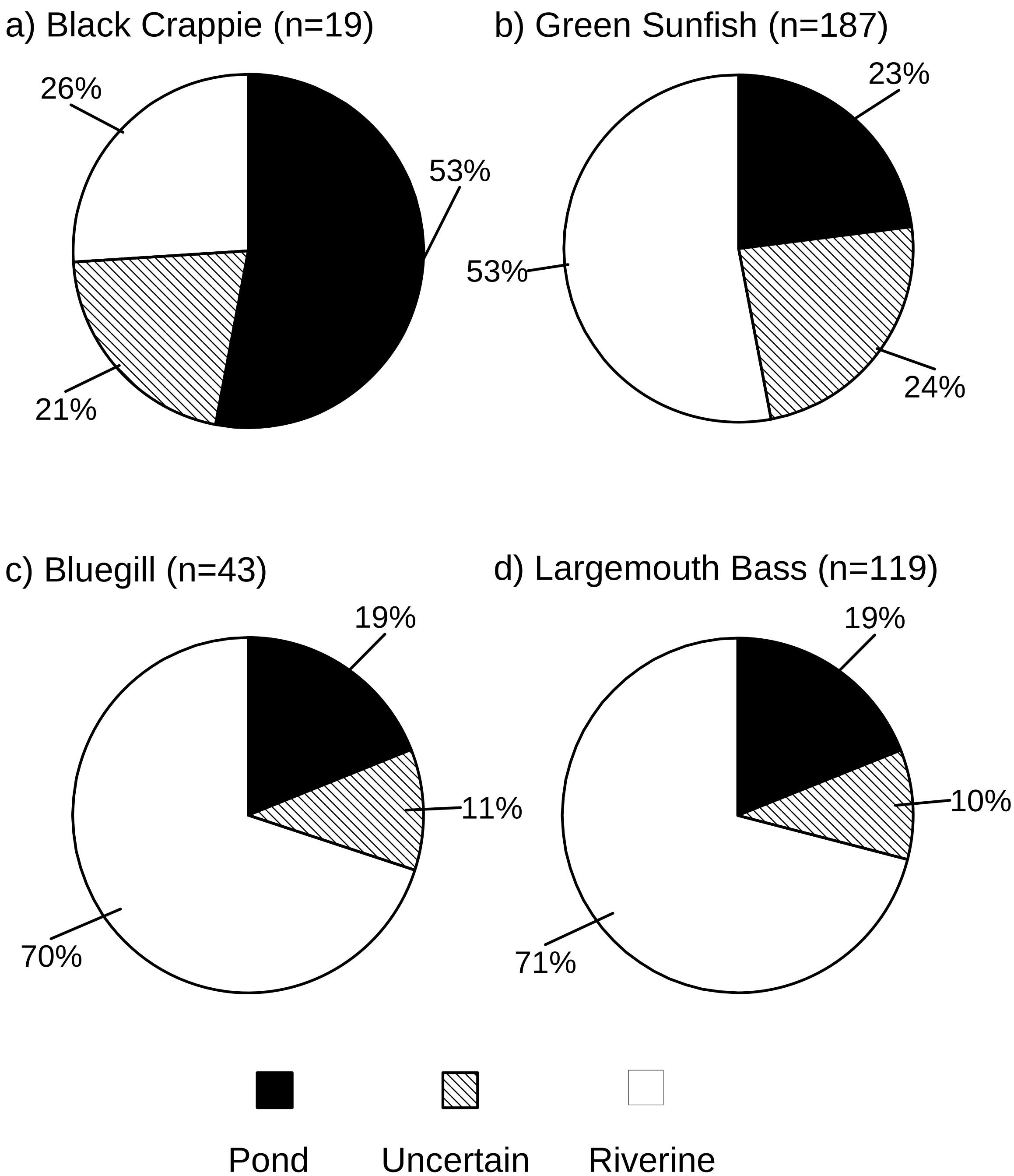


Figure D-1. Relative proportions of black crappie (a), green sunfish (b), bluegill (c), and largemouth bass (d) collected in Colorado River backwater and main channel habitats with pond, uncertain, and riverine otolith core signatures. Number of individuals analyzed (n) is indicated for each species as are percentages contained within each slice.

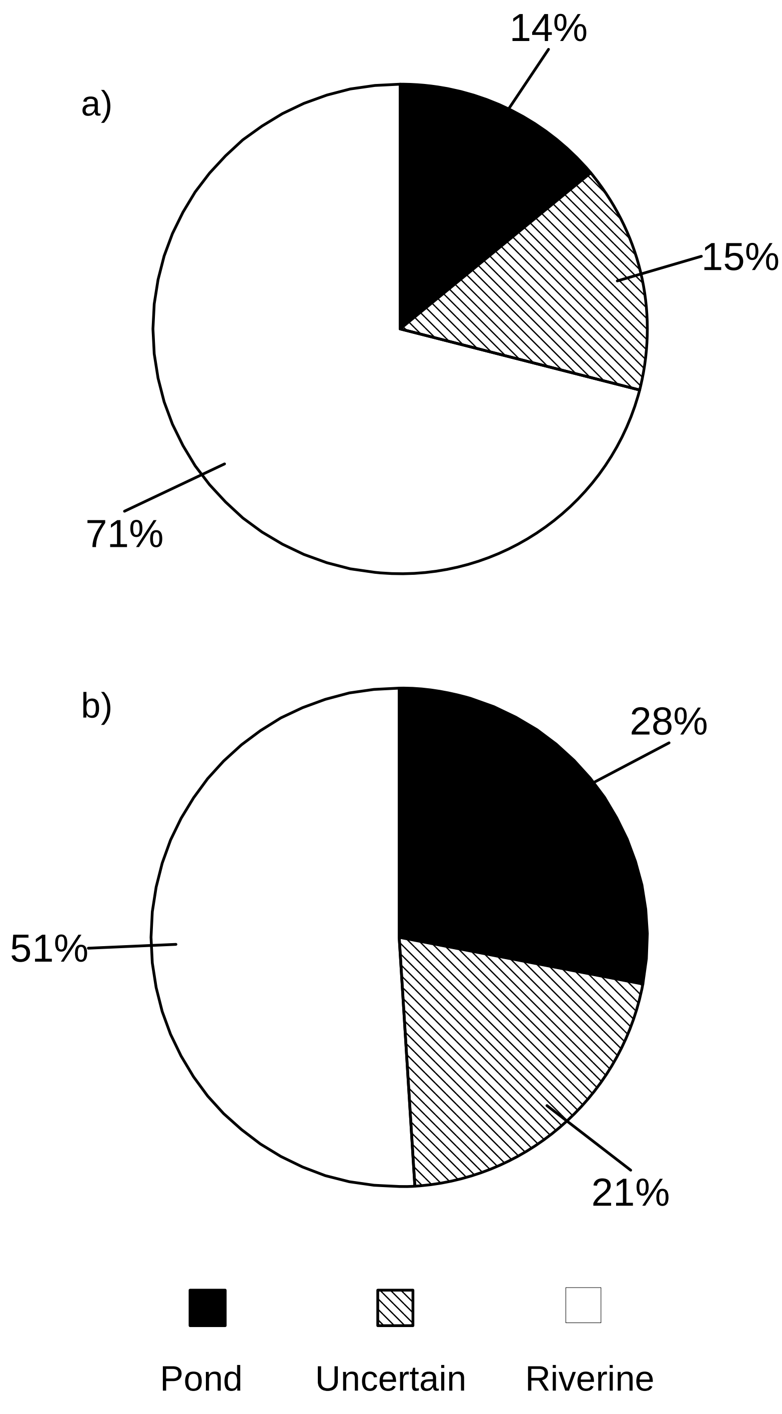


Figure D-2. Otolith core environmental signatures (pond, uncertain, or riverine) for centrarchids collected in Colorado River backwater and main channel habitats above (a) and below (b) the Gunnison River confluence. Numerical values indicate percentages contained within each slice. n=154 and n=214 fish collected above and below the Gunnison River confluence, respectively.

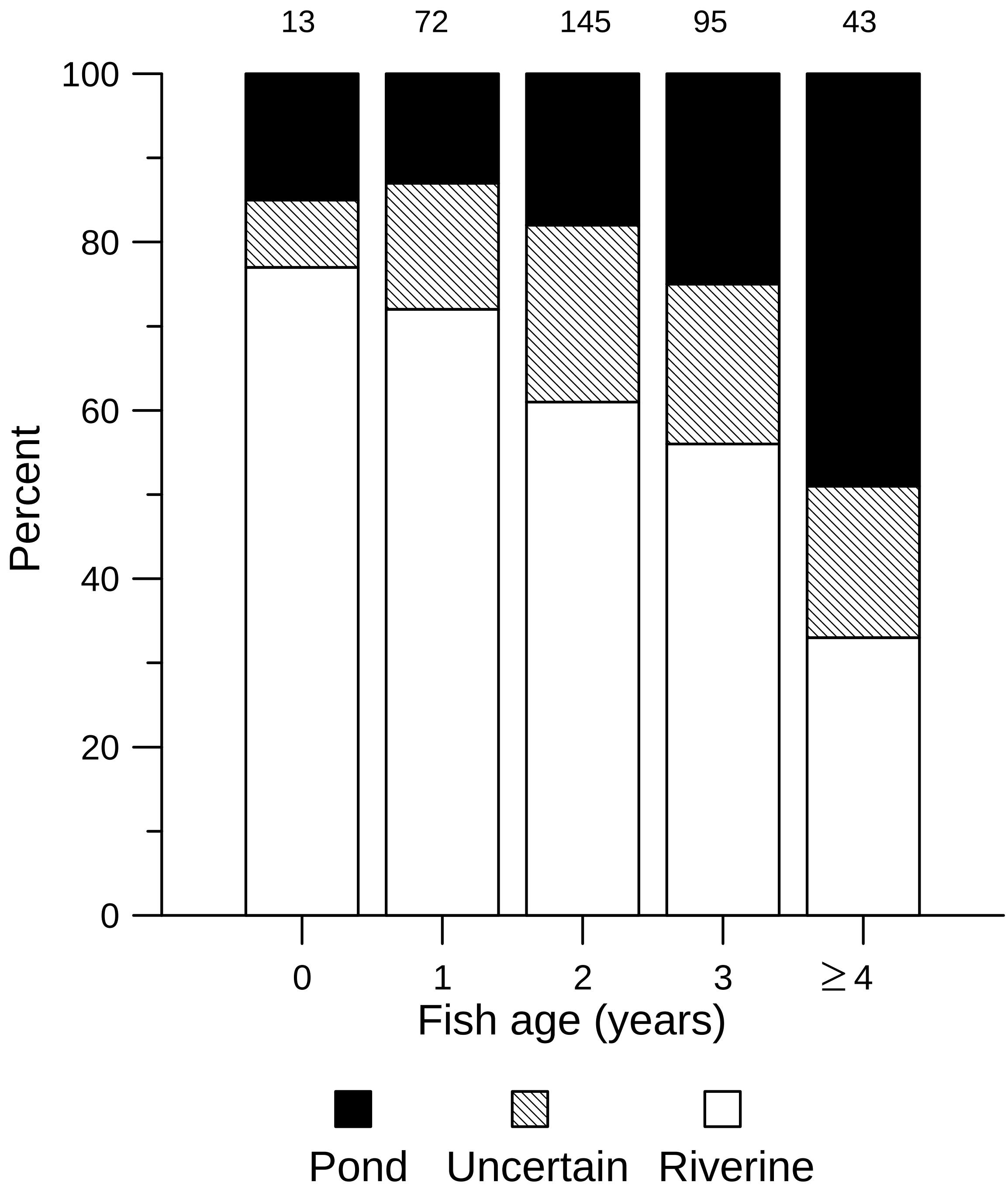


Figure D-3. Relative proportions of centrarchids collected in Colorado River backwater and main channel habitats with pond, uncertiain, and riverine otolith core signatures within fish age classes from age-0 to age ≥ 4 years. Values above bars indicate number of fish analyzed for each age class.

APPENDIX E

ISOTOPIC, ELEMENTAL & BIOENERGETICS STUDIES: APPLICATION OF ISOTOPIC AND ELEMENTAL TECHNIQUES TO IDENTIFY PROVENANCE OF FISHES AND TO FACILITATE BIOENERGETICS PROJECTIONS OF FOOD-WEB IMPACTS OF PISCIVORES IN RIVERS

PREPARED BY:
DR. BRETT M. JOHNSON, DR. GREG WHITLEDGE, MARIO SULLIVAN AND DAN GIBSON-REINEMER

Prepared for:

Patrick J. Martinez, Aquatic Research Biologist, Colorado Division of Wildlife

ISOTOPIC, ELEMENTAL & BIOENERGETICS STUDIES: APPLICATION OF ISOTOPIC AND ELEMENTAL TECHNIQUES TO IDENTIFY PROVENANCE OF FISHES AND TO FACILITATE BIOENERGETICS PROJECTIONS OF FOOD-WEB IMPACTS OF PISCIVORES IN RIVERS AND RESERVOIRS.

Period of Performance: 07/01/04 - 06/30/05

Prepared by:

Dr. Brett M. Johnson, Dr. Greg Whitledge, Mario Sullivan and Dan Gibson-Reinemer

Fisheries Ecology Laboratory
Department of Fishery and Wildlife Biology
Colorado State University, Fort Collins, CO 80523-1474
Voice (970) 491-5002 FAX (970) 491-5091

June 30, 2005

TABLE OF CONTENTS

INTRODUCTION

An understanding of trophic dynamics is fundamental to effective fishery management (Johnson and Martinez 2000). Knowledge of food web interactions is also essential for evaluating the importance of competitive and predatory relationships among native and nonnative fishes. This report summarizes the third year of research developing, refining and applying new methodologies for the study of trophic dynamics in rivers and reservoirs in Colorado. Results of work developing techniques to trace origins and movement patterns (provenance) of invasive nonnative fishes are also presented.

ISOTOPIC ANALYSIS OF SMB TISSUES

Isotopic signatures continue to support the hypothesis that smallmouth bass from the Yampa River derive most of their energy from crayfish (Figure 1, 2).

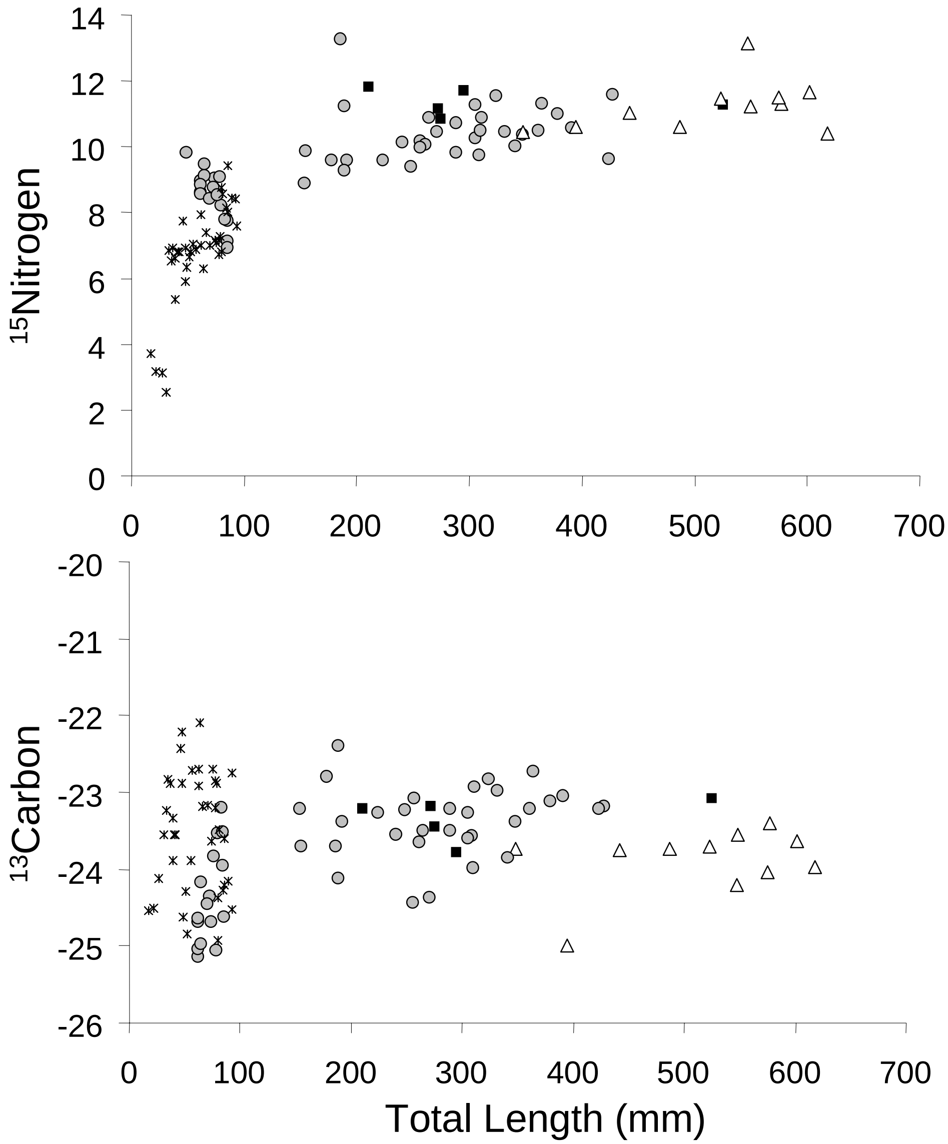


Figure E-1. Carbon and nitrogen stable isotope signatures in smallmouth bass (circles), northern pike (squares), channel catfish (triangles), and crayfish (stars) sampled from the Yampa River during September and October, 2001-2004.

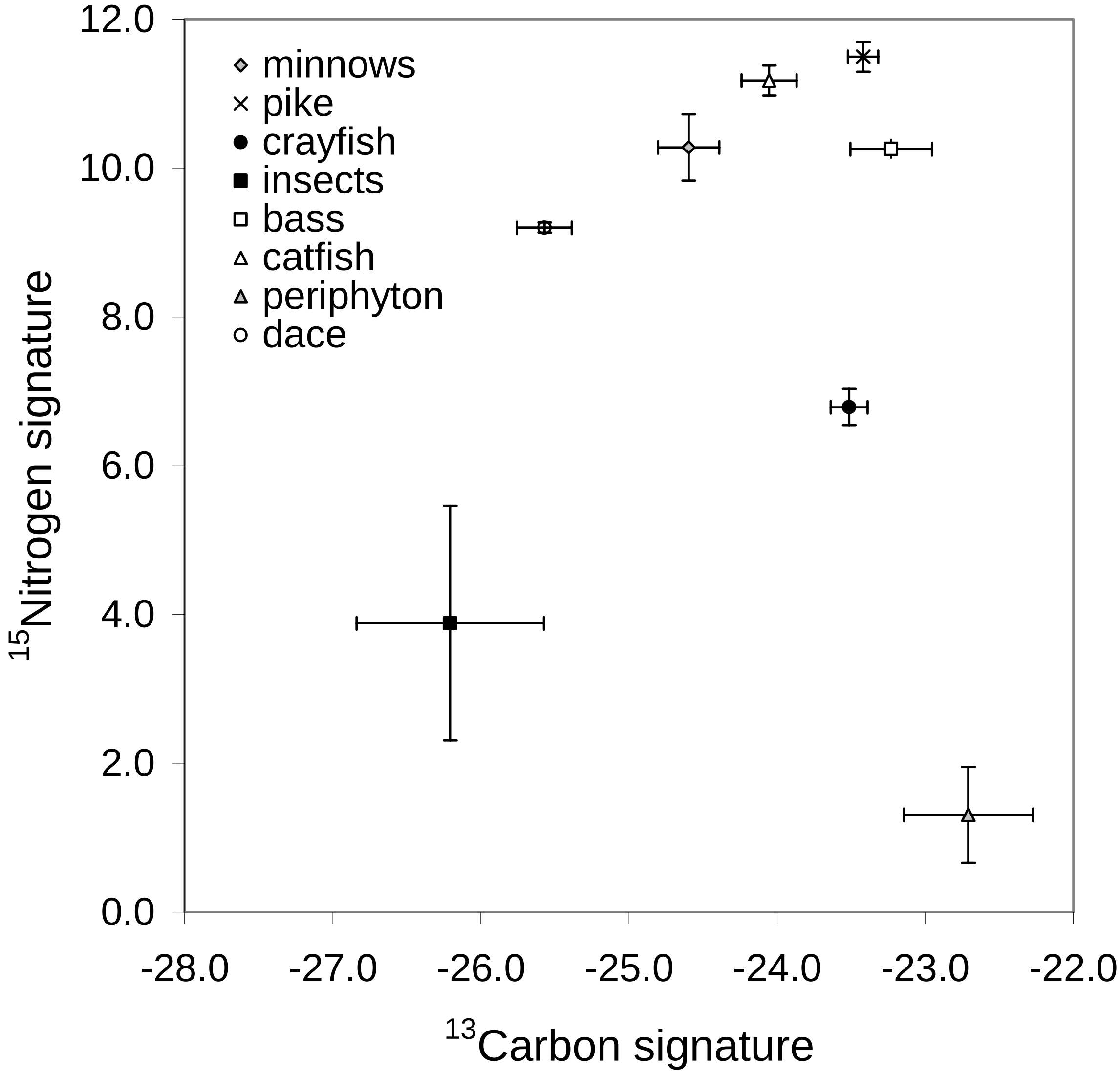


Figure E-2. Carbon-nitrogen isotope plot showing mean isotopic signatures of eight taxa collected from the Yampa River, Colorado. Error bars show ±SE.

ISOTOPIC AND ELEMENTAL ANALYSES OF SMB OTOLITHS

Thin sections of otoliths from 34 smallmouth bass (8 from Lake Powell, 15 from the Yampa River, 1 from Rifle Gap Reservoir, and 10 from the Colorado River in the Grand Valley) have been analyzed by LA-ICPMS to date. Otolith thin sections were analyzed for nine elements (^{44}Ca, ^{25}Mg, ^{55}Mn, ^{63}Cu, ^{66}Zn, ^{88}Sr, ^{137}Ba, ^{202}Hg, ^{208}Pb) using a Perkin Elmer ELAN 6000 ICPMS coupled with a CETAC Technologies LSX-500 laser ablation system. These elements were chosen based on their suspected abundance and because literature on Lake Powell water chemistry (sorry, I can't find this reference) indicated that elevated levels of Hg were present there. A transect was ablated with the laser on each otolith thin section extending from the otolith nucleus to its edge along the longest axis (beam diameter = 25 μm, scan rate = 10 μm/s, laser pulse rate = 10 Hz, laser energy level = 9 mJ, wavelength = 266 nm). A standard developed by the U.S.G.S. (MACS-1, $CaCO_3$ matrix) was analyzed every 5 samples to adjust for possible instrument drift. Isotopic counts were converted to elemental concentrations (ppm). Elemental concentrations were calculated based on integrations over entire laser transects, which incorporates temporal (both intra- and inter-annual) variation in otolith elemental composition for fish from each location.

All elements except Hg were detected in smallmouth bass otoliths (see table of data). Ranges of elemental concentrations for fish from the four locations overlapped for all elements except Sr. Sr concentrations were higher in all fish from Lake Powell compared to fish from the other 3 locations, indicating that Sr concentration will be useful for identifying Colorado River fish that originated in Lake Powell (ANOVA also indicates that mean Sr concentration is significantly higher for Lake Powell fish, $P<0.0001$). Inspection of ^{88}Sr intensity data along laser transects from the 10 Colorado River smallmouth bass otoliths indicated no evidence that any of these fish had emigrated from Lake Powell. The only other element that was significantly different among fish from the four locations was Ba, whose mean concentration was significantly higher in otoliths from Yampa River fish (ANOVA, $P<0.0001$). However, the range of Ba concentrations for Yampa River fish overlaps those of fish from the other locations, so this element will not be able to classify fish origin with 100% accuracy.

Recommended and pending next steps:

An additional 14 otolith thin sections from fish collected at Rifle Gap Reservoir will be analyzed very soon to better characterize elemental signatures for smallmouth bass from that location. These analyses were to have been completed last week, but problems with the laser prevented analyses from being conducted.

Otoliths from 40 smallmouth bass (10 from Rifle Gap Reservoir, 10 from the Yampa River, 5 from Lake Powell, and 5 each from the Colorado River below the Gunnison River confluence, the Colorado River in the Grand Valley above the Gunnison River confluence, and the Colorado River between Rifle and Rulison) have been prepared for analysis of stable oxygen isotope ratios ($\delta^{18}O$). This element was chosen for consideration because: 1) Water samples suggest differences in $\delta^{18}O$ among some locations (Lake Powell -12.23 $^{o}/_{oo}$; Yampa River -16.2 $^{o}/_{oo}$ ± 0.7 SE, n=2; Rifle Gap -15.7 $^{o}/_{oo}$; Colorado River -16.3 $^{o}/_{oo}$ ± 0.09 SE, n=32) and 2) For locations among which water $\delta^{18}O$ is not significantly different (e.g. Colorado and Yampa

Rivers), differences in water temperature may create distinct otolith $\delta^{18}O$ signatures, as otolith $\delta^{18}O$ is affected by both water temperature and water $\delta^{18}O$. The plan is to have these samples analyzed this summer; I have been trying to contact a U.S.G.S. lab in Denver about this analysis, but have been unable to reach them so far. If I can't contact them soon, I'll try another lab.

If otolith $\delta^{18}O$ analysis indicates significant differences among locations, additional water samples for $\delta^{18}O$ analysis (same as for hydrogen isotopes) should be collected, emphasizing possible source locations. I would also recommend that clean water samples be collected so that any differences in otolith elemental or isotopic signatures among locations can be related to differences in water chemistry. This isn't absolutely necessary, but would strengthen the argument that we're really sampling known provenance fish from each possible source location. Otoliths from any additional potential source locations should be added to our data set.

At this point, we're able to identify fish that have originated from Lake Powell, but not the other potential source locations. A subsample of the oldest available fish collected in the Colorado River could be analyzed by LA-ICPMS (for Sr concentration in particular) to provide additional data for determining what contribution (if any) Lake Powell has made to the smallmouth population in the Grand Valley. Emphasis on the oldest available fish is suggested to maximize the potential for an analyzed fish having the signature of the ultimate smallmouth bass source(s). Some younger fish should be included in analyses, however, to determine whether source(s) are still contributing smallmouth bass to the Colorado River or if these younger fish are primarily being produced in the river itself. Additional analyses of unknown provenance fish should be conducted if pending data analyses reveal other chemical markers capable of distinguishing among possible source locations.

DISCRIMINANT ANALYSIS TO IDENTIFY FISH ORIGINS IN COLORADO

The Colorado River system is home to several native fish species that have become threatened or endangered due to changes in the flow regime of the river and introduced species. Centrarchid fishes such as bass and sunfish are of particular concern to fishery managers because of their predation on juvenile native fish. Efforts to control the centrarchid fishes have been hampered by the fact that we know very little about their origins and movement (hereafter referred to as "provenance"). Establishing a means of distinguishing the chemical signatures of different bodies of water in and around the Colorado River would give managers a valuable tool for understanding fish provenance. I believe that otolith microchemistry has the potential to reveal information about the environmental history of a fish and thus allow researchers to deduce the area from which it came based upon the microchemical signature in the otolith.

The Colorado Division of Wildlife (CDOW) collected smallmouth bass from Lake Powell, Yampa River and Colorado River during the summer of 2004. Otoliths were removed and analyzed using laser ablation inductively coupled plasma mass spectrometry (LA-ICMPS) for Mg (X1), Mn (X2), Cu (X3), Zn (X4), Sr (X5), Ba (X6), Hg and Pb (X7) (Table 1). Mercury levels in all samples were below the detection limits of the machinery and hence excluded from further analysis. In all cases, there was no discernable trend in the patterns of elemental abundance. This strongly suggests that the fish sampled had not moved from one body of water to another, and therefore each elemental concentration is reported as a mean value averaged over the life of each fish.

Strontium tends to occur in the otolith at much higher levels than the other elements analyzed because of its ionic similarity to calcium, a major component of the otolith. It has also been one of the most important elements used in otolith studies. I began the analysis by performing tests of normality for Sr at each of the three locations. The results provided no evidence of a non-normal distribution of the data. Summary statistics for Sr indicate that the Yampa and Colorado Rivers have similar means and that there is unequal variance among the three locations (Table 2). Levine's test for homogeneity of the Sr data confirms the unequal variance (Table 3).

Considering the above tests, I decided that the dataset was suitable for analysis using discriminant function analysis. My goal is to use the seven elements analyzed to distinguish among the three locations. Ultimately, I would like to expand this to more than twenty locations but do not yet have data for those locations. This analysis serves as a pilot study of sorts for the application of this technique.

Significant differences were seen in the elemental composition of the three locations (Table 4). This indicates that the fish at the three locations have different chemical signatures in their otoliths and that discriminating among the three locations should be possible with a high degree of success. Out of the 32 fish analyzed in this study, 29 were accurately classified using posterior probabilities (Appendix II). All three misclassifications occurred when fish from the Yampa River or Lake Powell were classified as belonging to the Colorado River group.

Group classification was performed for both linear and quadratic discriminant analysis, using resubstitution and cross-validation. Quadratic DA provided error-free classification using the resubsitution method (Table 5). When cross-validated, however, the error rate rose to 55.71%, indicating that the results were unreliable (Table 6). Linear DA provided better results. The resubstitution method had an overall error rate of 8.93% (Table 7). Cross-validation of the linear DA yielded an overall error rate of 17.86%, which was much lower than that observed using quadratic DA (Table 8).

Cross-validation is an important step in determining the success of classification because it is independent of the individual being classified. In this example, 32 fish from 3 locations were classified according to group in the resubstitution method after the same 32 fish were used to build a model for the purpose of classifying; in this sense, the logic supporting the method is almost tautological. Cross-validation proceeds to classify a fish based upon what the other 31 fish display. Thus, the fish of interest plays does not affect the model that classifies the fish of interest.

Quadratic DA does not pool the covariance matrix estimates, whereas linear DA does. In the current example, cross-validation demonstrated that the overall error rate of ~9% in linear DA was more reliable than the 0% error rate achieved using quadratic DA. I would suggest that managers in a similar situation proceed with linear DA in order to have more confidence in the accuracy of their classification; however, I would also recommend that the decision be based upon a consideration of the variance within groups.

I used stepwise model selection in order to determine which of the seven elements analyzed were useful for discriminating among locations. Of the seven elements examined, only Sr and Ba were significant enough to be included in the model (Table 9). Both Sr and Ba were highly significant ($p<0.001$) in the first step of the stepwise analysis and no other elements achieved the level of significance necessary to be included in the model (see Appendix III for complete results). Other elements were likely excluded because they were either too homogenous among locations or too heterogeneous within locations to be of use. Strontium and barium were more constant at each location and tended to differ more at different locations.

With only two variables that were significant enough to be included in a model for discriminating among locations, canonical DA may not be as useful as in a case where many elements are used in the model. I chose to analyze the data using canonical DA in order to visually represent the data and to determine which canonical coefficients were most useful. The small variation in the Colorado River samples (group 3) becomes apparent in the graph of canonical correlations (Figure E-3). The locations display little, if any, overlap based upon this classification. The first canonical correlation factor contains most of the variation (Table 10).

Ultimately, I would consider the analysis successful. The primary significance of the results is that there is enough variation in otolith microchemistry among locations in Colorado for the technique to be considered for studies of fish provenance. My study will be similar in nature, but involve close to 20 locations. However, many of my fish will be reared in very homogeneous conditions, so that I expect within-site variation to be quite low. Thus I cannot make conclusions about the success rate in my study based upon the results of this analysis, but I have demonstrated that the technique is a valid method of discriminating among fish from different locations based solely on microchemical analysis of otoliths.

```
             Plot of Can2*Can1.  Symbol is value of loc.

Can2 ,
     ,
   3 ˆ
     ,                                                1
     ,       2
     ,
   2 ˆ      2         2
     ,
     ,          2    2
     ,     2
   1 ˆ                                                1
     ,                                               1      1
     ,                      22
     ,                      2                                 1
   0 ˆ             2      2             1
     ,                  2
     ,                 3     3       1
     ,
  -1 ˆ             2                  1
     ,                       3 3
     ,               2    33  3  3
     ,
  -2 ˆ
     ,                        3
     ,
     ,
  -3 ˆ
     ,
     Šfˆfffffffffffˆfffffffffffˆfffffffffffˆfffffffffffˆfffffffffffˆff
      -4          -2           0           2           4           6

                                    Can1
```

Figure E-3. Plot of the first two canonical correlation factors.

Table E-1. Concentrations (ppm) of eight trace elements in SMB otoliths collected from four locations; *nd=not detected.

Fish number	Length (mm)	Weight (g)	Age	Mg	Mn	Cu	Zn	Sr	Ba	Hg	Pb
Lake Powell											
LP042704001	258	212	2+	64	2	2	16	2503	12	nd	1
LP042704002	300	308	3+	65	1	3	12	2248	16	nd	1
LP042704003	361	604	4+	20	1	1	nd	1544	9	nd	0
LP042704004	232	148	2+	31	1	1	nd	2233	14	nd	0
LP042704005	231	138	2+	5	3	3	16	2407	21	nd	2
LP042704006	289	332	3+	1	1	10	1	2096	11	nd	2
LP042704008	251	184	2+	8	1	1	1	1622	11	nd	2
LP042704009	225	150	2+	12	0	0	11	1517	4	nd	0
mean				**26**	**1**	**3**	**10**	**2021**	**12**	**nd**	**1**
Yampa River											
YAR060104001	86	8	2+	2	14	11	9	1278	21	nd	2
YAR060104002	213	126	3+	5	11	0	nd	1278	18	nd	1
YAR060104003	157	48	2+	12	2	1	1	917	15	nd	0
YAR060104004	140	38	2+	13	52	1	nd	993	40	nd	1
YAR060104005	156	54	2+	27	3	1	nd	838	25	nd	0
YAR060104006	192	92	2+	18	6	1	nd	1256	18	nd	1
YAR060104007	253	268	4+	20	5	1	1	1232	16	nd	2
YAR060104008	225	166	3+	12	2	1	nd	992	29	nd	0
YAR060104009	127	28	2+	nd	17	1	3	1079	15	nd	0
YAR060104023	310	446	3+	68	15	11	17	1412	25	nd	25
YAR060104024	302	430	4+	13	2	1	2	1179	14	nd	2
YAR060104026	361	806	4+	19	nd	2	24	1458	21	nd	4
YAR060104027	379	744	7+	19	1	1	8	919	8	nd	2
YAR060104029	446	1540	13+	10	1	1	2	844	6	nd	1
mean				**18**	**10**	**2**	**7**	**1120**	**19**	**nd**	**3**

Table E-1. Continued. Concentrations (ppm) of eight trace elements in SMB otoliths collected from four locations; *nd=not detected.

Fish number	Length (mm)	Weight (g)	Age	Mg	Mn	Cu	Zn	Sr	Ba	Hg	Pb
Colorado River											
P-CL041904001	329	464	4+	22	1	0	1	1201	5	nd	1
RD-RP043004001	335	618	5+	14	6	1	nd	1105	6	nd	1
RD-RP043004002	319	530	4+	33	1	0	nd	1047	3	nd	1
P-GVIC050304001	300	386	4+	17	1	1	2	1015	6	nd	0
CL-RB050404013	298	438	3+	34	2	nd	2	1146	9	nd	1
GVIC-CL051404001	377	756	6+	2	1	0	3	1277	8	nd	1
GVIC-CL051404002	334	520	5+	12	1	1	2	1122	6	nd	0
GVIC-CL051404009	298	398	3+	17	1	nd	4	1203	6	nd	0
RD-RP051704016	292	384	4+	27	1	nd	8	1117	5	nd	1
RD-RP051704017	316	514	4+	10	1	2	nd	1104	10	nd	0
mean				19	1	1	3	1134	6	nd	1
Rifle Gap Reservoir											
RGR082604001	369	678	7+		nd	nd	nd	1494	5.91	nd	0.46
RGR041905001	199	90	.								
RGR041905002	221	120	.								
RGR041905003	294	300	.								
RGR041905004	229	215	.								
RGR041905005	183	65	.								
RGR041905006	181	75	.								
RGR041905007	150	35	.								
RGR041905008	131	20	.								
RGR041905009	167	40	.								
RGR041905010	161	45	.								
RGR041905011	121	20	.								
RGR041905012	220	115	.								
RGR041905013	379	820	.								
RGR041905014	223	110	.								

Table E-2. Mean and standard deviation for Sr data collected at each of the three locations used in the study.

Location	N	Mean	Standard deviation
Lake Powell	8	2021	401
Yampa River	14	1120	207
Colorado River	10	1134	77

Table E-3. Levine's test for homogeneity of Sr variance, ANOVA of absolute deviations from group means taken from SAS output.

Source	df	Sum of squares	Mean square	F-value	Pr>F
Location	2	365776	182888	17.94	<0.0001
Error	29	295615	10193		

Table E-4. Multivariate statistics and exact F statistics computed in SAS for the three locations used in the study.

Statistic	Value	F-value	Numerator df	Denominator df	Pr>F
Wilks' Lambda	0.10	7.18	14	46	<.0001
Pillai's Trace	1.28	6.14	14	48	<.0001
Hotelling-Lawley Trace	5.28	8.41	14	33.60	<.0001
Roy's Greatest Root	4.40	15.06	7	21	<.0001

Table E-5. Number of classifications into group (top number) and rate (bottom number) using quadratic DA with resubstitution.

		Location		
Resubstitution		**1**	**2**	**3**
	1	8	0	0
		100.00	0.00	0.00
Location	2	0	14	0
		0.00	100.00	0.00
	3	0	0	10
		0.00	0.00	100.00

Table E-6. Number of classifications into group (top number) and rate (bottom number) using quadratic DA with cross-validation.

		Location		
Cross-validation		**1**	**2**	**3**
	1	0 0.00	8 100.00	0 0.00
Location	2	1 7.14	13 92.86	0 0.00
	3	3 30.00	3 30.00	4 40.00

Table E-7. Number of classifications into group (top number) and rate (bottom number) using linear DA with resubstitution and proportional prior probabilities.

		Location		
Resubstitution		**1**	**2**	**3**
	1	7 87.50	0 0.00	1 12.50
Location	2	0 0.00	12 85.71	2 14.29
	3	0 0.00	0 0.00	10 100.00

Table E-8. Number of classifications into group (top number) and rate (bottom number) using linear DA with cross-validation and proportional prior probabilities.

Linear D.A.		**Location**		
Cross-validate		**1**	**2**	**3**
	1	6 75.00	0 0.00	2 25.00
Location	2	0 0.00	10 71.43	4 28.57
	3	0 0.00	0 0.00	10 100.00

Table E-9. Results from step 1 of the stepdisc procedure in SAS displaying the seven elements measured in the analysis and their significance levels.

Variable	R-square	F-value	Pr>F	Tolerance
Mg	0.043	0.64	0.532	1.000
Mn	0.170	2.96	0.068	1.000
Cu	0.101	1.62	0.215	1.000
Zn	0.088	1.39	0.264	1.000
Sr	0.735	40.28	<0.001	1.000
Ba	0.456	12.14	<0.001	1.000
Pb	0.063	0.98	0.387	1.000

Table E-10. Eignevalues obtained from proc candisc in SAS from data on fish from three locations in Colorado**.**

	Eigenvalue	Difference	Proportion	Cumulative
1	4.394	3.512	0.833	0.833
2	0.882		0.167	1.000

DIET ANALYSIS OF NONNATIVE FISHES IN THE YAMPA AND COR RIVERS

Additional findings from these analyses are provided in a draft manuscript entitled: "Smallmouth Bass: The Primary Predatory Threat to Recovery of the Native Fish Assemblage of The Yampa River, Colorado," submitted under separate cover.

Yampa River

Analysis of smallmouth bass stomachs is nearly complete. Analyses continued to show the importance of crayfish to smallmouth diets (Figure). Aquatic insects were also common in guts but fish constituted less than 10% of the diet by mass. Cannibalism resulted in a significant fraction of fish in the diet. The size range of fish ingested by smallmouth bass ranged about 10 to 40% (Figure).

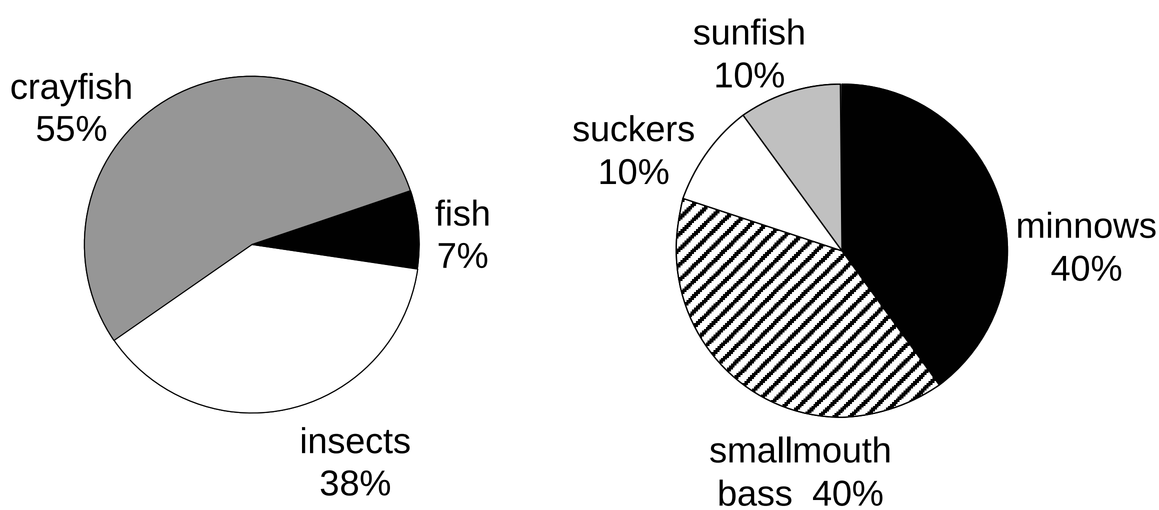


Figure E-4. Diet composition of 14 smallmouth bass collected from the Yampa River, CO, during June 2003 to June 2004. Left: overall diet percentages; right: composition of the fish fraction of the diet.

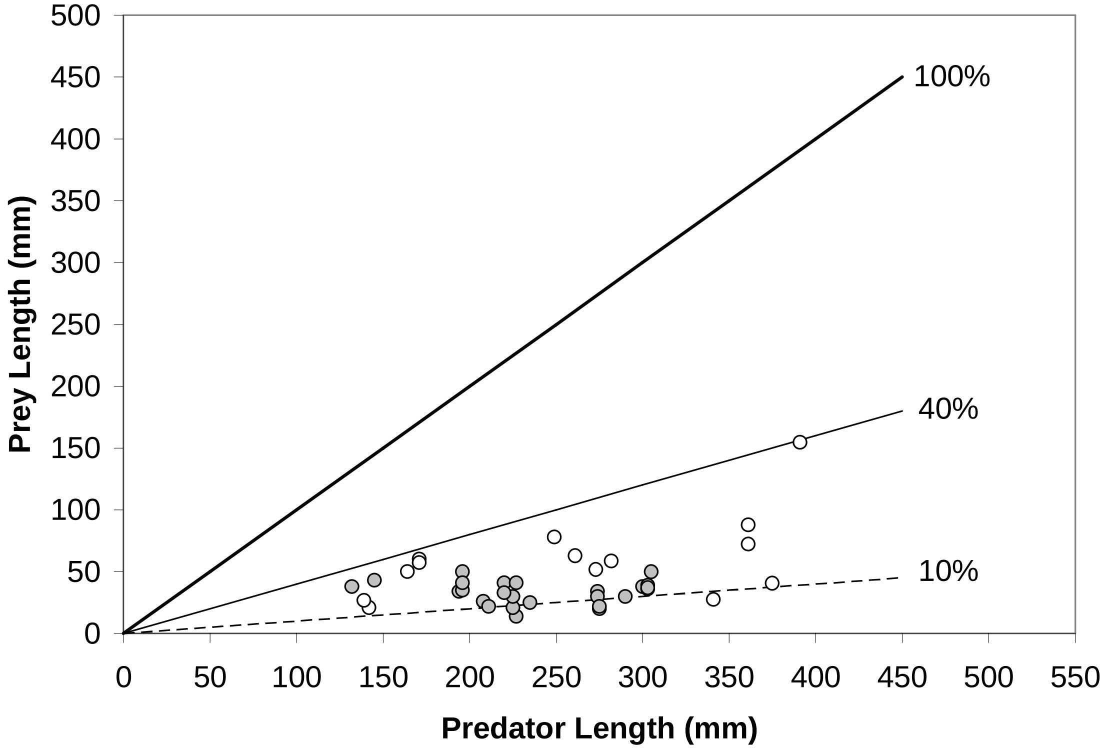


Figure E-5. Total lengths of prey in stomachs of a) 14 smallmouth bass collected from the Yampa River, CO (open circles) during June 2003 to June 2004, and b) 31 smallmouth bass collected from the Colorado River during summer, 2004 (closed circles). Diagonal lines show prey:predator length ratio.

Analysis of samples from northern pike and channel catfish is continuing.

Colorado River

Smallmouth bass in the Colorado River were considerably more piscivorous than in the Yampa River (Figure). About two thirds of the diet was composed of fish, and was exclusively minnows and suckers.

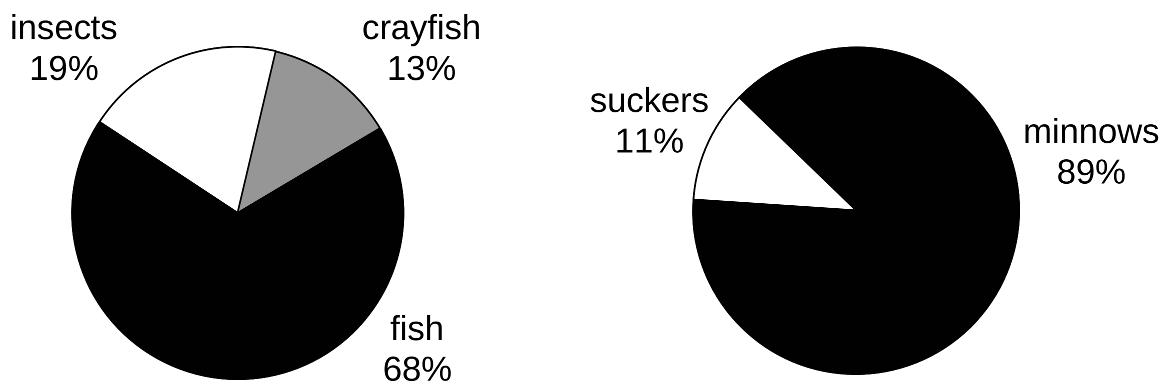


Figure E-6. Diet composition of 31 smallmouth bass collected from the Colorado River, CO, summer, 2004. Left: overall diet percentages; right: composition of the fish fraction of the diet.

Diet of the two channel catfish analyzed consisted entirely of aquatic insects (Ephemeroptera, Plecoptera, Trichoptera) and crayfish. Diet analysis of channel catfish samples is continuing.

BIOENERGETICS PROJECTIONS FOR SMALLMOUTH BASS

Findings from these analyses are provided in a draft manuscript entitled: "Smallmouth Bass: the Primary Predatory Threat to Recovery of the Native Fish Assemblage of the Yampa River, Colorado," submitted under separate cover.

RECOMMENDATIONS

1. Analysis of centrarchid, channel catfish, northern pike and yellow perch diet composition should continue to bolster sample sizes and to increase the range of sizes and times of year over which diet inferences are possible.

2. Bioenergetics projections of smallmouth bass consumption in the Yampa River, yellow perch consumption in Blue Mesa Reservoir, and centrarchid consumption in the upper Colorado River should be refined as new diet data become available.

3. Water, otolith and tissue samples should be collected from invading nonnative species suspected source and recipient waters to refine our ability to trace origins of translocated fish.

4. We should continue to work on manuscripts deriving from this research and submit them to scientific journals.

Appendix F

MEMO:
ANALYSIS OF OTOLITHS FROM THE YAMPA AND COLORADO RIVERS, LAKE POWELL, AND RIFLE GAP RESERVOIR
SUBMITTED BY DAN GIBSON-REINEMER, COLORADO STATE UNIVERSITY

ANALYSIS OF OTOLITHS FROM YAMPA RIVER, COLORADO RIVER, LAKE POWELL, AND RIFLE GAP

The results from Greg's data suggest that it is possible to discriminate among the four locations with relatively high accuracy. The overall, cross-validated success rate was approximately 85% (see below).

For the sake of convenience in SAS, I've labeled the four locations numerically as follows: Lake Powell (1), Yampa River (2), Colorado River (3), and Rifle Gap (4). In the table below, the worst accuracy occurred when Lake Powell fish were classified as Colorado River fish. However, all of the Colorado River fish were correctly identified as originating from the Colorado River. Rifle Gap appears to have a distinct signature, with 13 of 15 otoliths correctly identified. Of the two misclassifications, one was to Lake Powell and one to the Yampa River.

The DISCRIM Procedure
Classification Summary for Calibration Data: WORK.GREGDISC
Cross-validation Summary using Linear Discriminant Function

Number of Observations and Percent Classified into loc

From loc	1	2	3	4	Total
1	6	0	2	0	8
	75.00	0.00	25.00	0.00	100.00
2	0	11	2	1	14
	0.00	**78.57**	14.29	7.14	100.00
3	0	0	10	0	10
	0.00	0.00	**100.00**	0.00	100.00
4	1	1	0	13	15
	6.67	6.67	0.00	**86.67**	100.00
Total	7	12	14	14	47
	14.89	25.53	29.79	29.79	100.00
Priors	0.25	0.25	0.25	0.25	

Error Count Estimates for loc

	1	2	3	4	Total Rate			
						0.2500	0.2143	0.0000
0.1333	**0.1494**							
Priors	0.2500	0.2500	0.2500	0.2500				

The above analysis was based on 7 elements: Mg, Mn, Cu, Zn, Sr, Ba, and Pb. I did a step-wise selection procedure to determine the elements that were significantly different among locations. I used a selection level of .05 for entry and .10 for removal. Using these criteria, only Mg, Sr, and Ba (in that order) were significant enough to be used; the remaining elements were not. The question of whether to include elements that are not significant is a tough one that I'll be working on over the next few months.

The STEPDISC Procedure
Stepwise Selection: Step 4
Statistics for Removal, DF = 3, 41

Variable	Partial R-Square	F Value	Pr > F
Mg	0.7524	41.52	<.0001
Sr	0.7376	38.42	<.0001
Ba	0.4603	11.65	<.0001

No variables can be removed.

Statistics for Entry, DF = 3, 40

Variable	Partial R-Square	F Value	Pr > F	Tolerance
Mn	0.0160	0.22	0.8838	0.5514
Cu	0.0784	1.13	0.3467	0.6100
Zn	0.0153	0.21	0.8905	0.6027
Pb	0.0373	0.52	0.6737	0.6105

No variables can be entered.

Big Picture

One of the more important questions I recall you asking was whether you could find a marker for Rifle Gap. Given the results, this seems to indicate that Rifle Gap fish can be identified with a high degree of accuracy- at least when you only have the three alternative locations used here.

I'll work on tracking down any information on water chemistry from these locations, if Greg collected any, and any additional information on more precise locations from which the fish were collected.

Plot of Can2*Can1. Symbol is value of loc.

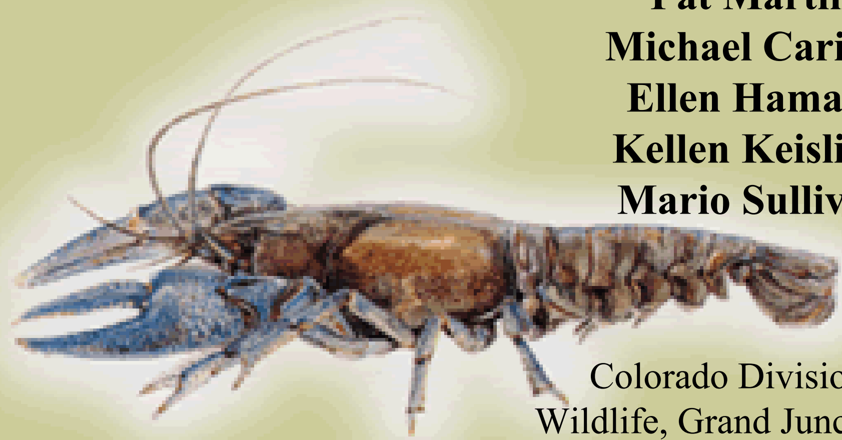
```
Can2 ,
     ,
   5 ˆ                                                  1
     ,
     ,
     ,                                                                            1
     ,
   4 ˆ                                                             1
     ,
     ,
     ,
     ,                                                       1          1
   3 ˆ
     ,
     ,
     ,
     ,
   2 ˆ                                         1
     ,                                          1
     ,                                           1
     ,                                                         4
     ,
   1 ˆ                               3
     ,                         2
     ,                         2
     ,                                  3                           4
     ,                           2          3                 4         4
   0 ˆ                     2     2 2  33
     ,                            3      3
     ,                       2           3
     ,                              3       3                           4
     ,                                                                         4
  -1 ˆ       2          2     22
     ,
     ,      2                                                       4        4
     ,                                            2                                   4
     ,                                                                4
  -2 ˆ          2
     ,                                                                  4       4
4
     ,                                                                                4
     ,                                                                              4
     ,
  -3 ˆ
     ,

Šˆffffffffˆffffffffˆffffffffˆffffffffˆffffffffˆffffffffˆffffffffˆffffffffˆffffffffˆffffff
ffˆ
     -5       -4       -3       -2       -1        0        1        2        3        4
5

                                           Can1
```

Appendix G

POWERPOINT:
A PRELIMINARY EXAMINATION OF CRAYFISH IN THE YAMPA RIVER IN NORTHWESTERN COLORADO

A preliminary examination of crayfish in the Yampa River in northwestern Colorado

Pat Martinez
Michael Carillo
Ellen Hamann
Kellen Keisling
Mario Sullivan

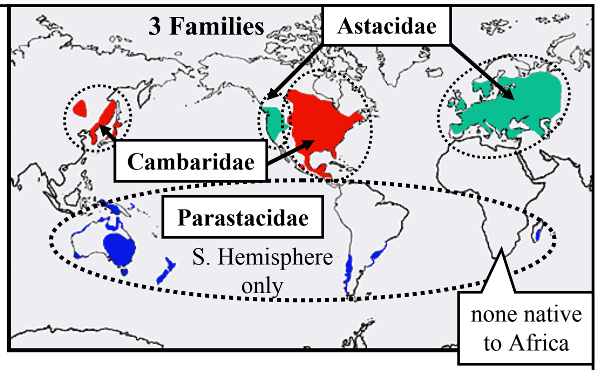


Colorado Division of Wildlife, Grand Junction

Introduction:

- **NO crayfish species are native to Colorado River Basin**
- crayfish not reported in Yampa River (YAR) in 1975-1976
- apparent explosion of verile crayfish (VCF) in 2002
    - onset & persistence of extreme drought
    - reduction of small-bodied fish
    - expansion of SMB range & abundance
- crayfish demographics required for food-web assessments
- was VCF explosion a bioenergetic response to temperature?
- are VCF exacerbating nonnative piscivore populations?
- might VCF prey upon/compete with native fishes?

Crayfish facts:



-**Kingdom** Animalia

-**Phylum** Arthropoda

-**Subphylum** Crustacea

-**Class** Malacostraca

-**Order** Decapoda

-**Infraorder** Astacidea = crayfishes & lobsters

- 540 species worldwide, 319 species in U.S. & Canada
- found in lotic & lentic environments, omnivorous
- serve as food for fish, wildlife, humans
- crayfish widely introduced in N.A. & elsewhere; invasive

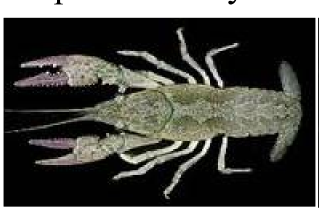
Colorado crayfish: 6 native species

Cambarus diogenes
Devil crayfish

Procambarus simulans

Orconectes causeyi
[Calasieu] crayfish

Orconectes immunis
Papershell crayfish



Orconectes neglectus
Ringed crayfish

Orconectes virilis
Northern crayfish

VCF *Orconectes virilis* trivia:

- other common names are **fantail** or northern crayfish
- **largest native range** of all crayfish in U.S. & Canada
- adaptable to **lotic & lentic** environments
- up to 5in./130mm total length=2.5in./60mm **carapace length**
- hide/excavate under rocks, debris & mud; **non-burrowing**
- mature in one year; produce 200-500 eggs; may **live 4 years**
- **polytrophic**; may be consumer/prey or predator in food web
- high densities **reduce snails, molluscs**
- may eat fish eggs; **prey on fish larvae & small fishes**

Establishing sampling methodology:

- literature & internet
- long-term abundance monitoring
- size structure (length & weight)
- numbers or biomass per unit area
- various sampling options available (consider water depth)
  - **traps** (baited, largest crayfish)
  - **handnets, dip nets** (larger crayfish)
  - **electrofish** (burrows & under rocks)
  - **quadrat** (flight response)

- Dr. Chris Myrick → Dr. Maria J. Ellis, Spring Rivers Ecological Sciences, LLC, Cassel, California

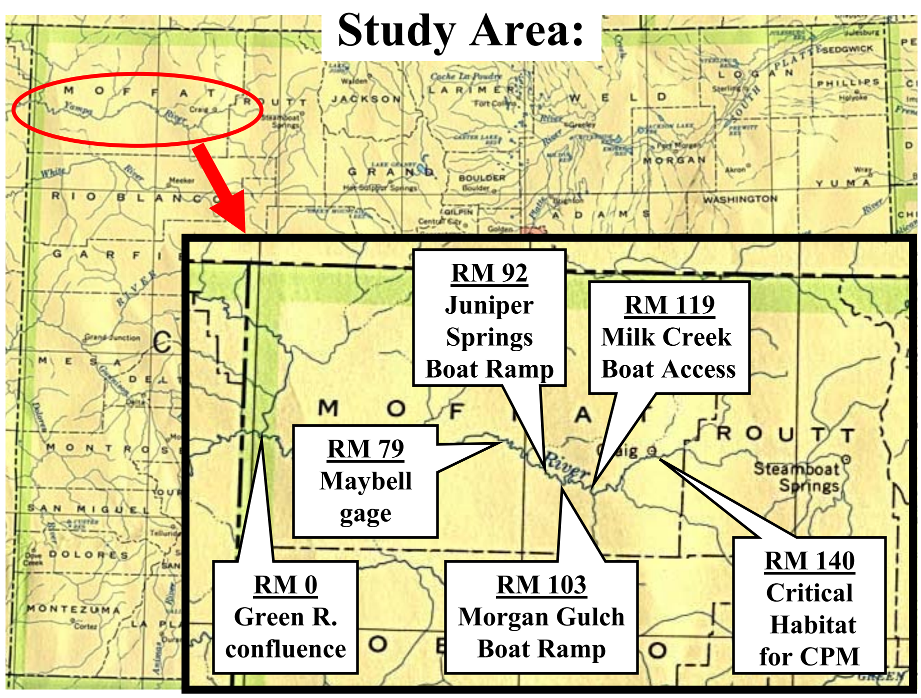
Selection of sampling sites & dates:

- target major instream habitat types
- relatively short segments to minimize variability
- long-term access (road or watercraft, public land)
- CO State Parks & BLM boat launch sites
- upstream of boat ramps

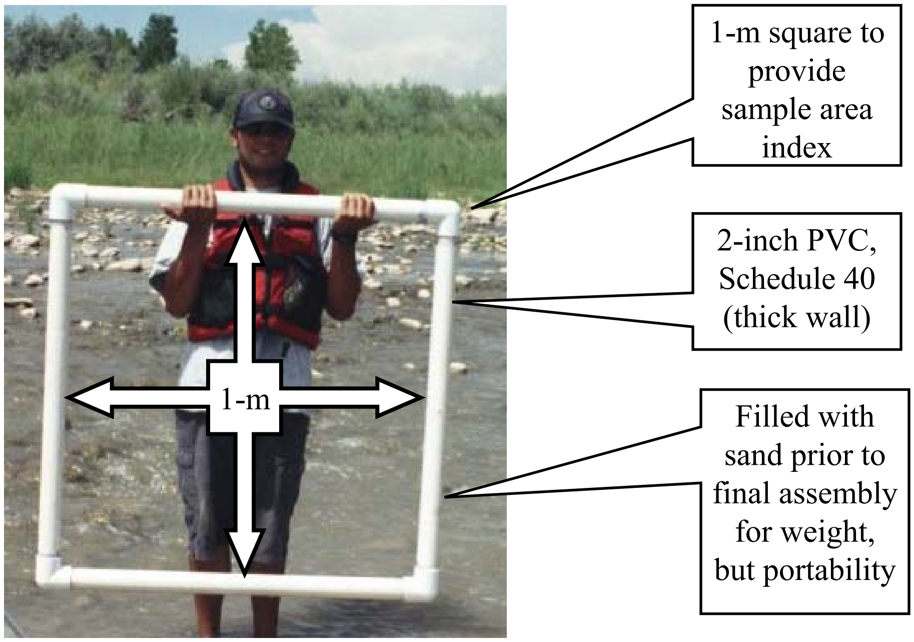
- flow must allow access to entire channel
- water must be clear for visibility
- tolerable water temperature for crew
- crayfish reproduction complete
- standing crop reflective of crayfish biomass

} **AUGUST**

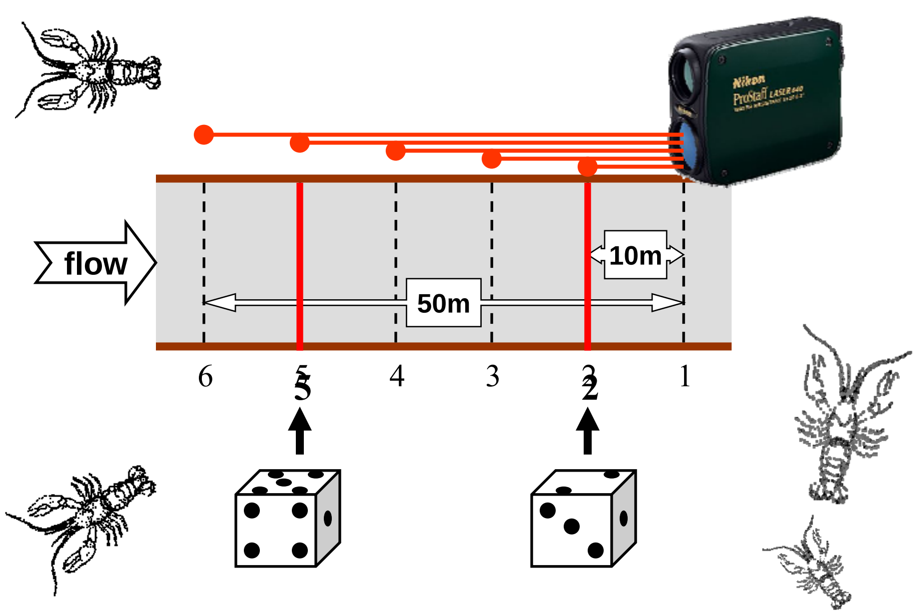
Study Area:



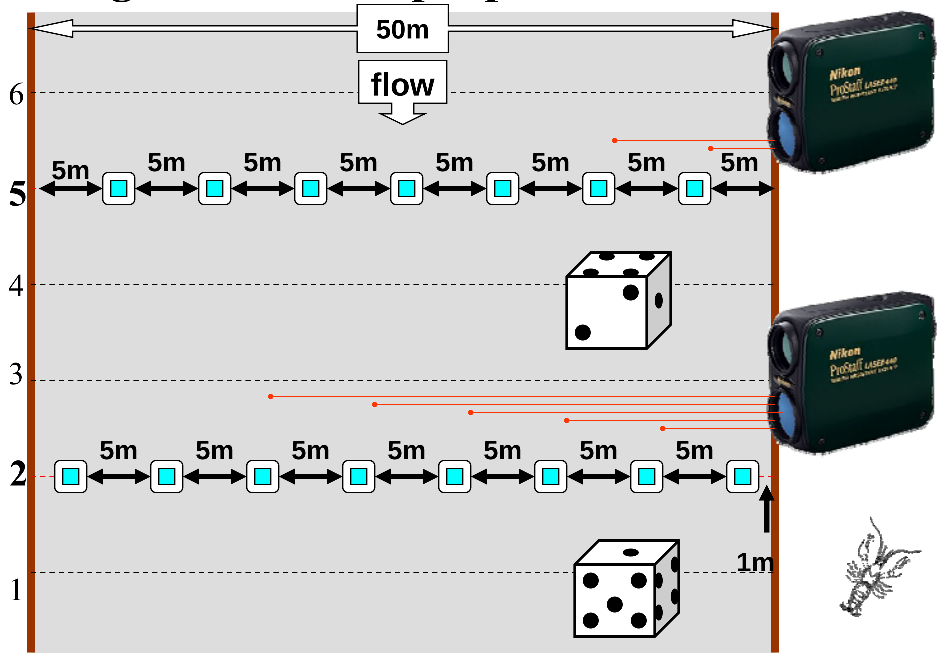
1-m square crayfish sample plot:



Selection of sampling transects:



Assignment of sample plots:



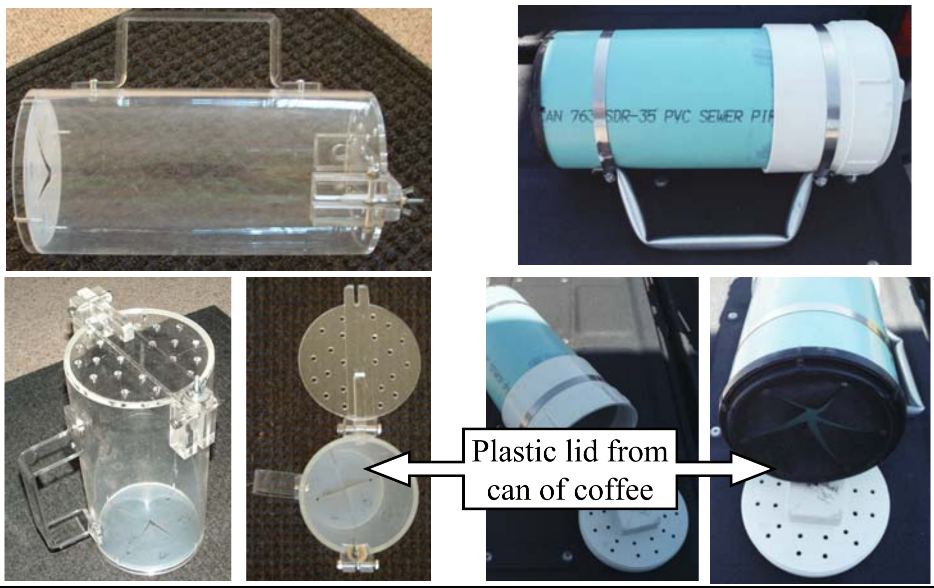
Sampling sites 22-24 August 2005:

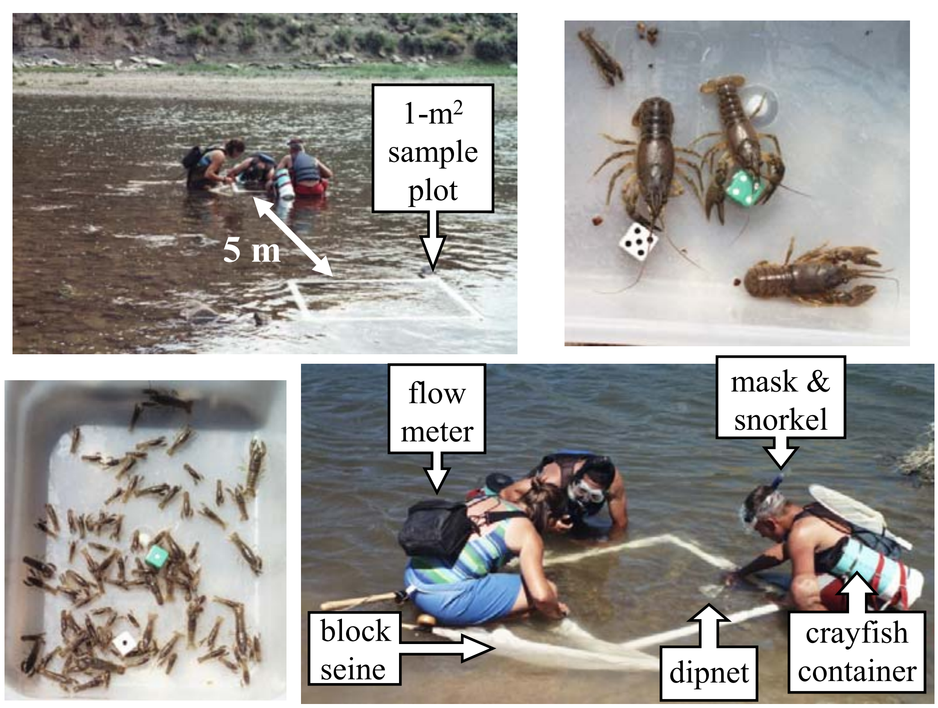
Station parameters	Juniper Springs	Morgan Gulch	Milk Creek
Rivermile	92.2	103.1	118.6
Habitat	**Run**	**Pool**	**Riffle**
Channel width (m)	61&56	62&72	40&39
Flow (cfs) @ Maybell	151	148	165
Temperature °C	19.9	21.1	21.4
Mean depth (cm)	33	46	45
Mean velocity (ft./sec.)	1.24	0.63	1.27
Primary substrate	GR/CB	GR/SA	CB/RK

Crayfish container:

¼-in X 6-in. dia. cast acrylic

6-in. dia. sewer pipe



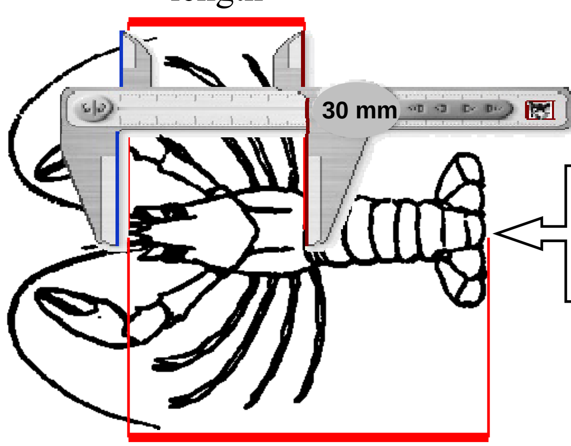


Number of crayfish:

XS	Sample plot													Cat.	Mis.	Tot.
	1	2	3	4	5	6	7	8	9	10	11	12	13			
Juniper Springs																
T2	0	0	0	0	0	4	5	2	3	14				25	3	28
T5	4	0	1	2	1	4	2	2	4	24				40	4	44
Morgan Gulch																
T1	16	13	20	9	0	0	0	0	1	3	1			35	28	63
T6	108	4	5	7	26	4	6	7	3	3	4	6	3	157	29	186
Milk Creek																
T2	85	29	20	7	4	0	2							110	37	147
T3	77	26	9	2	5	0	44							116	47	163
All stations →														**483**	**148**	**631**

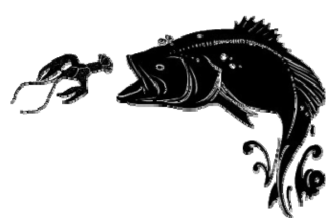
Measuring crayfish length:

Carapace length

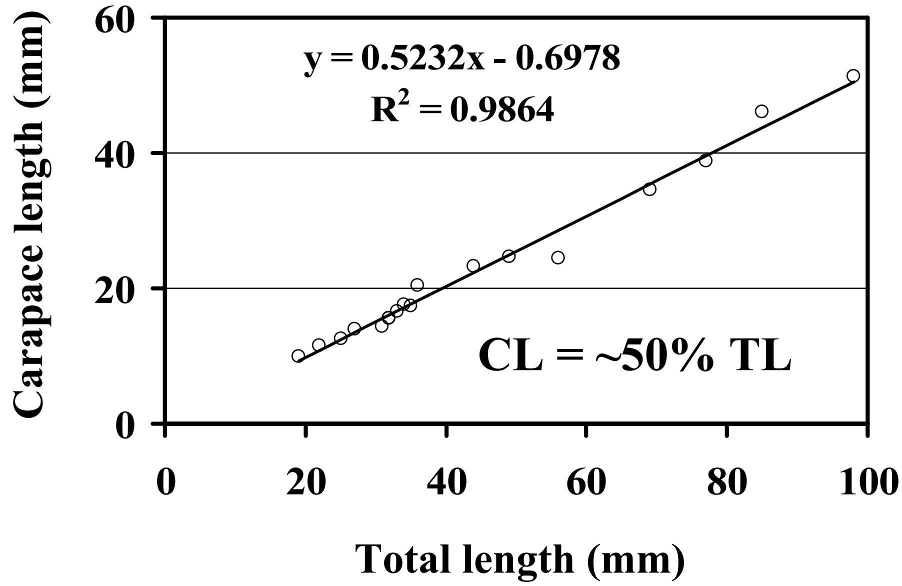


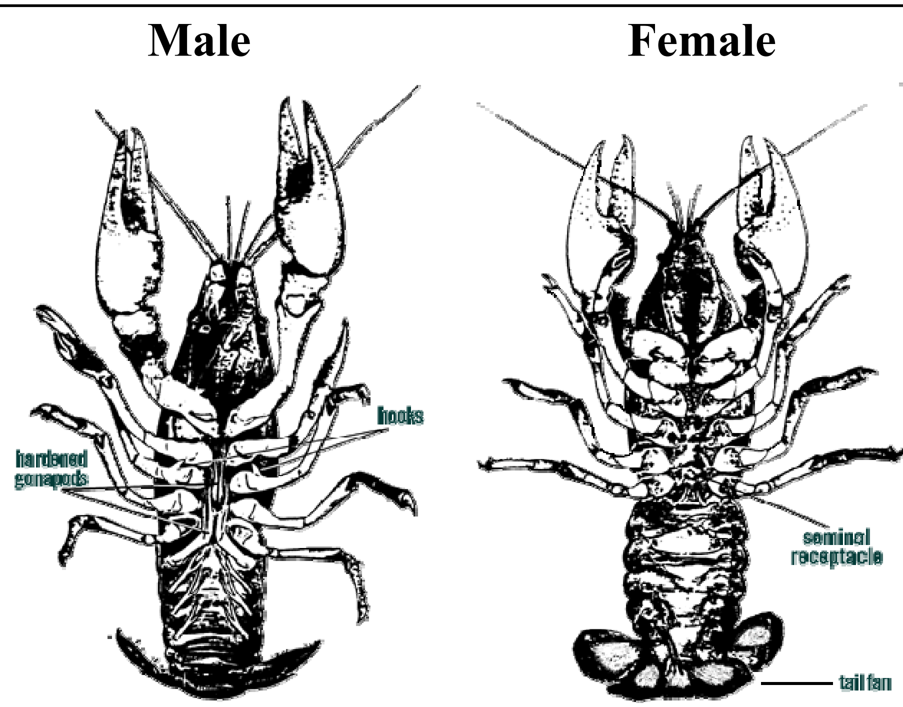
Total length

Total length difficult due to handling; may be erosion of telson

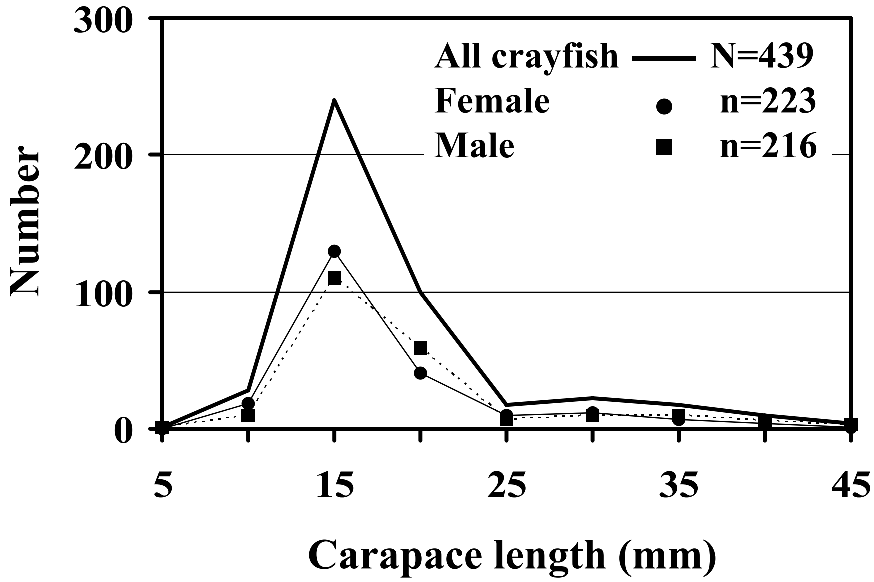


Total length vs. carapace length:

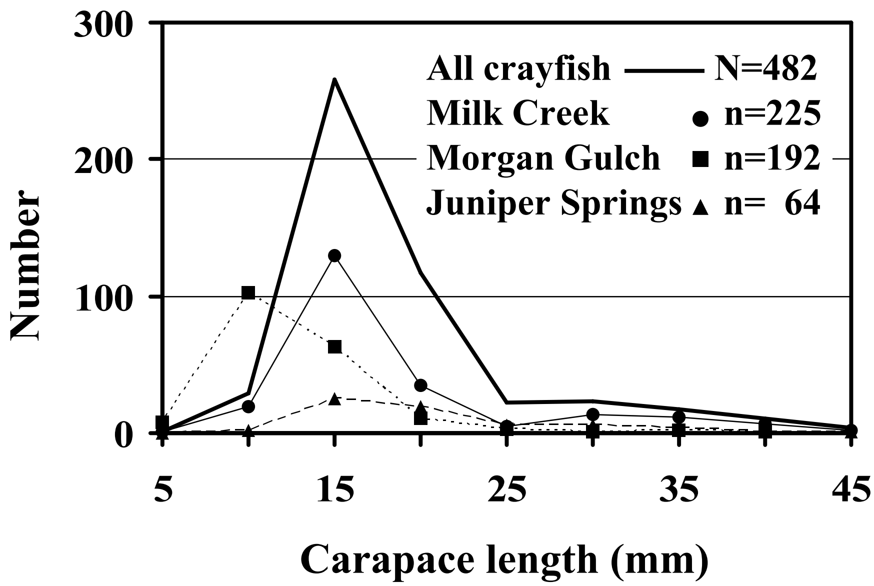




Crayfish sex vs. size composition:



Size structure by station:

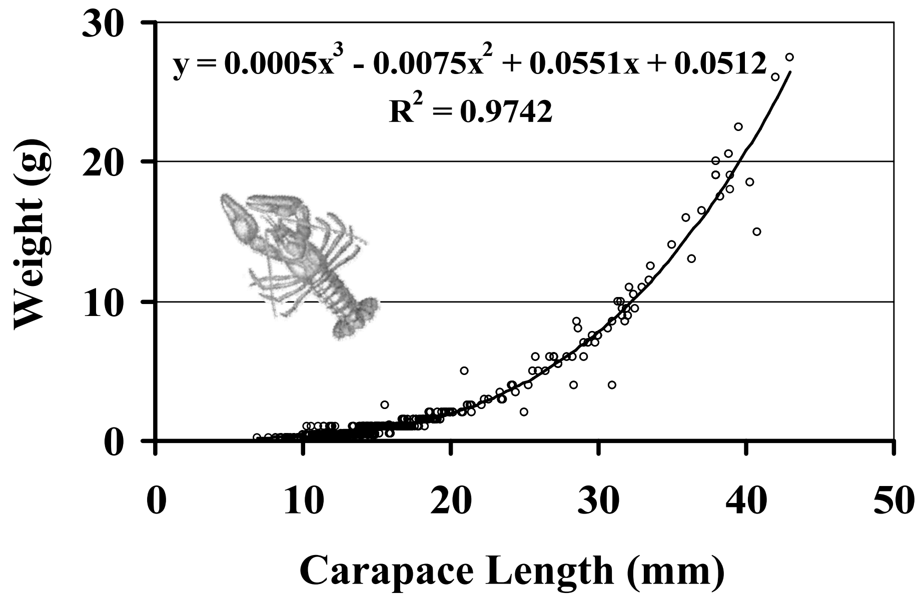


Density & size of crayfish:

Habitat	Percent*	No./m²	Weighted density	Mean carapace length in mm	Weighted CLmm
Juniper Springs					
RUN	64%	3.6	2.3	18.2	11.7
Morgan Gulch					
POOL	30%	10.4	3.1	15.1	4.5
Milk Creek					
RIFFLE	6%	22.1	1.3	16.3	1.0
100%		**Wtd. no./m² = 6.7**		**Wtd. CL = 17.2 mm**	

*Anderson, R. 2003. Riverine fish flow investigations. Federal Aid Project F-289-R6 Progress Report. Colorado Division of Wildlife, Fort Collins. 100 pp.

Carapace length vs. weight:



Estimating VCF numbers & biomass:

- YAR mean width = 60m; length = 80 miles (130 km)
- ~7.8M m^2 of stream @ 6.7 NCF/m^2 = **~52M VCF**
- Mean VCF CL = 17.2mm @ 1.3 g = **~9g/m2**
- ~400K VCF/km = ~540 kg/km = **90 kg/ha**

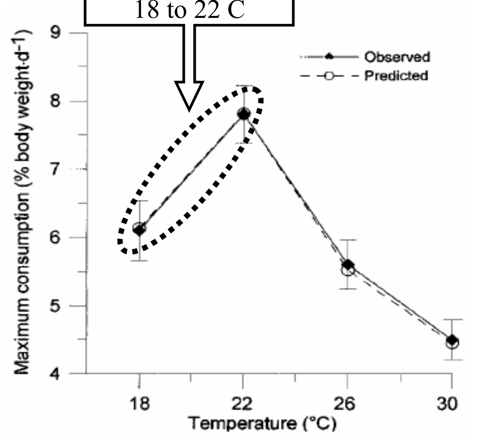
Standing crop + annual production vs. SMB predation (kg/ha):

YAR	All fish >150mm	[native fishes]	Fish <150mm	VCF	**SMB prey**
p/b	0.34		1.25	0.58	
SC+AP	100	[40]	32	142	**45**
SMB pred.	**~50%**	**[>100%]**	**>100%**	**~30%**	

- <u>was VCF explosion a bioenergetic response to temperature?</u>

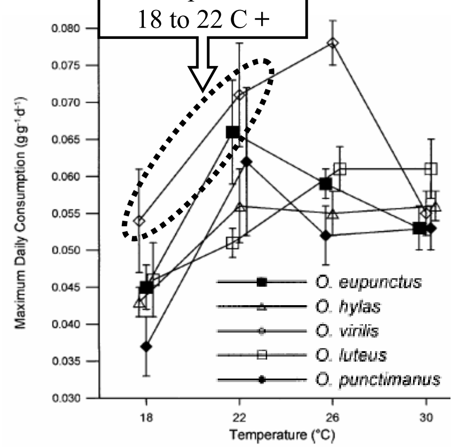
Rapid increase in consumption from 18 to 22 C

SMB



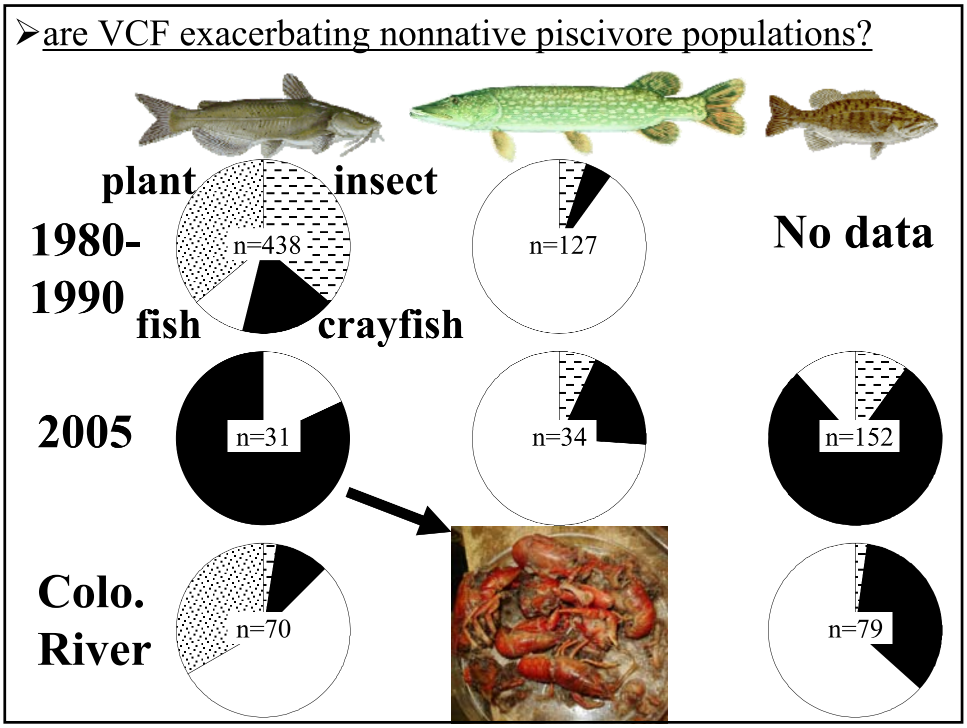
Similar increase in consumption from 18 to 22 C +

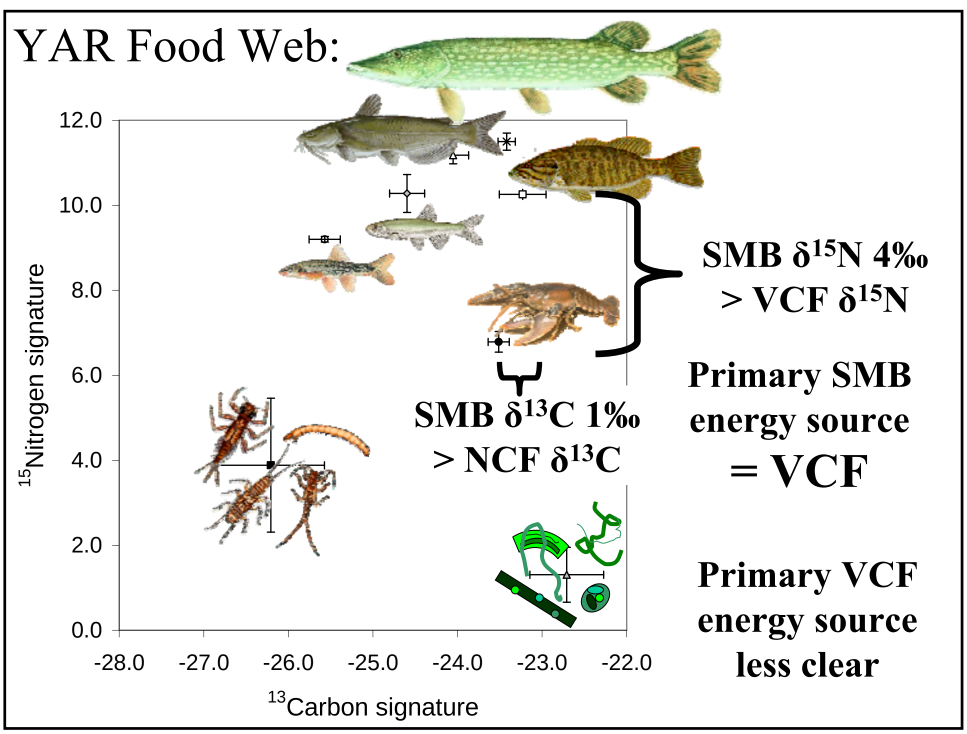
VCF



Whitledge, G. W., R. S. Hayward, R. D. Zweifel, and C. F. Rabeni. 2003. Development and laboratory evaluation of a bioenergetics model of subadult and adult smallmouth bass. Transactions of the American Fisheries Society 132:316-325.

Whitledge, G. W., and C. F. Rabeni. 2003. Maximum daily consumption and respiration rates at four temperatures for five species of crayfish from Missouri, U.S.A. (Decapoda, *Orconectes* spp.). Crustaceana 75 (9):1119-1132.


➢are VCF exacerbating nonnative piscivore populations?
plant
insect
1980-
1990
n=438
n=127
No data
fish
crayfish
2005
n=31
n=34
n=152
Colo.
River
n=70
n=79


YAR Food Web:
12.0
10.0
8.0
6.0
4.0
2.0
0.0
-28.0
-27.0
-26.0
-25.0
-24.0
-23.0
-22.0
15Nitrogen signature
13Carbon signature
SMB δ15N 4‰
> VCF δ15N
SMB δ13C 1‰
> NCF δ13C
Primary SMB
energy source
= VCF
Primary VCF
energy source
less clear

➢might crayfish compete with/prey upon native fishes?

- http://www.usgs.gov/invasive_speciesplw/crayfish.html

  - juvenile desert sucker *Catostomus clarkii* & sonora sucker *Catostomus insignis* < 7mm long vulnerable to VCF predation

  - suckers lost weight in association with VCF in lab

- Carpenter, J. 2005. Competition for food between an introduced crayfish & two fishes endemic to the Colorado River basin. Environmental Biology of Fishes 72:335-342.

  - VCF competed for food & reduced growth of flannelmouth sucker *Catostomus lattipinnis* in lab

  - Gila chub *Gila intermedia* less affected by VCF

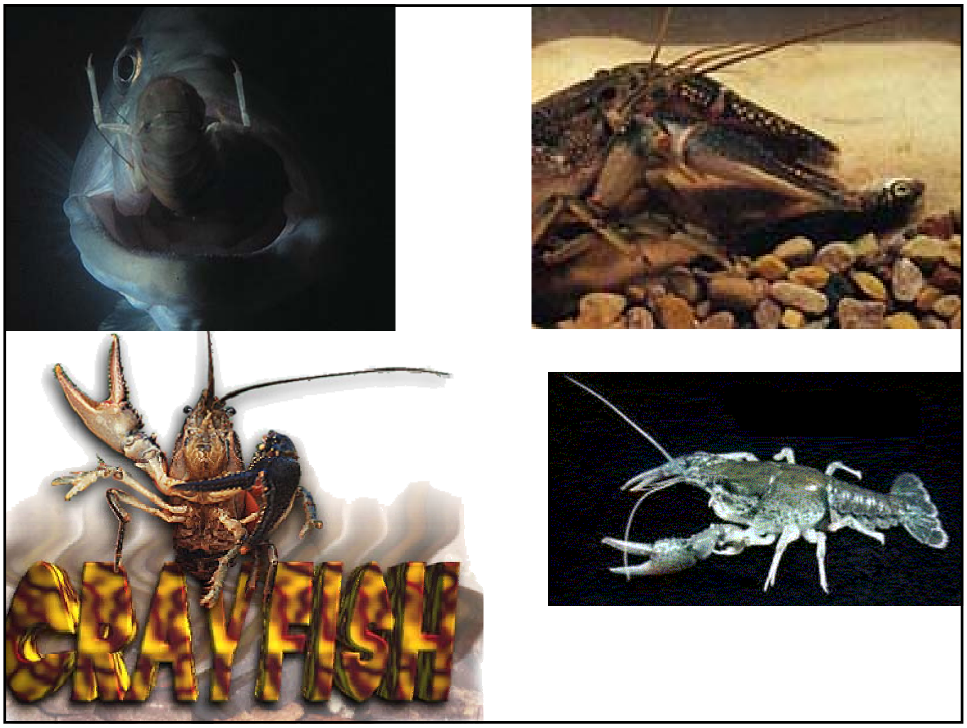
  - VCF likely compete with suckers & chubs via interference competition = agnostic or territorial behavior

Recommendations:

- Further evaluate crayfish sampling methods & data to establish protocol to monitor VCF in YAR & elsewhere to track population trends & study role in riverine food webs

- Nonnative crayfish should be of great concern given VCF benefit to riverine nonnative fishes & evidence of VCF predation/competition with native fishes of UCRB

- Review crayfish stocking activity, policies, & regulations to slow spread of VCF & to prevent inadvertent, illicit or intentional introduction of new crayfish species into UCRB


CRAYFISH