DISSERTATION

INTERACTING EFFECTS OF PHYSICAL AND BIOLOGICAL FACTORS ON

RECRUITMENT OF AGE-O COLORADO SQUAWFISH

Submitted by
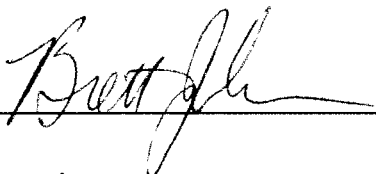
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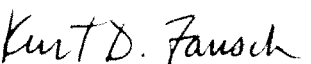
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WE HEREBY RECOMMEND THAT THE DISSERTATION

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ABSTRACT OF DISSERTATION

EFFECTS OF PHYSICAL AND BIOLOGICAL FACTORS ON

RECRUITMENT OF AGE-O COLORADO SQUAWFISH

Recruitment is central to population ecology because the abundance of young

individuals often drives dynamics of subsequent life stages. However, understanding

mechanisms that regulate recruitment is challenging because it integrates numerous life-

history processes including timing and success of reproduction, and growth and dispersal

of young. Recruitment processes are particularly difficult to understand in riverine

ecosystems that are physically disturbed by the presence and operations of impoundments

and biologically altered by numerous non-native species. I used a combination of

laboratory and field investigations to evaluate the importance of physical and biological

factors that may affect recruitment of early life stages of endangered Colorado squawfish

Ptychocheilus lucius in the Green River, a regulated river of the Colorado River basin.

Colorado squawfish embryos reared in three constant (18, 22, 26°C) and three

fluctuating temperatures (18, 22, and 26°C; diel fluctuation ± 2.5°C) had relatively high

hatch rates of 54-79%, whereas hatching success was only 38% at constant 30°C. There

was no significant difference in hatch between constant and fluctuating temperatures.

Survival of larvae to 7-d was 52-88% in water temperatures ranging from 18-26°C but

was only 13% at constant 30°C. Survival of larvae was 10-31% higher in fluctuating than

in constant temperatures. Incidence of abnormalities was 2-22% at 18-26°C and 100% at

30°C. Differences in abnormality rates were not detectable between constant and

fluctuating temperatures. Time to initiation of hatch, swim bladder inflation, and

exogenous feeding was shorter at higher temperatures. Differences in lengths of larvae at hatching and at 7-d at the various test temperatures were small and not considered ecologically significant. Tolerance of a relatively wide range of warm water temperatures by Colorado squawfish embryos and larvae may reflect the historically variable Colorado River environments in which the species evolved. Cold summer water temperatures caused by mainstream dams have eliminated Colorado squawfish from portions of its historic Colorado River basin range. Water temperatures that more closely reflect historic regimes are necessary to restore self-sustaining populations of Colorado squawfish in those areas.

Growth and survival response of Colorado squawfish larvae up to 42 d-old was determined under fluctuating and constant 18, 22, 26°C, and 30°C water temperatures and at five different food abundance levels. Optimal growth was at the highest water temperatures and food abundance levels. High growth was observed at temperatures > 25°C with moderate food abundance; growth was lowest under lowest food rations and highest temperatures. Survival of larval Colorado squawfish was optimal at 26.2°C and 235 nauplii·fish⁻¹·day⁻¹; model solutions indicated that survival was > 90% at temperatures ranging from 20 to 30°C with moderate food abundance. Survival was lower at 18°C even in the presence of high food abundance and was lowest when food abundance was low and temperature was high. Neither growth nor survival was significantly different between fluctuating or constant regimes. Colorado squawfish larvae denied food for 5, 10, or 15 d after first feeding could have begun (6 d), had survival greater than 87% which was equivalent to continuously fed controls. Survival of fish denied food for 17.5 d after feeding could have begun declined from 84% before

feeding to 57% after feeding. Survival of fish denied food for > 20 d after first feeding was low. Point of no return was between 17.5 and 20 d. Low, stable flows that simulate natural hydrographs may enhance growth, survival, and recruitment of early life stages of Colorado squawfish by increasing water temperature and food abundance in regulated rivers of the Colorado River basin.

Otolith analysis may be a tool to evaluate various hypotheses about recruitment regulation in wild populations of Colorado squawfish. Otolith microstructure of Colorado squawfish was investigated under different temperature and feeding regimes to determine validity of daily increment aging and patterns of otolith growth. Sagittae and lapilli formed prior to hatching. Otolith increments were deposited daily after hatching in fish in constant 22°C and fluctuating ($\pm$ 2.5°C d^{-1}) 18, 22, and 26°C temperatures. Otolith increments were clearer and counts were more accurately estimated in fish reared in fluctuating than in constant temperatures. Lapillus diameters of slow growing Colorado squawfish were significantly larger than similar-sized fast growing fish, indicating that fish and otolith growth rates were not proportional. Somatic growth of starved larvae generally ceased immediately but otolith growth continued for up to 15 d. Otolith growth was reduced for up to 5 d after starved fish began to feed. Timing of starvation and reduced growth may not be accurately recorded by reduced otolith increment spacing. Otolith increments deposited during starvation periods were of low contrast and may indicate periods of low food abundance in the wild. Otolith analysis will be useful for elucidating age, growth, and recruitment patterns of young Colorado squawfish.

Recruitment of age-0 Colorado squawfish in the Green River, Colorado River basin, was investigated with otolith analysis to evaluate relative importance of physical

and biological factors at intra-annual and annual time scales. Distributions of hatching dates in 1991 and 1992 indicated that larvae in cohorts that hatched early survived poorly to fall. Growth rate comparisons suggested that the few early hatched fish that survived were a fast-growing subset of the fish present in the same cohort in summer. I attributed this to size-selective predation mortality by introduced fishes. Larvae most likely to survive to fall were hatched late and grew relatively slowly. Slow growth rates and high survival that are incongruous with patterns for early-hatched larvae were probably due to environmental factors and to natural mortality of large predaceous red shiners *Cyprinella lutrensis* in mid- to late-summer. An independent individual-based computer simulation model which had a gape-limited red shiners as predators and Colorado squawfish larvae as prey produced similar size-selective patterns. Results of model simulations also suggested that fish with moderate growth rates were more than twice as likely to survive as fish with low-growth rates, underscoring the biological significance of growth. Alternative hypotheses to explain recruitment patterns such as starvation and competition were not proximate explanations for the size-selective patterns observed. A physical process, stochastic flooding reduced growth rates of Colorado squawfish and combined with size-selective predation to cause very low recruitment in the lower Green River in 1992. Otherwise, recruitment was unaffected by discharge and temperature regimes in the summers of 1991 and 1992. Linear plateau regression models predicted no negative effect of mean July-August discharge level on annual abundance of Colorado squawfish juveniles except at relatively high discharge. Low abundance of juvenile Colorado squawfish in 1991 and 1992 when size-selective patterns were evident suggested that predation may regulate recruitment in some years.

These results suggest that Colorado squawfish are able to recruit substantial populations of age-0 fish under a wide variety of environmental conditions even when habitat may be limited. Results also suggest that non-native fishes may have a substantial impact on recruitment of age-0 Colorado squawfish. Discharge management that emphasizes habitat enhancement should be supplemented with strategies to reduce effects of non-native fishes.

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INTRODUCTION

Recruitment is central to population ecology because the abundance of young

individuals often drives dynamics of subsequent life stages (Roughgarden et al. 1988).

Understanding mechanisms that regulate recruitment is challenging because it integrates

many disparate life-history processes, including timing and success of reproduction and

growth and dispersal of young. Moreover, most aquatic organisms with larval and adult

phases have highly variable recruitment because their high fecundity, coupled with small

variations in regulating processes, cause large differences in survival of larvae (Hjort

1914, Thorson 1950, Fogarty et al. 1991).

Recruitment may often be regulated by interacting physical and biological

processes, which change in relative importance across temporal and spatial scales. In

marine systems and freshwater lakes, physical processes such as nearshore currents

produced by wind may transport larvae into or away from suitable habitats and thereby

influence local or regional recruitment (Frank and Leggett 1981, Roughgarden et al.

1988). In rivers, regimes of discharge, temperature, and sediment transport influence

timing of reproduction by adults and the quality and quantity of critical habitat for larvae

(Petts 1984). Distribution, abundance, and size-structure of the population of

reproducing adults determines the supply of larvae upon which other biological factors

act (Thorson 1950, Gaines et al. 1985, Underwood and Fairweather 1989). These factors

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include predation, competition, and starvation, which often interact with environmental conditions that influence growth to regulate recruitment of larvae (May 1974, Hunter 1981, Houde 1987, Bailey and Houde 1989).

Most populations of endangered Colorado squawfish *Ptychocheilus lucius* of the Colorado River basin may be recruitment-limited (Tyus 1991). In Green River, which is the largest remaining population, annual densities of juveniles in fall (recruits) vary from near zero up to 75 fish/100 m^2 of backwater habitat (Tyus and Haines 1991). However, the relative effects of discharge regime, habitat alterations, and introduced fishes on recruitment among and within years is poorly understood. I designed laboratory experiments (chapters 1-3) and a field study (chapter 4) to evaluate the relative importance of physical and biological factors on recruitment of age-0 Colorado squawfish.

Experiments described in chapter one (Bestgen and Williams 1994) were used to evaluate effects of different water temperatures, and constant and fluctuating temperature regimes, on hatching success of Colorado squawfish embryos and survival of larvae to 7 d. In chapter two (Bestgen 1996), growth and survival of larvae was measured at four different water temperatures levels, constant and fluctuating temperature regimes, and at five different levels of food abundance. In addition, an experiment to determine starvation resistance of squawfish larvae was also conducted. Chapter three (Bestgen and Bundy; submitted to *Transactions of the American Fisheries Society*) details experiments to validate the use of otoliths in determining age and growth rate of Colorado squawfish larvae and juveniles reared at different water temperatures with constant and fluctuating regimes, and different food conditions.

I then used information from laboratory experiments to evaluate effects of physical and biotic processes that may explain intra-annual and annual recruitment patterns of age-0 Colorado squawfish observed in the field. Field investigations (chapter four) employed otolith analyses to estimate hatching dates and growth rates of wild Colorado squawfish. This information was used to evaluate whether differential and size-selective patterns of mortality were evident in cohorts of larvae hatched at different times in the summers of 1991 and 1992. Finally, conclusions of field investigations were evaluated with an individual-based model and a long-term recruitment data set.

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CHAPTER ONE

EFFECTS OF FLUCTUATING AND CONSTANT TEMPERATURES ON

EARLY DEVELOPMENT AND SURVIVAL OF COLORADO SQUAWFISH

ABSTRACT

A laboratory study was conducted to determine the effects of four constant temperatures (18, 22, 26, and 30°C) and three fluctuating temperatures (18, 22, and 26°C; diel fluctuation ± 2.5°C) on early development and survival of Colorado squawfish *Ptychocheilus lucius*, which is listed as an endangered species by the U. S. Department of Interior. Average hatch in constant and fluctuating temperatures was 72% at 18°C, 67% at 22°C, 62% at 26°C and 38% (constant temperature only) at 30°C. There was no significant difference in hatch between constant and fluctuating temperatures. Average survival of larvae to 7 d in constant and fluctuating temperatures was 68% at 18°C, 64% at 22°C, 83% at 26°C, and 13% (constant temperature only) at 30°C. Survival of larvae at 30°C may be confounded by relatively low hatch of embryos and poor condition of larvae. Survival of larvae was 10-31% higher in fluctuating than in constant temperatures. Incidence of abnormalities was 2-22% at 18-26°C and 100% at 30°C. Differences in abnormality rates were not detectable between constant and fluctuating temperatures. Time to initiation of hatch, swim bladder inflation, and exogenous feeding was shorter at higher temperatures. First feeding occurred about 31 h earlier in fluctuating temperatures than in constant temperatures. Differences in lengths of larvae at hatching (d 1) and at d 7 at the various test temperatures were small and not considered ecologically significant. Tolerance of a relatively wide range of warm water temperatures by Colorado squawfish embryos and larvae may reflect the historically variable Colorado River environments in which the species evolved. Cold summer water temperatures caused by mainstream dams have eliminated Colorado squawfish from portions of its historic Colorado River basin range. Water temperatures that more closely reflect historic

regimes are necessary to restore self-sustaining populations of Colorado squawfish in

those areas.

The Colorado squawfish *Ptychocheilus lucius* was listed as an endangered species in 1967 (U. S. Department of Interior, 1973). Colorado squawfish formerly were widespread and abundant throughout warmwater reaches of the Colorado River basin, but are presently restricted to the upper basin upstream of Lake Powell in the Green, Colorado, and San Juan rivers and their larger tributaries (Behnke and Benson 1983; Platania et al. 1991). Decline of Colorado squawfish has been attributed to introduction of nonnative fishes that compete with, or prey upon early life stages (Miller 1961; Williams et al. 1989, Carlson and Muth 1989). Mainstream dams constructed throughout the Colorado River basin beginning in 1913 have also negatively affected distribution and abundance of endemic Colorado squawfish (Holden 1979; Tyus 1991a). Low summer water temperatures caused by hypolimnetic releases from these dams may curtail reproduction of Colorado squawfish and reduce growth and survival of young fish (Vanicek et al. 1970; Holden 1979; Hamman 1981; Marsh 1985; Kaeding and Osmundson 1988).

Previous laboratory studies have evaluated the effect of temperature on hatch of Colorado squawfish embryos, but not on larval survival. Hamman (1981) induced spawning of hatchery Colorado squawfish at 18-19°C and hatched < 3% of embryos at 12-13°C and 20-21°C. Embryos from wild Colorado squawfish that were artificially produced by hormone injections or spawned naturally in raceways had hatch rates of 30-90%, when incubated at temperatures of 20-24°C. Marsh (1985) incubated Colorado squawfish embryos at 5, 10, 15, 20, 25 and 30°C and found hatch was highest (2%, and 27% in two trials) at 20°C. He also documented lower hatch (0%, and 9%) and highest incidence of abnormalities at 25°C, and no hatch at the other temperatures. Optimum

embryo incubation temperature for Colorado squawfish was determined to be 20-22°C
(Hamman 1981; Marsh 1985).

The narrow optimum temperature range for Colorado squawfish embryo
incubation suggested by laboratory studies was not expected given the highly variable
temperature regimes found during the spawning season of this species. Colorado
squawfish in the unregulated Yampa River, Colorado, may spawn when water
temperatures range from 16 to 26°C (Nesler et al. 1988, Tyus 1990). The relatively wide
temperature range during presumed reproduction by Colorado squawfish in the wild
suggests that the optimal temperature range for embryo incubation found in previous
laboratory studies may be too narrow. This study was conducted to determine effects of
water temperature and of constant and fluctuating temperatures on hatch of embryos, and
on survival and development of Colorado squawfish larvae.

Methods

Colorado squawfish embryos were obtained from the Dexter National Fish
Hatchery and Technology Center, Dexter, New Mexico. Embryos used in the 18-26°C
treatments in 1991 were from wild fish captured from the Colorado River, near Grand
Junction, Colorado. The wild adults were held in ponds and allowed to ripen naturally
until 14 June, which is 0-4 weeks earlier than wild Colorado squawfish spawn in upper
Colorado River basin (Nesler et al. 1988; Tyus and Haines 1991). Embryos used in the
30°C treatment in 1992 were from a mixed, upper Colorado River broodstock held in the
Dexter hatchery; these fish were allowed to ripen naturally in ponds until 29 May.
Colorado squawfish were artificially spawned following injections with common carp

Cyprinus carpio pituitary (males) and human chorionic gonadotropin (females; Hamman 1981). The resulting embryos were shipped to Fort Collins, Colorado, in water at 18-21°C within 24 h.

Four groups of 30 viable embryos each were randomly assigned to each experimental treatment, placed in incubation chambers, and acclimated to test temperatures at about 2°C/h. Embryos were incubated in chambers constructed from 76-mm inside-diameter polyvinyl chloride (PVC) pipe with 560-um nylon screen attached inside and halfway down the 100 mm long pipe section with silicone caulk. Chambers were placed in a bath and filled with water delivered via a fine bore pipette. Holes were drilled at the base of the chamber wall to allow water to flow out. Embryos were incubated at nominal constant temperatures of 18, 22, 26, and 30°C and nominal fluctuating temperatures of 18, 22, and 26°C (diel fluctuation of ± 2.5°C). One group of embryos was incubated in each of four test chambers at each of the seven constant and fluctuating temperature treatments. Additional embryos and larvae were held in a fifth chamber at each treatment so that observations of development and fish length could be made without disturbing fish in the other four incubation chambers.

Diel fluctuating temperatures were tested because diel temperature fluctuations of 5°C or more are common in the Yampa and Green rivers, Colorado and Utah, during the spawning season of Colorado squawfish (late June to early August, Nesler et al. 1988; personal observation KRB). Our fluctuating temperature cycle mimicked the natural cycle as follows: lowest temperatures occurred from 0100 to 0700 h (e.g., 19.5°C for the 22°C fluctuating treatment); warming occurred at a constant rate from 0700 to 1300 h; temperatures were highest from 1300 to 1900 h (e.g., 24.5°C for the 22°C fluctuating

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treatment); and cooling occurred at a constant rate from 1900 to 0100 h. Water temperatures in the constant and diel fluctuating regimes were maintained close to the desired nominal or nominal $\pm$ 2.5°C diel range (Table 1). Head reservoirs provided uniformly heated water to each incubation chamber. Flow-through conditions in each incubation chamber (complete exchange rate every 7.6 min) maintained dissolved oxygen at 5-6 mg/L, and prevented accumulation of waste products. Photoperiod was 14 h light:10 h dark. Some embryos became infected with fungus in the first 16 h after tests began and embryos in all test chambers were immediately treated with 1% formalin for 5 min and, 2 h later, with 5 mg/L malachite green for 30 min. A few embryos (< 5%), died early in the study, perhaps from fungus or developmental deficiencies. They were replaced with viable embryos from the appropriate fifth incubation chamber. After the first 16 h, no embryos were replaced. Fungus was not noted after treatment with malachite green and all subsequent egg mortality was attributed to treatment effect. Treatment of embryos with fungicide was not seen as a confounding factor because all embryos were treated similarly. Embryos with an opaque, irregularly-shaped chorion were pronounced dead; mortality was verified under a dissecting microscope (30x).

Embryos were monitored 6-12 times daily and first, 50%, and 100% hatch were recorded. Immediately after hatching, each larva was measured (total length, TL, to the nearest 0.1 mm) with a dissecting microscope fitted with a calibrated ocular micrometer. Measured larvae were counted and placed in 0.5 L jars in water baths at treatment temperature until hatching was completed. Surviving larvae were then counted and returned to their incubation chambers.

Daily observations to determine age at swim bladder inflation and first feeding were made with a dissecting microscope. Age at swim bladder inflation and first feeding in each treatment was taken as the age at first observed occurrence in an individual larva in the treatments. Newly hatched brine shrimp nauplii *Artemia sp.* were presented to larvae twice daily ad libitum and presence or absence of food in the gut was documented 1 h later. Larvae were maintained in experimental treatments for 7 d after hatching and then counted and measured. Larvae were maintained in the fifth incubation chamber for all treatments until first feeding was observed. All larvae were examined daily for obvious abnormalities (i.e., lack of eyes, spinal deformities, swim bladder anomalies).

Percent hatch, percent survival of larvae to d 7 (of embryos that hatched), and percent of larvae with abnormalities (of embryos that hatched), were calculated from the four replicates in each treatment. Percentages were transformed (arcsine and square-root) to stabilize statistical variances and normalize the data. Main effects of temperature at three levels (18, 22, 26°C) and constant versus fluctuating temperature regimes and their interaction (temperature x regime) were analyzed with fixed-effects two-factor analysis of variance (ANOVA). The Tukey test for multiple comparisons was used if the overall F-test was significant ($P \leq 0.05$). Statistical analyses were conducted with the SAS statistical package (SAS Institute 1988). The data for the 30°C constant temperature treatment were excluded from statistical analyses because all larvae were deformed.

Results

Hatch.-- Mean hatch at 18, 22, and 26°C in constant and fluctuating regimes was 54-79% (Table 1) and did not differ significantly among treatments for main effects or for

the interaction (Table 2). When data for constant and fluctuating regimes were combined, hatch was higher at 18°C than at 22 or 26°C. Hatch at 30°C was 39%.

Survival.--Mean survival of larvae to d 7 in the 18-26°C treatments was 52-88% (Table 1), and the overall ANOVA F-test was significant for both main effects (temperature and regime), but not for the interaction (Table 2). Survival was highest at 26°C and was significantly different from the 18 and 22°C treatments; neither of the latter temperature treatments were significantly different from each other. Survival was higher in each fluctuating temperature treatment than in the corresponding constant temperature. Most of the significance of the overall F-test for regimes was due to higher survival in the 18°C fluctuating than in the 18°C constant treatment. Survival of larvae to d 7 in the 30°C treatment was 12.5%.

Abnormalities.--Abnormalities in the 18-26°C treatments was 2-22% (Table 1), and the overall F-test was significant due to temperature level but not to regime or interaction effects (Table 2). Lowest incidence of abnormalities was in the 26°C (4%, regime combined) and 18°C (12.5%) treatments, which were not significantly different. Mean percent abnormalities was higher in the 22°C treatments (19%); this rate was significantly different only from that observed in the 26°C treatments. All larvae hatched in 30°C were abnormal.

Timing of developmental events.--At a given temperature level, times to first hatch, 50% hatch, and 100% hatch were generally similar for fluctuating and constant regimes (Table 1). For example, time to initiation of hatching differed by 41 h between 18 and 26°C treatments (regimes combined). However, times for each hatching event interval between the 18 and 26°C treatments (regimes combined) were nearly equal (41-

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42.5 h), suggesting that once hatching began at any temperature, times to midpoint and end of hatching were about equal across all temperatures. In general, swim bladder inflation and first feeding occurred sooner at higher temperatures and also at fluctuating temperatures.

Lengths of larvae.--Length at hatch (Table 1) differed significantly for temperature level but not for regime, or interaction (Table 2). Lengths were significantly different in 18 and 26°C treatments, but not in the 22 and 26°C or 18 and 22°C treatments. Lengths at d 7 differed significantly for regime and interaction but not for temperature level. Most interaction significance was due to faster growth in the fluctuating regime at 22°C.

Discussion

Hatch and survival.--Results presented here generally corroborate those of previous laboratory studies. I also found hatch and larval survival were generally higher over a wider range of temperature conditions than previously reported. This may reflect the low density of embryos and the rapid exchange of water in our incubation chambers which minimized fungal infection and oxygen deprivation. Differences in hatch and survival at 18-26°C and 30°C may partly reflect the fact that these treatments were done in different years with embryos from different broodfish.

High hatch and survival of larvae were observed in all treatments except 30°C, a temperature rarely recorded in river channels in the upper Colorado River basin. Summer water temperatures of 30°C were found in the former range of Colorado squawfish in the lower Colorado River (Dill 1944). However, it is likely that Colorado squawfish there

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spawned earlier in the spring when water temperatures were lower. Although little is known about the reproductive ecology of Colorado squawfish in that area, spring spawning is also supported by collection of 32 mm (likely age-0) Colorado squawfish in May in Arizona (Sigler and Miller 1963).

Hatch was highest at 18°C (regimes combined) and lowest at 26°C, but survival to 7 d post-hatch was lowest at 18°C and highest at 26°C. If overall reproductive output (product of % hatch and % survival of larvae) to 7 d is considered, the "optimum temperature" for reproduction by Colorado squawfish may be 18-26°C. The lower temperature limit for incubation is unknown but survival was < 3% among embryos incubated at 12-13°C (Hamman 1981). The upper temperature limit for incubation is probably near 30°C. Our results indicate reproduction is precluded at 30°C by the high rate of abnormalities. Marsh (1985) found no hatching of Colorado squawfish embryos spawned artificially at 18°C and transferred to 10, 15, or 30°C; however, he did not acclimate his embryos, which increased the likelihood of temperature shock and subsequent embryo mortality.

The higher hatch at lower temperatures and higher post-hatch survival at higher temperatures observed in this study may suggest that these are differences in physiological tolerances of embryos and larvae and may represent adaptions to particular habitats. Colorado squawfish may spawn in the 16-26°C main channel habitat (Nesler et al. 1988; Tyus and Haines 1991). Larvae emerge from the substrate after an undetermined period, drift downstream, and occupy 25-28°C, low-velocity channel margin habitats. Field evidence suggests that larger (older) Colorado squawfish larvae and juveniles prefer backwater habitat (Tyus 1991b; Tyus and Haines 1991, Haines and

Tyus 1991), where warmer water promotes faster growth and better survival. Furthermore, movements of marked age-0 Colorado squawfish suggest they seek warmer water (Tyus 1991b).

If optimal embryo hatch and larval survival temperatures are indeed different, then hatch and larval survival may not be independent at some temperatures due to the experimental design used in this study. In the 30°C treatment, which is marginal for hatch, survival of larvae may be confounded by the low number and poor condition of embryos that hatched. Survival of larvae hatched in cooler temperatures and then acclimated and reared at 30°C might have been higher than that of larvae hatched and reared at 30°C.

Hatch was not significantly different in fluctuating and constant regimes. Survival of larvae to d 7 was higher in fluctuating temperatures (11-31%), due largely to high survival in the fluctuating regime and low survival in the constant regime in the 18°C treatment.

Abnormalities.--Abnormalities were apparent in all fish from the 30°C treatment, but incidence was lower at 18-26°C and similar to that observed by Marsh (1985) at 20 (11%) and 25°C (26 %). Abnormalities observed in all treatments in this study were usually so severe (pronounced spinal deformity, ballooning of the abdominal cavity) that these fish would not survive in the wild.

Developmental rates and growth.--Rates of development of Colorado squawfish embryos and larvae varied directly with water temperature. Reasons for the markedly longer hatching interval at constant than at fluctuating regimes at 18 and 22°C are unknown. Fluctuating temperatures at 18 and 22°C may have also shortened the time to

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swim bladder inflation and exogenous feeding. Despite statistically significant differences in lengths of larvae in some treatments, it is improbable that the small differences observed for either newly hatched (difference among mean lengths in treatments < 15%) or 7 d post-hatch larvae (difference < 5%) are of ecological significance. Different endogenous feeding rates between treatments may be the reason for differences in fish lengths.

Times to developmental events and length measurements were recorded irregularly in the 30°C treatment due to low hatching success and poor condition of larvae and may be less accurate than those reported for the other treatments. Time of first hatch and swim bladder inflation may not represent the norm due to rare events (e.g., very early hatch or air bladder inflation in a single fish). The times to first feeding that I report are more reliable because most fish in a treatment chamber began to feed almost simultaneously, thus reducing the potential influence of rare events.

Management implications.--Colorado squawfish apparently have complex adaptive strategies for reproduction in historically fluctuating riverine habitats of the Colorado River basin and may use discharge level and variation, and water temperature, as primary environmental cues to initiate reproduction (Nesler et al. 1988; Tyus 1990). In its unregulated state, flow and temperature regimes in the Colorado River basin varied dramatically across seasons and years. Given such variability, eurythermicity of embryos and early larvae is not surprising.

Reduced summer water temperature is thought to be partially responsible for extirpation of Colorado squawfish in river reaches immediately downstream from dams (Holden 1979; Behnke and Benson 1983; Marsh 1985). Reestablishment of self-

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sustaining populations of Colorado squawfish in reaches affected by dams may require

restoration of temperatures that more closely approximate historical regimes (Tyus and

Karp 1989). Effects of a diverse and abundant nonnative fish community on Colorado

squawfish should also be considered. Integrated laboratory and field research is needed

to better determine the role of temperature and other factors that control migration,

spawning, early development, and survival of Colorado squawfish in the Colorado River

basin.

Acknowledgments

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Table 1.--Percent hatch and survival. and early development (mean and SE; $\underline{N}$ = 4 replicates) of Colorado squawfish reared at four constant and three diel fluctuating ($\pm$ 2.5°C) temperatures.

	Nominal temperature and regime[a]						
	18°C		22°C		26°C		30°C[b]
Biological variables	Constant	Fluctuating	Constant	Fluctuating	Constant	Fluctuating	Constant
Mean percent hatch	79 (2.5)	65 (5.0)	60 (7.8)	74 (8.7)	69 (4.8)	54 (10.5)	38 (6.5)
Mean percent survival to 7 d posthatch	52 (4.5)	83 (4.3)	58 (5.8)	69 (7.9)	78 (4.1)	88 (4.9)	13 (4.4)
Mean percent abnormal[c]	15 (7.5)	10 (3.6)	14 (2.5)	22 (6.3)	2 (2.6)	6 (3.0)	100
Mean time (h) to:							
Initiation of hatching[d]	110 (8.1)	124 (9.7)	81 (5.7)	97 (1.3)	77 (1.6)	75 (0.3)	58
50% hatching[d]	134 (2.3)	144 (0.5)	115 (5.4)	105 (2.0)	96 (0.5)	98 (1.0)	68
End of hatching[d]	154 (6.8)	152 (4.8)	134 (13.0)	128 (4.8)	110 (4.8)	111 (4.9)	81

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Table 1 Continued.

	Nominal temperature and regime[a]						
	18°C		22°C		26°C		30°C[b]
Biological variables	Constant	Fluctuating	Constant	Fluctuating	Constant	Fluctuating	Constant
Hatching interval (h)[e]	44	28	53	31	33	36	23
Inflation of air bladder[f]	186	172	183	143	139	141	
First feeding[f]	229	194	183	167	163	122	
Mean total length (mm) at:							
Hatching ($\underline{N}$=10)	4.8 (0.3)	5.2 (0.2)	5.3 (0.1)	5.6 (0.2)	5.5 (0.2)	5.5 (0.2)	
7 d posthatching ($\underline{N}$=10)	9.7 (0.1)	9.8(0.1)	9.6 (0.2)	10.2 (0.1)	10.0 (0.1)	10.0 (0.1)	

[a]Nominal and mean (SE) temperatures as follows: Constant: 18°C = 19.1 (0.02); 22°C = 21.8 (0.06); 26°C = 25.6 (0.18); 30°C = 30.0 (0.14). Diel fluctuating (minumum and maximum): 18°C = 15.7 (0.14) and 20.5 (0.20); 22°C = 19.5 (0.14) and 24.7 (0.09); 26°C = 23.5 (0.21) and 28.3 (0.09).

Table 1 Continued

[b]Hatching time observations limited to one incubation chamber, lack of other event and length measures due to poor condition of embryos.

[c]Fish with obvious external abnormalities (i.e., scoliosis or lordosis; improper swim bladder inflation; incomplete eye development).

[d]Hours postfertilization.

[e]Time to end of hatching minus time to first hatch.

[f]Hours posthatch.

Table 2.--Probability values for overall, temperature level and fluctuating and constant regime main effect, and interaction F-tests, for hatch, larval survival, abnormalties, and length at hatch and 7 d post-hatch, of early life stages of Colorado squawfish.

| | Biological Variable | | | | |
Source	Hatch	Survival	Abnormalities	Length, hatch	Length, 7-d
Overall F-test	0.18	0.001	0.05	0.05	0.008
Main effects:					
Temperature	0.35	0.01	0.008	0.02	0.18
Regime	0.39	0.001	0.29	0.13	0.04
Interaction	0.09	0.13	1.000	0.68	0.02

CHAPTER TWO

GROWTH, SURVIVAL, AND STARVATION RESISTANCE OF COLORADO SQUAWFISH

LARVAE ESTIMATED BY RESPONSE SURFACE METHODOLOGY

ABSTRACT

Growth and survival response of larval Colorado squawfish, *Ptychocheilus lucius*,

under fluctuating 18, 22, and 26°C (5°C diel fluctuations) and constant 18, 22, 26°C, and

30°C temperature conditions and ration size corresponding to 12.5, 28, 64, 142, 320 brine

shrimp nauplii fish⁻¹ day⁻¹ was determined from laboratory experiments. Optimal growth

of 0.305 mm total length (TL) d⁻¹ was predicted at 31°C and 342 nauplii fish⁻¹ day⁻¹; high

growth of 0.281-0.297 mm TL d⁻¹ was found at temperatures of 25.7-27.6°C with 250-

293 nauplii fish⁻¹ day⁻¹. Lowest growth was under lowest food rations and highest

temperatures. Growth of Colorado squawfish larvae declined 4 to 7% for each degree

drop in temperature from 22 to 18°C, even in the presence of 142 and 320 nauplii fish⁻¹

day⁻¹. Neither growth nor survival was significantly different between fluctuating or

constant regimes. Survival of larval Colorado squawfish was optimal and 95% at 26.2°C

and 235 nauplii fish⁻¹ day⁻¹; model solutions indicated that survival was > 90% at

temperatures ranging from 20 to 30°C with food abundance > 180 nauplii fish⁻¹ day⁻¹.

Survival was lower at 18°C even in the presence of high food abundance and was lowest

when food abundance was low and temperature was high. Highest mortality occurred

more than 20 days after experiments began and mortalities occurred sooner in higher than

lower temperatures. Colorado squawfish larvae denied food for 5, 10, or 15 d after first

feeding could have begun (6 d), had survival greater than 87% which was equivalent to

continuously fed controls. Survival of fish denied food for 17.5 d after feeding could

have begun declined from 84% before feeding to 57% after feeding. Survival of fish

denied food for 20 d after first feeding could have begun was < 6%; survival was < 1%

after feeding was begun. Point of no return was between 17.5 and 20 d. Low, stable

flows that simulate natural hydrographs may enhance growth, survival, and recruitment of early life stages of Colorado squawfish by increasing water temperature and food abundance in regulated rivers of the Colorado River basin.

The Colorado squawfish, *Ptychocheilus lucius*, is a large, predaceous cyprinid endemic to the Colorado River Basin. Although formerly widespread and abundant, populations are now limited to the San Juan, Colorado, and Green rivers and their major tributaries in the upper Colorado River basin (Behnke & Benson 1983, Platania et al. 1991). The species is listed as endangered by the U. S. Fish and Wildlife Service. Decline of Colorado squawfish has been attributed partly to nonnative fishes that compete with or prey on early life stages (Miller 1961, Williams et al. 1989, Carlson & Muth 1989). Mainstream dams constructed throughout the Colorado River Basin since 1913 have also negatively affected distribution and abundance of Colorado squawfish (Vanicek et al. 1970, Holden 1979, Tyus 1991). Low summer water temperatures caused by hypolimnetic releases from these dams may curtail reproduction of Colorado squawfish and reduce growth, survival, and recruitment of early life stages (Vanicek et al. 1970, Kaeding & Osmundson 1988, Tyus & Haines 1991).

Several hypotheses have been forwarded regarding mechanisms which control recruitment of fishes early in life (Houde 1987). Starvation, presence and duration of a critical period during ontogeny, and biotic interactions such as competition and predation may all affect larval survival. Outcomes of these processes often depend on larval size and growth rates, which may in turn be affected by environmental factors such as water temperature, temperature fluctuations, and food abundance (Houde 1987, Weatherly & Gill 1987, Miller et al. 1988). Understanding effects of these environmental factors on growth, survival, and starvation resistance may be essential in determining the complexity of mechanisms that control recruitment of Colorado squawfish in the wild.

Research on fishes in the upper Colorado River Basin has emphasized effects of altered flow regimes on distribution and abundance of endangered fishes such as Colorado squawfish (Tyus & Karp 1989, Tyus & Haines 1991). However, few inferences can be made about direct effects of temperature, habitat availability, and food abundance on growth, survival, and starvation resistance of larval Colorado squawfish because scant empirical laboratory or field data are available that describe the ecology of early life stages. Black & Bulkley (1985) studied effects of constant 15, 20, 25 and 30°C temperatures on growth of yearling Colorado squawfish and found fastest growth at 25°C when provided with unlimited food. However, food availability may be low in fluctuating riverine backwaters of the Colorado River Basin and optimal growth conditions for larvae may be different than for yearlings. Therefore, laboratory studies were conducted to determine growth and survival response of larvae in constant and fluctuating temperature regimes at several different temperature and food abundance levels. Starvation resistance of Colorado squawfish larvae was also examined.

Methods

Growth and survival experiments.--Colorado squawfish embryos were obtained from Dexter National Fish Hatchery and Technology Center, Dexter, New Mexico (Hamman 1981). Test animals were held in 2.0 L aquaria (20 cm x 10 cm x 12.5 cm deep). Flow-through conditions in each aquarium maintained dissolved oxygen at 5-6 mg/L, and prevented accumulation of waste products. Photoperiod was 14 h light:10 h dark.

Healthy two-day old larvae were randomly selected and counted into groups of 20, randomly assigned to one of seven experimental treatments, placed in aquaria, and acclimated to test temperatures at a rate of about 2°C/h. Larvae were subjected to fluctuating or constant temperatures (hereafter called regimes), with nominal constant temperatures of 18, 22, 26, or 30°C and nominal fluctuating temperatures of 18, 22, or 26°C (diel fluctuation of ± 2.5°C, 5°C total). Embryos hatched in 1991 were used for the 18, 22, and 26°C fluctuating and 22°C constant-temperature-treatment experiments, and 1992 embryos were used for the 18, 26, and 30°C constant and a second 22°C constant temperature treatment experiments.

Fluctuating temperatures were tested because diel temperature fluctuations of 5°C or more are common in the Yampa and Green rivers in Colorado and Utah during late June through August (Nesler et al. 1988; United States Geological Survey records, published annually). The fluctuating temperature cycle mimicked the natural cycle as follows: lowest temperatures occurred from 0100 to 0700 h (e.g., 19.5°C for the 22°C fluctuating treatment); warming occurred at a constant rate from 0700 to 1300 h; temperatures were highest from 1300 to 1900 h (e.g., 24.5°C for the 22°C fluctuating treatment); and cooling occurred at a constant rate from 1900 to 0100 h. Observed mean water temperatures (SE) in the constant and diel fluctuating regimes were as follows: constant nominal 18°C = 18.2 (0.07), 22°C = 21.95 (0.05), 26°C = 26.0 (0.02), and 30°C = 30.2 (0.04); diel fluctuating temperatures (mean minimum and maximum) were 18°C = 15.7 (0.14) and 20.5 (0.20), 22°C = 19.5 (0.14) and 24.7 (0.09), and 26°C = 23.5 (0.21) and 28.3 (0.09).

Larvae were fed measured aliquots of nauplii of brine shrimp *Artemia sp.*, in morning and evening at nominal rates of 6.25, 14, 32, 71, and 160 nauplii fish[-1] feeding[-1]; total daily food abundance is reported throughout. Periodic measurement of brine shrimp hatch indicated that nominal feeding rates were not significantly different ($P = 0.15$) than target rates. Smallest brine shrimp nauplii were able to pass through the netting, although it was qualitatively estimated that escapement was < 10%. Feeding rates were adjusted daily when mortality occurred. Fish did not begin feeding until 4 d after treatments began (6 d old) but were subjected to experimental treatment conditions prior to feeding so that developmental rates would be consistent with test temperatures.

Each treatment combination of temperature (18, 22, 26, or 30°C), regime (fluctuating or constant), and food abundance (12.5, 28, 64, 142, or 320 nauplii fish[-1] day^{-1}) was replicated three times, except that the 22°C constant regime treatment (with five ration levels) was conducted in both 1991 and 1992 to test for annual variation, and no 30°C fluctuating treatment was conducted due to lack of space. Thus, a total of 120 experimental units was used.

Survival in aquaria was monitored daily by making counts of larvae until successive counts were the same. Dead fish were removed, labelled, and preserved in 100% ethanol. Experiments were ended when 750 degree days were accumulated: 41.7 d for 18°C treatments (fluctuating and constant regimes had similar thermal units); 34 d for 22°C treatments; 28.8 d for 26°C treatments; and 25 d for the constant 30°C. Remaining larvae were counted, preserved, and measured (nearest 0.1 mm TL). Mean growth rate (mm d^{-1}) for each replicate was based on the surviving fish.

Statistical analysis.--A response surface model was fit to experimental data where growth (G) and survival (S) were response variables and regime, temperature, and food abundance were the independent variables. Growth (mm d^{-1}) data were analyzed with PROC RSREG (SAS Institute, 1988) to obtain least-squares estimates of model coefficients; survival data (as logit S = ln (Y/N-Y), where Y = number surviving out of 20 (N) individuals) were modeled in PROC GENMOD to obtain maximum likelihood estimates of model coefficients and standard errors. Regression models had the form

$$\underline{Y} = \beta_0 + \beta_1\underline{X}_1 + \beta_2\underline{X}_2 + \beta_3\underline{X}_3 + \beta_{11}\underline{X}_1^2 + \beta_{22}\underline{X}_2^2 + \beta_{33}\underline{X}_3^2 + \beta_{12}\underline{X}_1\underline{X}_2$$

$$+ \beta_{13}\underline{X}_1\underline{X}_3 + \beta_{23}\underline{X}_2\underline{X}_3 + \beta_{123}\underline{X}_1\underline{X}_2\underline{X}_3 + \epsilon,$$

where in this instance,

$\underline{Y}$ = G or logit(S);

$\underline{X}_1$ = water temperature;

$\underline{X}_2$ = food abundance;

$\underline{X}_3$ = fluctuating or constant regime;

β_0 = intercept;

$\beta_1, \beta_2, \beta_3$ = coefficients of linear terms;

$\beta_{11}, \beta_{22}, \beta_{33}$ = coefficients of quadratic terms;

$\beta_{12}, \beta_{13}, \beta_{23}, \beta_{123}$ = coefficients of cross products;

ϵ = random error, normal (G model) or binomial (S model) distribution.

Response surface analysis permitted examination of the joint effects on a response of different levels of one or more independent variables. Rationale for response surface designs and analyses is detailed in Box & Draper (1987). Recent applications to

experimental data and supporting discussions are in Clancy & King (1993) and Scholz et al. (1994).

Global growth and survival response models were first constructed with all independent variables with final growth model selection based on Mallows C_p and final survival model selection based on Akaike's information criterion (AIC) adjusted for overdispersed data (QAIC), sensu Anderson et al. (1994). The unique optimum for each response variable with respect to the independent variables was achieved by setting the partial derivatives equal to zero and solving. If optima were outside the range of experimental conditions, ridge analysis was used to determine the location of a ridge on the surface where the predicted response increases or decreases at the fastest rate; the starting point for ridge analysis was midway between the highest and lowest values of each independent variable in the experiment. Lack-of-fit (LOF) tests, residual plots, and model F-statistics were computed to determine the adequacy of the growth model fit to the data. F-statistics were used to determine if functions satisfied the Box-Wetz criterion, which states that if the regression F-value exceeds 10 times the critical F-value, the function is adequately estimated. The critical F-value with 5 and 114 degrees of freedom, and P = 0.01, was 3.18, which results in a Box-Wetz criterion of 31.8. Deviance statistics were used to assess fit of the data to the survival model and to determine if maximum likelihood estimates were appropriate. Significant interaction terms were plotted and analyzed prior to interpreting main effects. Heterogeneity χ^2 tests (Zar 1984) were used to identify outlier replicates for survival of larval Colorado squawfish under various ration and temperature level treatments. Sums of these tests for all experimental

treatments were used as a goodness-of-fit test for the full model for censored and uncensored data (pers. comm., G. White, Colorado State University).

Starvation resistance experiments.--Starvation resistance of Colorado squawfish was tested by delaying feeding of larvae after they were capable of feeding exogenously (6 d post-hatch) and comparing survival and growth to a control group. Thirty fish were placed in each of 18 1-L beakers, and three replicate beakers were randomly assigned to six treatments where first feeding was delayed 0 (control), 5, 10, 15, 17.5, or 20 days. Survival in each beaker was monitored daily. Fish were fed brine shrimp nauplii *ad libitum* twice daily after feeding began. Growth was monitored by sampling and measuring larvae from additional cultures of fish that were first fed at the same treatment intervals used for delayed feeding trials. Beakers were cleaned as necessary and aerated to maintain dissolved oxygen at about 6 mg L^{-1}. Survival and growth rates before and after first feeding were compared to a continuously fed control group.

Results

Growth response.--Growth of Colorado squawfish larvae was not significantly different in constant and fluctuating regimes ($P= 0.93$), so data for the same temperatures were pooled in subsequent analyses. Response surface analysis indicated Colorado squawfish larvae achieved maximum growth at the highest food abundance and temperature (Fig. 1, Table 1). Mallows C_p selected a model that included the main effects temperature and food abundance, their respective squared terms, and their interaction (Table 2). The final model had an $R^2 = 0.976$ and RMSE $= 0.012553$. Food abundance explained most growth rate variation (partial R^2 for food abundance, its squared term and

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food x temperature interaction = 0.916). The LOF test was highly significant ($P <$ 0.0001), but residual analysis did not indicate model LOF. The regression growth model F-value of 933.7 far exceeded the Box-Wetz criterion of 31.8, which suggested adequate model fit.

Optimization suggested a maximum growth rate of 0.305 mm TL d^{-1} at 31°C and food abundance of 345 nauplii fish^{-1} day^{-1}, both of which are just outside of the upper range of values tested in this experiment (30°C and 320 nauplii fish^{-1} day^{-1}). Ridge analysis also suggested that growth of Colorado squawfish larvae increased fastest at highest temperature and food abundance (Table 3), but indicated that growth response was fairly static (0.281-0.297 mm TL d^{-1}) for temperatures ranging from 25.7-27.6°C and food abundance of 250 to 293 nauplii fish^{-1} day^{-1}. Thus, optimization and ridge analysis showed growth of Colorado squawfish larvae was highest between 25.7 and 31°C and at 250 to 342 nauplii fish^{-1} day^{-1}. Model solutions showed that larval Colorado squawfish growth declined 4.5-7% for each degree drop in temperature from 22 to 18°C at maximum food abundance. Growth decreased fastest along a ridge defined by temperature of 24°C and by food abundance of 166 to 13 nauplii fish^{-1} day^{-1}.

A significant food x temperature interaction ($P < 0.0001$) was likely due to reduced growth rates of Colorado squawfish larvae in treatments at 18°C and food abundance of 142 and 320 nauplii fish^{-1} day^{-1}, when compared to growth rates of larvae in the same food abundance levels for 22, 26, and 30°C (Fig. 2). Growth rates at 18°C with food abundance of 142 and 320 nauplii fish^{-1} day^{-1} were 20 and 31% less than growth rates averaged for the three higher temperatures with the same food abundance. Growth rates at 12.5, 28, and 64 nauplii fish^{-1} day^{-1} at 18°C were comparable to those achieved at

higher temperatures. Significant interactions may invalidate interpretation of model main effects unless interactions are orderly ones (Ott 1988). The interaction here is assumed to be orderly because growth at higher food rations at 18°C is always less than other temperatures, so straightforward interpretation of main effects is appropriate.

Survival response.--Survival of Colorado squawfish larvae was not significantly different in constant and fluctuating regimes (P= 0.67), and QAIC did not select models with regime or any of its interaction terms included, so data for the same temperatures were pooled in subsequent analyses. Response surface analysis and QAIC indicated survival of Colorado squawfish larvae was best predicted by a model that included food abundance (as ln), temperature, squared terms, and the interaction (Figure 3). Use of log transformed food abundance was justified by improved survival model fit over that given by untransformed values. Log food abundance x temperature interaction was the most important explanatory variable as determined by a stepwise regression selection procedure, followed by log food abundance and its squared term, temperature squared, and temperature (Table 2). The response surface and model solutions indicated survival > 90% when temperature ranged from 20 to 30°C and when food abundance was > 180 nauplii fish^{-1} day^{-1}. Actual survival values were about 90% or higher at experimental food abundances of 142 and 320 nauplii fish^{-1} day^{-1} at temperatures of 22, 26 and 30°C (Table 1). Survival was slightly reduced at 18°C, the lowest temperature tested, even in the presence of high food abundance and was lowest overall when food abundance was low and temperature was high.

Optimization analysis suggested survival was maximized at 95% at 26.2°C and 235 nauplii fish^{-1} day^{-1}. Survival was lowest when food abundance was least, and especially so at the highest temperatures.

Significant deviance (D) in the survival model ($D = 218.81$, df = 114, $P < 0.0001$) indicated extra-binomial variation (overdispersion) in experimental data. Heterogeneity χ^2 tests (Zar 1984) on sets of replicate data (Table 4) identified six outlier observations as a potential cause of overdispersion. Subsequent re-analysis of censored data improved deviance ($D = 172.76$, df = 108, $P = 0.0001$) but not to an appreciable degree. The sum of the χ^2 tests on sets of replicates, which is a goodness-of-fit test for the overall model, indicated an inadequate fit of the model to the data ($\chi^2 = 176.68$, df = 100, $P < 0.0001$) for uncensored data and an improved but marginally inadequate model fit for censored data ($\chi^2 = 115.28$, df = 94, $P = 0.067$). Re-analysis of censored data indicated that substantial extra binomial variation was still present. Therefore, maximum likelihood parameter estimates and quasi-likelihood theory adjusted standard errors (inflated to account for extra-binomial variation) using all data were deemed more appropriate than estimates with censored data. Similar χ^2 analysis of the 22°C constant temperature treatment data indicated no difference in survival between years ($\chi^2 = 1.675$, df = 1, p = 0.196).

Plots of mortality as a function of time in various treatments indicated that most fish died 20 d or more after treatments began (Fig. 4), although high mortality occurred sooner in most 30°C treatments. In general, mortality was highest at the lowest food abundance (Fig. 3). Within a single food abundance level mortality was also higher at

warmer temperatures than lower ones. Distribution of mortalities in treatments where survival was > 90% were evenly spread through time.

The food x temperature interaction was significant ($P < 0.0001$) and indicated that within the lowest two food abundance treatments (12.5 and 28 nauplii fish^{-1} day^{-1}), survival decreased as temperature increased (Fig. 5). At 64 nauplii fish^{-1} day^{-1}, survival in 30°C was 25% less than average survival in the other three temperature treatments which had similar survival. As for growth, the survival response interaction was an orderly one that allowed interpretation of main effects.

Starvation resistance.--Survival of Colorado squawfish was about 95% for treatments where larvae were denied food for 0 (control), 5, or 10 d (Fig. 6). Survival of larvae denied food for 15 d after normal first feeding declined from 93% to 87% after first feeding. Survival of larvae denied food for 17.5 d declined from 84 % before to 63% after feeding began, but stabilized at 57% at the end of the experiment. Only 6% of Colorado squawfish larvae denied food for 20 d survived to the end of the delay period, and only 1% survived after food was offered. Point of no return is defined here as the time over which a batch of larvae are denied food, are then offered food, and have cumulative mortality $\geq$ 50%. Point of no return for Colorado squawfish larvae was between 17.5 and 20 d.

Lengths of fish in all treatments increased during the first 5 d, then growth slowed if fish were not fed (Fig. 7). Fish denied food for 0 or 5 d grew at very similar rates. Fish in treatments where feeding was delayed 10, 15, or 17.5 d increased growth immediately following initiation of feeding. Growth rate trajectories observed for fish in these treatments were similar to those observed in treatments where fish were denied food for 0

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and 5 d. Fish were, of course, smaller when denied food for 10 or more days. Most fish

in the 20 d treatment died prior to feeding so no observations of post-feeding growth were

possible.

Discussion

Growth response.--Growth of Colorado squawfish larvae based on model results

was optimum at 31°C and food abundance of 345 nauplii fish^{-1} day^{-1}, conditions which

were similar to the highest ones tested (30°C and 320 nauplii fish^{-1} day^{-1}). Because the

predicted temperature maximum is warmer than the highest one studied, it is not possible

to determine if the true optimum for growth of larval Colorado squawfish is 31°C. Ridge

analysis indicated nearly equivalent growth of 0.281-0.297 mm TL d^{-1} at temperatures

from 25.7 to 27.6°C, so it is doubtful that growth response increases significantly at

temperatures much above 30°C. Environmental observations suggest that early life stages

of Colorado squawfish occupy habitat where water temperatures were as high as 28-32°C

(Dill 1944, Tyus & Haines 1991, KRB, pers. obs.). Thus, the estimated optimal growth

temperature of 31°C may be realistic.

Significant LOF was detected for the growth response model, in spite of R^2 =

0.98. The relatively large number of replicates per treatment after pooling regimes

resulted in 100 degrees of freedom for the pure experimental error term (divisor in F-ratio

LOF test) and 14 degrees of freedom to estimate LOF (numerator in F-ratio). The large

sample size and small pure experimental error mean square probably resulted in relatively

high power to detect a small amount of LOF. Residual plot analysis is useful to detect

model LOF if, as in this study, the number of observations is large with respect to the

number of parameters being estimated (Box & Draper 1987). Residual plot analysis did not detect inadequacies in model fit. The response surface growth model F-statistic far exceeded the Box-Wetz criterion (Box & Draper 1987). Thus, the growth response model was considered an adequate representation of the experimental data.

Fluctuating temperature regime had no effect on growth or survival compared to constant temperature conditions in this study. Some studies of effects of fluctuating temperature regimes on growth of fishes showed positive effects whereas others showed none (references in Vondracek et al. 1989, Woiwode & Adelman 1991). If fluctuating temperature has real effects, they may be life stage and species specific, or dependent upon specific conditions associated with cycles of feeding and temperature change or the amplitude of oscillations. Bestgen & Williams (1994) found that survival of endogenously feeding 7 d old Colorado squawfish larvae was 10-31% higher, and swim bladder inflation and first feeding occurred sooner, in fluctuating than in constant temperatures. The observed effects may have been due to life stage specificity for certain environmental conditions. Lack of an effect of fluctuating temperatures on growth or survival of larval Colorado squawfish in this study may be due to fluctuations that were too small or to physiological differences in slightly older and exogenously feeding larvae.

Optimal temperatures for growth of larval and yearling life stages of Colorado squawfish appear to be different. Growth of Colorado squawfish larvae in this study was optimal at $31°C$ and generally high at temperatures above $25.7°C$. However, Black & Bulkley (1985) found maximum growth of 0.26 mm TL d^{-1} for yearling Colorado squawfish (45 mm TL) at $25°C$, but lower growth of 0.06, 0.19, and 0.17 mm TL d^{-1} at 15, 20, and $30°C$, respectively. Such differences in temperature optima during ontogeny

(i.e. larval and yearling) are not uncommon and may be a mechanism for habitat segregation in fishes (Magnuson et al. 1979, McCauley & Huggins 1979).

Interaction analysis and growth model solutions suggested that Colorado squawfish larval growth rates declined at temperatures below 22°C, even when abundant food was available. This may suggest that digestion rates or food conversion efficiency was low at such temperatures. Basal metabolic rates and food requirements in fishes are typically higher at higher temperatures. This generalization was supported here as growth of Colorado squawfish larvae was low at 30°C when coupled with low food abundance levels of 12.5, 28, and 64 nauplii fish^{-1} day^{-1}, compared to all other test temperatures.

Survival response.--Survival of Colorado squawfish larvae was 90% or more in most treatments with food abundance of 142 and 320 nauplii fish^{-1} day^{-1} at all test temperatures. Survival declined slightly at 18°C, the lowest temperatures tested, even with high food abundance, and was lowest overall at low food abundance levels and high temperatures. Elimination of a single and unexpected survival observation of 30% at 18°C and 320 nauplii fish^{-1} day^{-1} increased mean survival to 88% for the remaining observations in that treatment. The outlier observation is partly the cause of decreased survival at higher food abundances in the model response surface (Fig. 3) with temperature apparently responsible for the remainder of the effect.

Normal maximum likelihood standard errors for parameter estimates are often underestimated when data are overdispersed. Thus, quasi-likelihood theory incorporates a variance inflation factor (here = square root of deviance/df, SAS Institute Inc. 1993) when calculating the standard errors to account for the extra-binomial variation. Thus,

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predictive power of the survival model is reduced because of larger quasi-likelihood theory standard errors and resulting wide confidence intervals.

The large amount of extra-binomial variation noted for larval Colorado squawfish survival was surprising, especially given the good fit of data in the growth model which were derived from the same fish in the same experimental conditions. Survival variation could be attributable to differences in experimental protocol or to variation in fish condition or quality between years. However, experimental equipment, conditions, and protocol were identical across years. Further, growth and survival in one set of experimental conditions (22°C constant temperature regime) replicated across years indicated no significant year effect, suggesting that experimental conditions and fish condition and quality were similar. It is possible that data overdispersion caused by variable survival is due to inherent genetic variation present in these fish. In the wild, individuals would select environmental conditions such as water temperature for which they are most adapted. In these laboratory tests, no selection was allowed and individuals were forced to occupy ambient conditions for which they may have been mal-adapted, thus resulting in variable survival.

Variable survival of larval Colorado squawfish in replicates (indicated by significant or nearly significant χ^2 tests for sets of replicates, Table 4) occurred at 18, 22 and 26°C and at low as well as high food levels. These data support the notion that survival variability within replicated data was not due to a particular food and temperature combination (e.g., low food abundance at cold temperatures) but may instead be a function of genetic variability. Survival across replicates was consistent at 30°C in most food abundance treatments (P-values ranging from 0.765 to 0.931) except one (n =

28 nauplii fish^{-1} day^{-1}, $P = 0.126$), perhaps indicating that mechanisms responsible for survival differences (e.g., genetic variation) were less important at this temperature. Lower number of replicates may also be responsible for lower survival variation at 30°C.

Survival of Colorado squawfish larvae during this laboratory study was higher than the 28-36% survival observed by Hamman (1989) for similar-sized fish reared in earthen hatchery ponds for 48-51 d at 19.4-23.8°C. Low food density caused by chemical treatment for eubranchiopod infestations may have been the cause for lower survival in that study.

Comparison of larval survival for razorback sucker *Xyrauchen texanus*, another endangered Colorado River Basin cypriniform fish, and Colorado squawfish suggested highest rates for both species at highest food abundance levels ($>$ 58 nauplii fish^{-1} day^{-1}, for razorback suckers (conversion of their 50 nauplii l^{-1}) and $\geq$ 142 nauplii fish^{-1} day^{-1} for Colorado squawfish) at 18°C, the only temperature tested by Papoulias & Minckley (1990). Survival of Colorado squawfish was 67% at 12.5 nauplii fish^{-1} day^{-1} compared to 27% for razorback suckers at 11.7 nauplii fish^{-1} day^{-1}. Lower survival for the larger razorback sucker larvae was likely due to longer test duration (50 vs. 42 d) compared to Colorado squawfish, and higher nutritional needs. Highest mortality for both species occurred 20 d or more after initiation of experiments.

Differences observed in optimum temperatures for growth (31°C) and survival (26.2°C) were not expected, but were probably not important because the response surface model solutions and experimental observations suggested survival was high at temperatures ranging from 20 to 30°C. Differences in optimum food abundance for growth (342 nauplii fish^{-1} day^{-1}) and survival (235 fish^{-1} day^{-1}) suggests that growth

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continued to increase with increasing food abundance, but that survival reached a high plateau at a lower food abundance.

Starvation resistance.--Colorado squawfish larvae denied food for up to 17.5 d after feeding was first possible (23.5 d post-hatch) were resistant to starvation, but survival was low for fish denied food for 20 d (26 d post-hatch). Time to 50% mortality was estimated to be between 17.5 and 20 d after feeding could begin, or 23.5 to 26 d post-hatch. Starvation resistance in fish larvae is generally a function of body size at hatch; larger fish are more resistant than smaller ones (Miller et al. 1988). Predicted time to 50% mortality of 19 d post-hatch (equation in Miller et al. (1988) not corrected for temperature) for 6.5 mm TL Colorado squawfish larvae was comparable to empirical estimates of 23.5-26 d post-hatch observed in this study. The 19 to 23 d time period to reach 50% mortality for 9-10 mm TL razorback sucker larvae (Papoulias & Minckley 1990) was much less than the anticipated 30 d based on equations in Miller et al. (1988), although predicted times for larvae of this size may be inaccurate. Colorado squawfish larvae generally did not show latent growth rate effects when deprived of food up to 17.5 d.

Ecological applications.--Results of this study indicate that maximizing temperature and food abundance in backwater habitat should benefit growth and survival of Colorado squawfish in early life. Because relatively small changes in growth and survival rates may cause substantial changes in annual recruitment levels of fishes (Houde 1987, Crowder et al. 1992, Rice et al. 1993), enhancing temperature and food abundance should also benefit recruitment of Colorado squawfish. Number and area of backwaters, and growth and abundance of age-0 Colorado squawfish are all negatively

correlated with discharge levels in upper Green River (Tyus & Haines 1991), so lower summer flows in regulated streams should benefit growth and survival of larvae. It is important to note that these backwaters are ones within the bankfull channel that are formed as high spring flows recede and should not be confused with inundated lowlands that result from overbank flooding. Backwaters that have relatively low exchange rates with cooler main channels have greatest potential to provide warm, food-rich environments, but are also the most susceptible to drying under fluctuating flow regimes. Thus, non-natural river fluctuations such as those caused by diel power-production water releases from dams should be minimized in order to maximize backwater temperature and productivity.

Diel temperature fluctuations in backwaters may motivate movements of early life stages of Colorado squawfish from warm backwaters during the day to relatively warm main channels at night when backwaters are cool (Tyus 1991). Such movement patterns may maximize diel degree day accumulation and growth potential of Colorado squawfish. Growth of larval Colorado squawfish may be limited in river reaches where main channel temperature regimes are < 22°C.

Starvation resistance times for Colorado squawfish larvae are probably shorter in the wild than in the laboratory because of increased energetic costs of negotiating large and swift rivers, avoiding predators, and securing suitable nursery habitat. High and extended spring runoff, floods, and river fluctuations caused by dams are among the factors that may affect ability of larvae to find and maintain positions in productive backwater habitat and avoid starvation.

Further research is needed to link results of growth, survival, and starvation resistance studies to annual, seasonal, or daily discharge and temperature regimes in main channels of the Colorado River Basin. Interactions between feeding, growth rates of early life stages of Colorado squawfish under various environmental conditions, and competition and predation are largely unstudied but may play a significant role in structuring recruitment patterns of Colorado squawfish in regulated rivers of the Colorado River Basin.

Acknowledgements

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Table 1. Mean growth (G, mm TL d^{-1}, (SE)) and survival (S, number of survivors/20 per replicate, (SE), number of experimental replicates) of Colorado squawfish larvae under various nominal temperature and ration (brine shrimp nauplii $fish^{-1}$ d^{-1}) treatments. Fluctuating and constant temperature treatments were pooled because no significant differences were detected.

Ration		Temperature (°C)			
		18	22	26	30
12.5	G	0.061 (0.0021)	0.071 (0.0029)	0.065 (0.0041)	0.043 (0.0058)
	S	0.67 (0.0543)	0.45 (0.0565)	0.24 (0.0352)	0.13 (0.0167)
		n=6	n=9	n=6	n=3
28	G	0.093 (0.0025)	0.092 (0.0018)	0.094 (0.0021)	0.063 (0.0058)
	S	0.86 (0.0455)	0.87 (0.0323)	0.73 (0.0279)	0.48 (0.0928)
		n=6	n=9	n=6	n=3
63	G	0.134 (0.0022)	0.138 (0.0012)	0.140 (0.0056)	0.129 (0.0061)
	S	0.82 (0.0587)	0.92 (0.0264)	0.90 (0.0387)	0.63 (0.0167)
		n=6	n=9	n=6	n=3
142	G	0.170 (0.0039)	0.204 (0.0046)	0.217 (0.0042)	0.215 (0.0048)
	S	0.91 (0.0239)	0.92 (0.0188)	0.93 (0.0357)	0.93 (0.0167)
		n=6	n=9	n=6	n=3
320	G	0.201 (0.0055)	0.273 (0.0055)	0.303 (0.0066)	0.291 (0.0013)
	S	0.78 (0.1085)	0.94 (0.0176)	0.95 (0.0224)	0.92 (0.0167)
		n=6	n=9	n=6	n=3

Table 2. Least squares (growth model) and maximum-likelihood (survival model) estimates and significance probabilities for coefficients from quadratic response surface regression models relating growth and survival responses to water temperature and food abundance.

Response variable or interaction	df	Coefficient estimate	SE	P	R^2
Growth[a]					
Intercept	1	-0.262717	0.041603	<0.00001	0.976
Temperature (T)	1	0.028677	0.003491	<0.00001	
Food (F)	1	0.000716	0.00007096	<0.00001	
T^2	1	-0.000637	0.00007167	<0.00001	
F^2	1	-0.000002443	0.00000013	<0.00001	
T x F	1	0.000031316	0.00000227	<0.00001	
Survival[b]					
Intercept	1	-6.5160	3.5272	0.0647	
Temperature (T)	1	0.2953	0.2563	0.2511	
lnFood (lnF)	1	2.0144	0.7498	0.0086	
T^2	1	-0.0169	0.0053	0.0012	
lnF^2	1	-0.4452	0.0749	<0.0001	
T x lnF	1	0.1084	0.0188	<0.0001	

[a] Growth model statistics: df = 5, 114, model F = 933.7 (F = 3.18 at P = 0.01), lack of fit P <0.0001, root mean square error = 0.012553; response is mm d^{-1}.

[b] Survival response is logit (survival). Food abundance is natural log (food). Deviance/df (n = 114) = 1.9194, inflation factor for standard errors was the square root of deviance/df = 1.3854.

Table 3. Predicted ridge maxima for growth of larval Colorado squawfish estimated by response surface analysis of effects of water temperature and food ration.

Growth Response	Factor Values	
(mm d^{-1}, (SE))	Temperature (°C)	Ration (nauplii fish^{-1} d^{-1})
0.236 (0.0028)	24.2	166
0.246 (0.0028)	24.3	181
0.254 (0.0028)	24.5	196
0.262 (0.0028)	24.7	210
0.269 (0.0027)	25.0	224
0.276 (0.0027)	25.4	237
0.281 (0.0026)	25.7	250
0.286 (0.0026)	26.2	261
0.290 (0.0026)	26.6	272
0.294 (0.0027)	27.1	283
0.297 (0.0028)	27.6	293

Table 4. Heterogeneity χ^2 analysis of larval Colorado squawfish survival in replicates within various nominal temperature and ration treatments (data are χ^2 value, df, and p-value) for uncensored (above) and censored (below, not repeated if NS) data sets. Outliers (n = 6) identified by significant χ^2 values ($P \leq 0.05$) for sets of replicates were removed and data were re-analyzed. Sums of individual χ^2 tests for replicates, which are goodness-of-fit tests for the full model for uncensored ($\chi^2 = 176.7$, df = 100, $P < 0.0001$) and censored ($\chi^2 = 115.28$, df = 94, $P = 0.067$) data, show effect of outlier removal.

Ration	Temperature (°C)			
(nauplii fish^{-1} d^{-1})	18	22	26	30
12.5	7.95, 5, 0.159	18.59, 8, 0.017	4.05, 5, 0.543	0.29, 2, 0.866
	-----	10.63, 7, 0.156	-----	-----
28	10.21, 5, 0.069	12.98, 8, 0.113	2.39, 5, 0.794	4.14, 2, 0.126
	-----	-----	-----	-----
63	13.80, 5, 0.017	13.10, 8, 0.109	10.0, 5, 0.075	0.14, 2, 0.931
	3.12, 3, 0.374	-----	-----	-----
142	4.10, 5, 0.535	7.13, 8, 0.523	12.32, 5, 0.031	0.54, 2, 0.765
	-----	-----	8.85, 4, 0.065	-----
320	41.64, 5, <0.001	8.47, 8, 0.389	4.40, 5, 0.493	0.44, 2, 0.804
	2.35, 3, 0.504	-----	-----	-----

Fig. 1. Response surface of Colorado squawfish larval growth (mm TL d^{-1}) in replicated

treatments as a function of water temperature and food abundance. Mean (SE)

growth for each treatment combination and the response surface model

coefficients are in Tables 1 and 2; the star represents the optimal growth response

as a function of the independent variables.

Temperature (°C)

Ration (nauplii fish^{-1} d^{-1})

Growth (mm TL fish^{-1} d^{-1})

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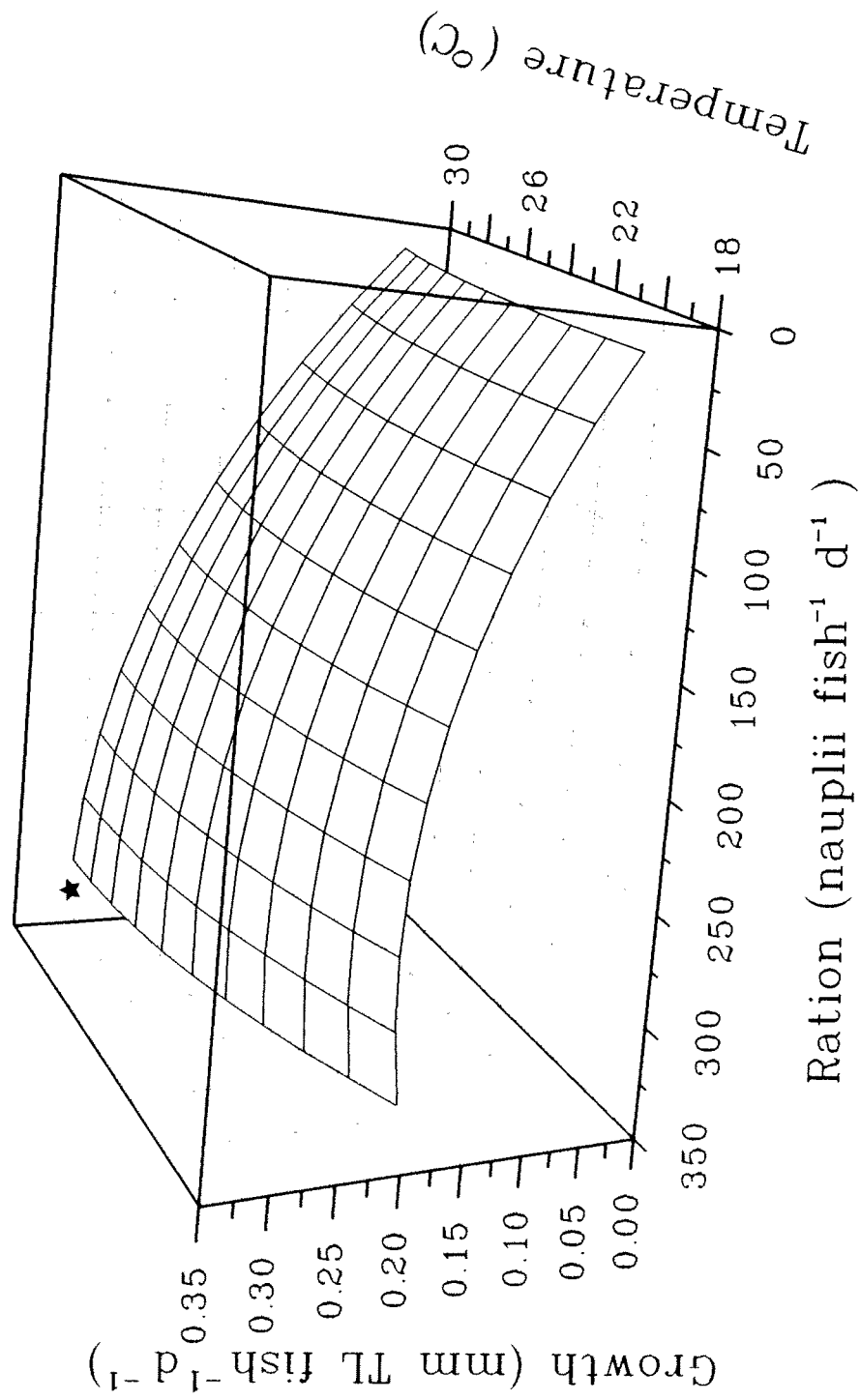
Fig. 2. Main and interaction treatment effects on mean growth of Colorado squawfish

larvae in water temperatures of 18, 22, 26, and 30°C and food abundance levels of

12.5, 28, 63, 142, and 320 nauplii fish^{-1} day^{-1}. Mean (SE) growth for each

treatment combination and the response surface model coefficients are in Tables 1

and 2.
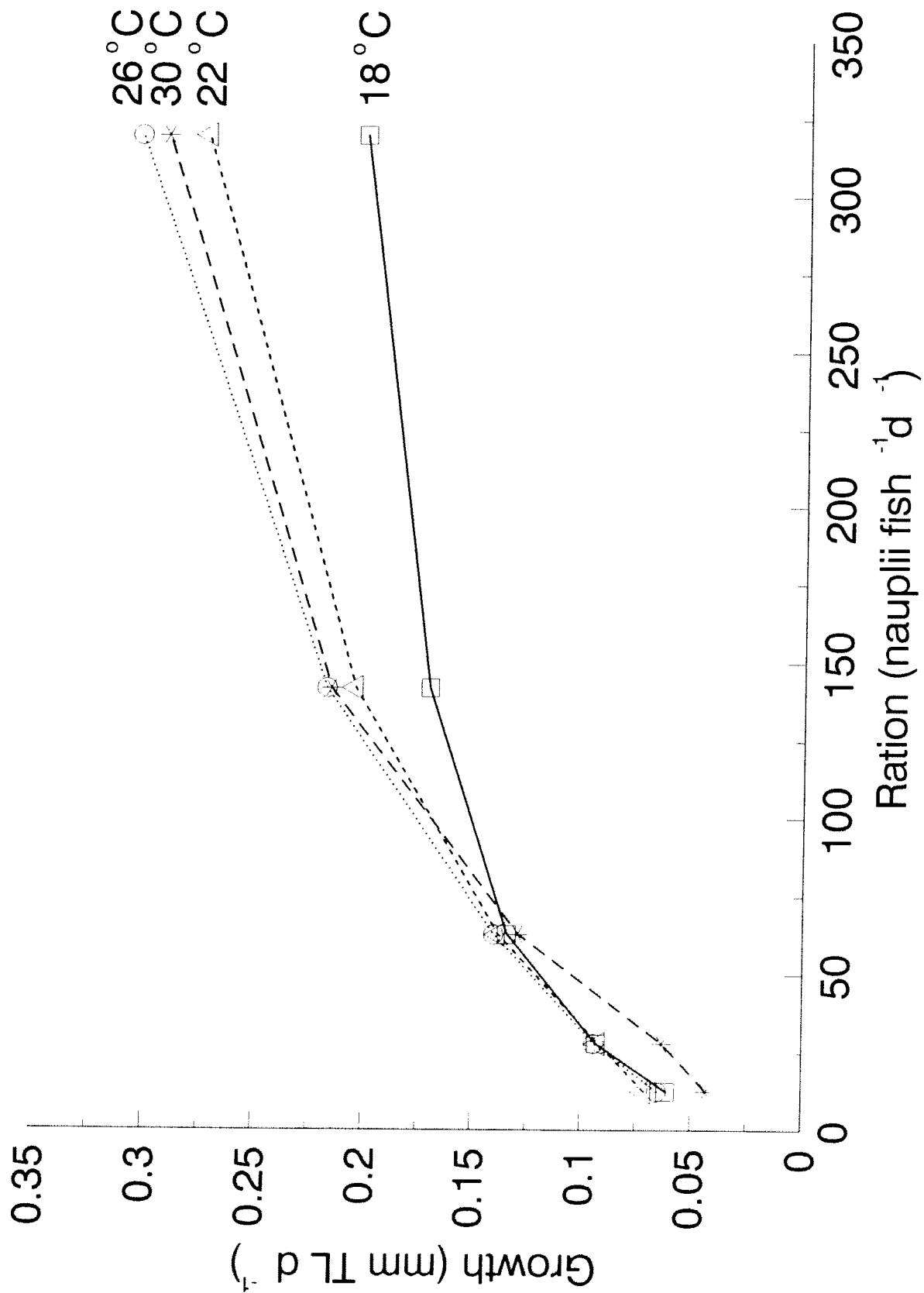
Fig. 3. Response surface of Colorado squawfish larval survival in replicated treatments as

a function of water temperature and food abundance (ln scale). Mean (SE)

survival for each treatment combination and the response surface model

coefficients (as logit (S)) are in Tables 1 and 2; the star represents the optimal

survival response as a function of the independent variables.
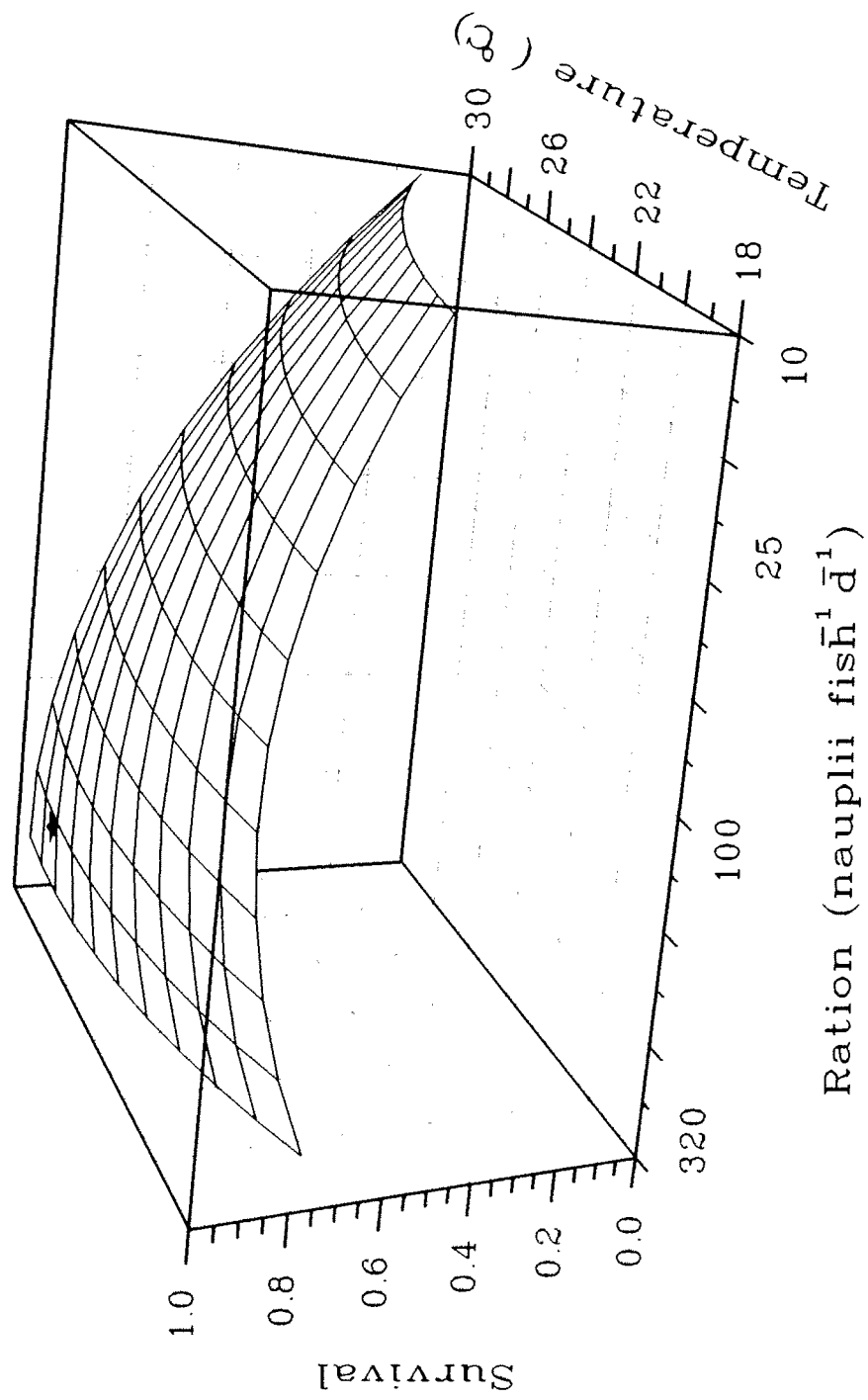
Fig. 4. Temporal distributions of mortality of Colorado squawfish larvae in replicated

treatments in water temperatures of 18, 22, 26, and 30°C and food abundance

levels of 12.5, 28, 63 nauplii fish⁻¹ day⁻¹. Percent mortality (n=180 fish total) in

each treatment combination is shown in the upper right corner. Mean mortality

was <10% in treatments with food abundance of 142 and 320 nauplii fish⁻¹ day⁻¹

and were not shown; mortalities in those were evenly distributed temporally.

Number of mortalities

12.5 naupliii fish^{-1} d^{-1} · 28 naupliii fish^{-1} d^{-1} · 63 naupliii fish^{-1} d^{-1}

18°C · 33% · 14% · 18%

22°C · 55% · 13% · 8%

26°C · 76% · 27% · 10%

30°C · 87% · 52% · 37%

Days after hatching
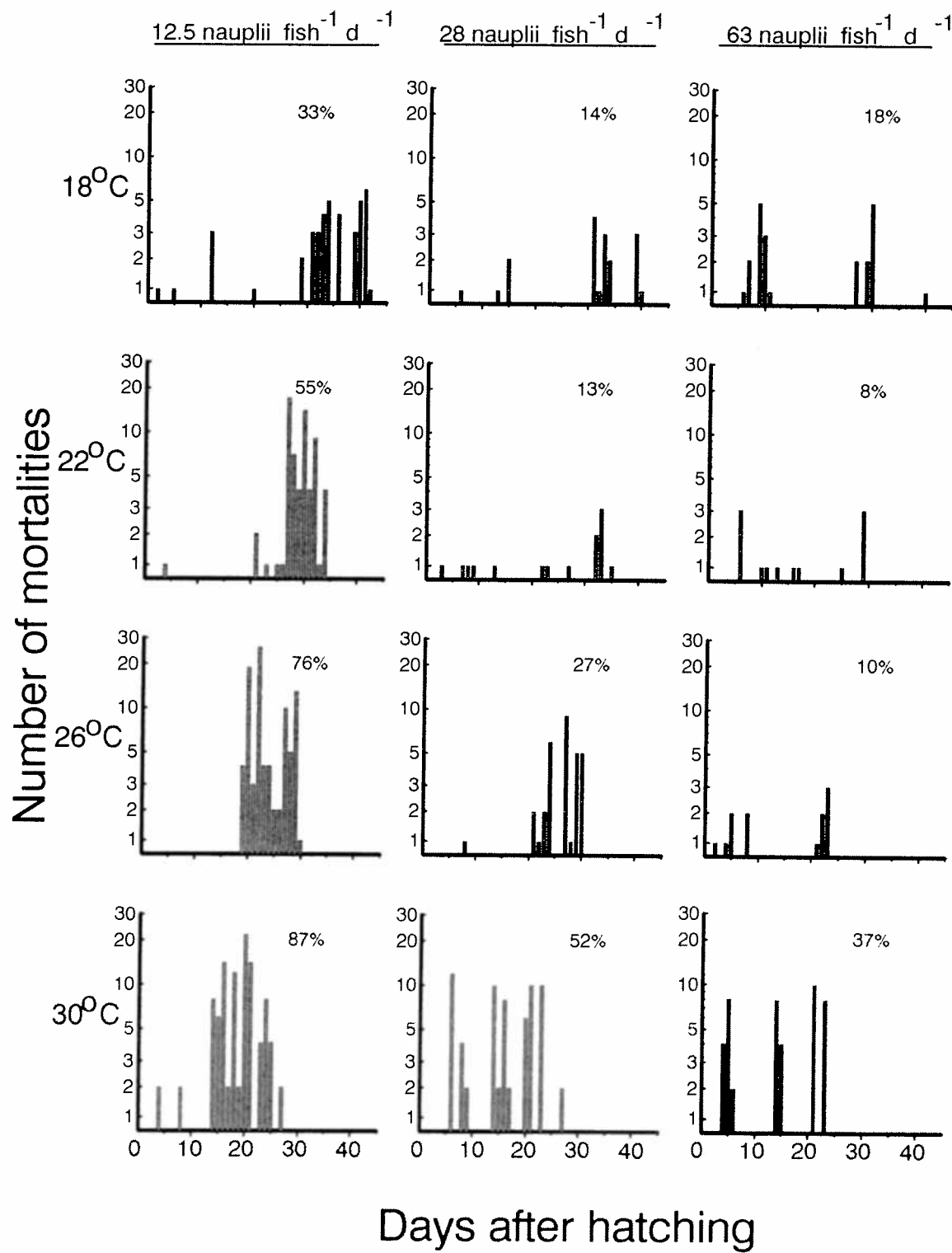
Fig. 5. Main and interaction treatment effects on mean Colorado squawfish larval

survival in water temperatures of 18, 22, 26, and 30°C and food abundance levels

of 12.5, 28, 63, 142, and 320 nauplii fish^{-1} day^{-1}. Mean (SE) survival for each

treatment combination and the response surface model coefficients are in Tables 1
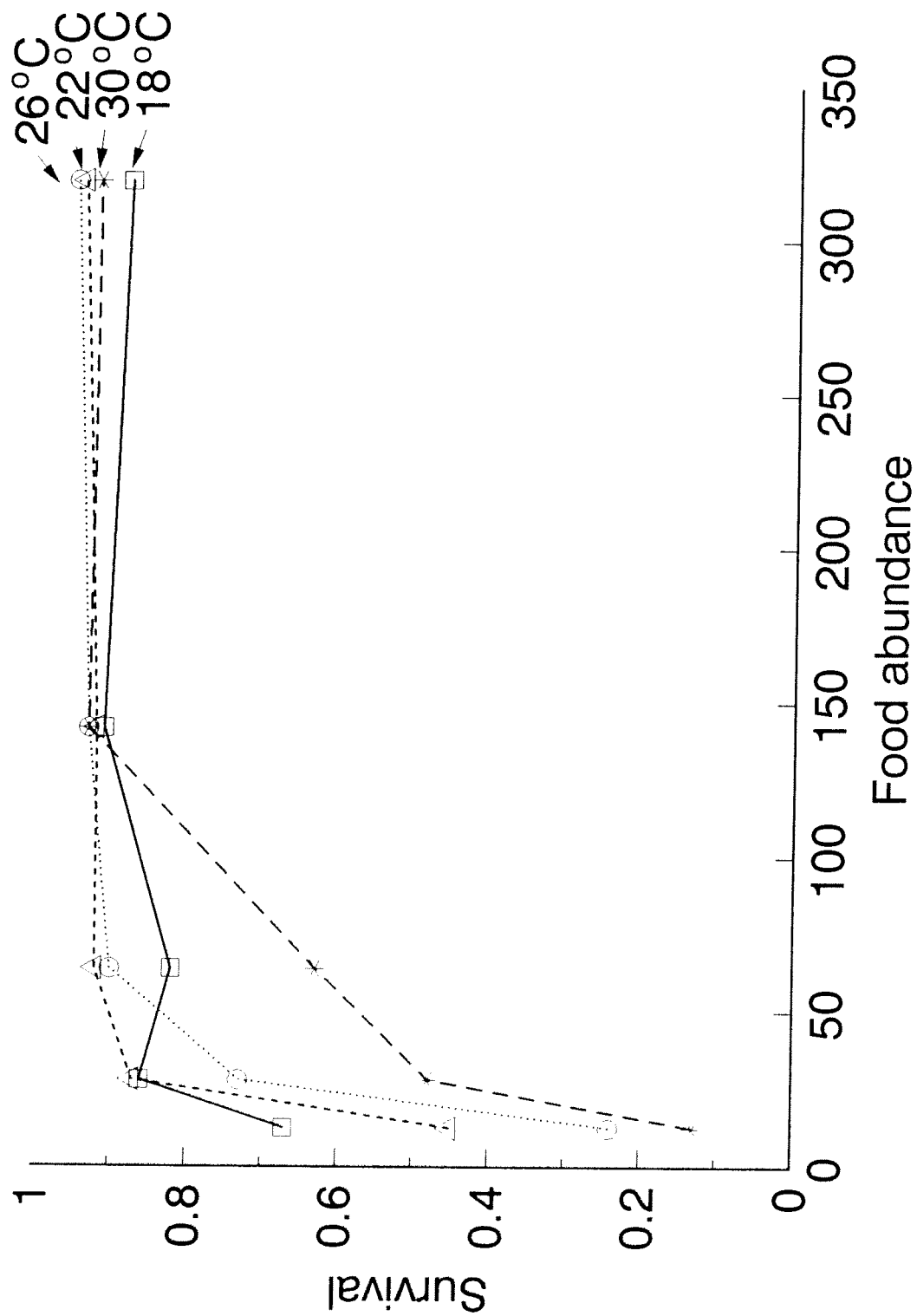
and 2.

Fig. 6. Survival of Colorado squawfish larvae (mean of three replicates) denied food for

0 (control), 10, 15, 17.5, and 20 d after fish were first able to feed; 5 d survival

was nearly identical to control and was not plotted.
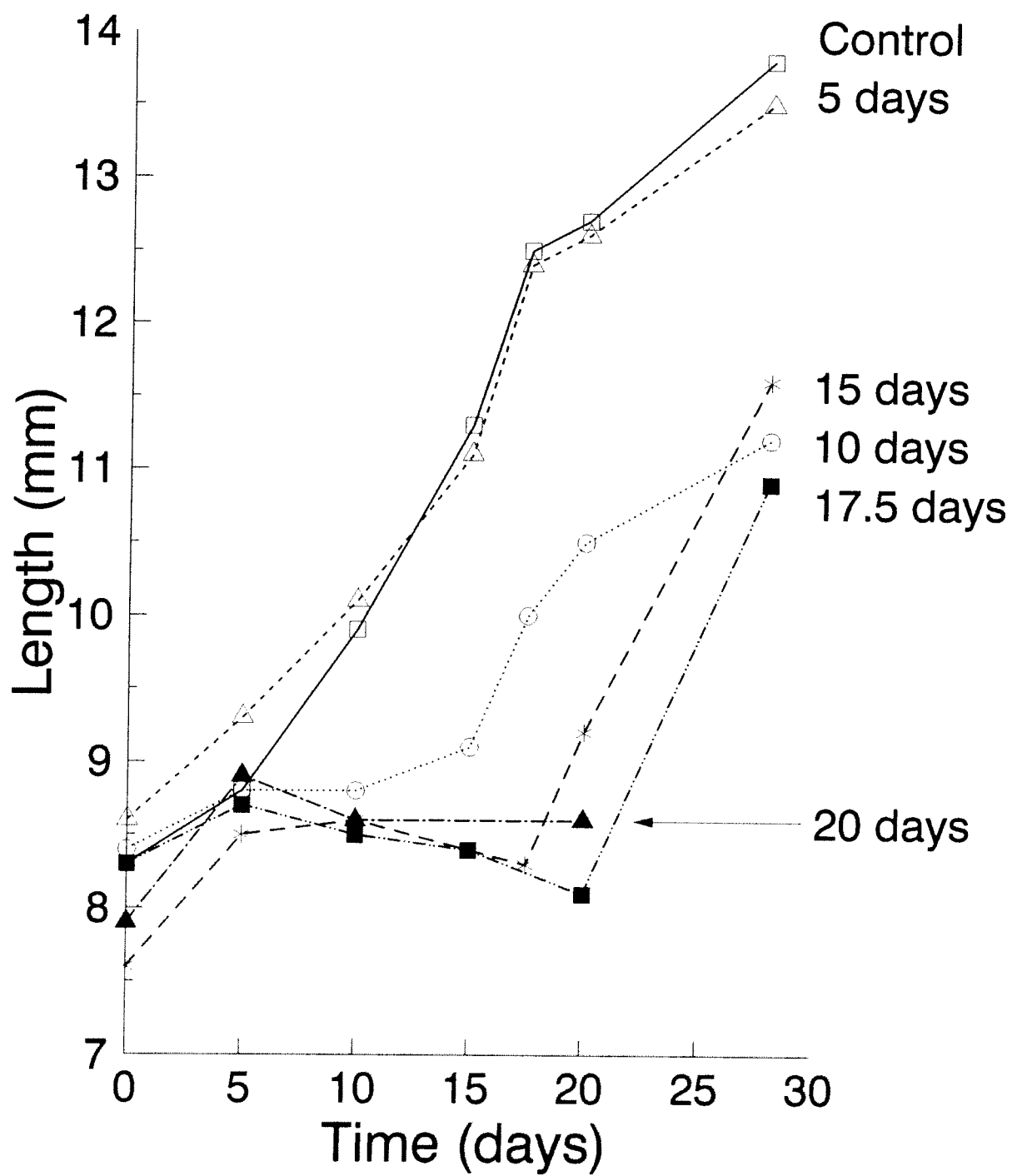
Fig. 7. Growth of Colorado squawfish larvae (n = 5-10 individuals) denied food for 0

(control), 5, 10, 15, 17.5, and 20 d after fish were first able to feed.
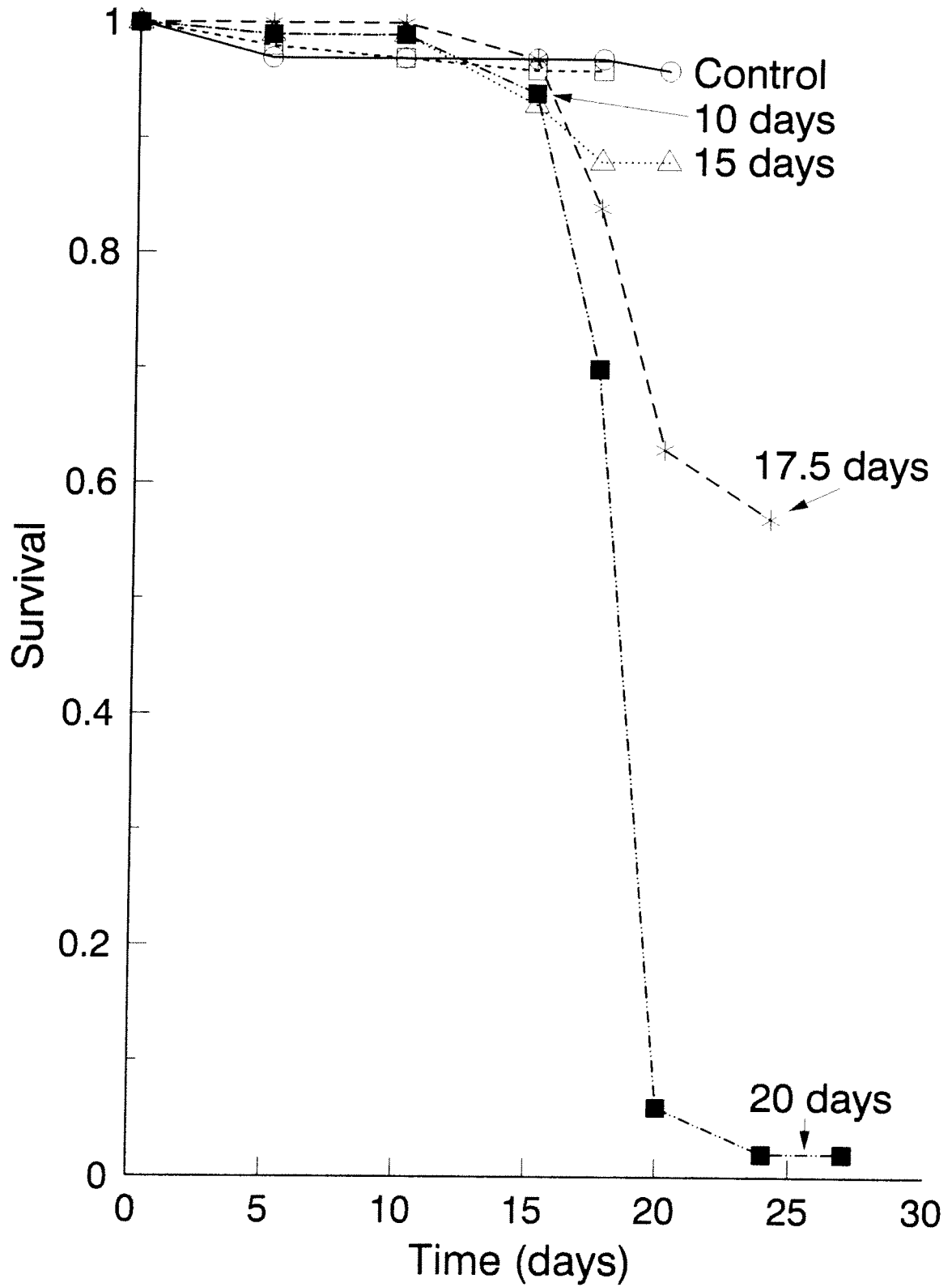
CHAPTER THREE

ENVIRONMENTAL FACTORS AFFECT DAILY INCREMENT DEPOSITION PATTERNS AND OTOLITH GROWTH IN YOUNG COLORADO SQUAWFISH (*PTYCHOCHEILUS LUCIUS*)

ABSTRACT

Otolith microstructure of endangered Colorado squawfish, *Ptychocheilus lucius*, was investigated under different temperature and feeding regimes to determine validity of daily increment aging and patterns of otolith growth. Sagittae and lapilli formed prior to hatching. Otolith increments were deposited daily after hatching in fish in constant 22°C and fluctuating ($\pm 2.5°C \cdot d^{-1}$) 18, 22, and 26°C temperatures. Otolith increments were clearer and counts were more accurately estimated in fish reared in fluctuating than in constant temperatures. Otolith growth was significantly less in 18°C than in 22 or 26°C indicating a direct effect of temperature, independent of somatic growth rate. Lapillus diameters of slow growing Colorado squawfish were significantly larger than similar-sized fast growing fish, indicating that fish and otolith growth rates were not proportional. Somatic growth of starved larvae generally ceased immediately but otolith growth continued for up to 15 d. Otolith growth was reduced for up to 5 d after starved fish began to feed. Timing of starvation and reduced growth may not be accurately recorded by reduced otolith increment spacing. Bias of length back-calculations for individuals and populations based on the assumption of proportional otolith and somatic growth should be evaluated before use with Colorado squawfish. Otolith increments deposited during starvation periods were of low contrast and may indicate periods of low food abundance in the wild. Increased otolith growth due to increased water temperature and first-feeding may signal timing of habitat shifts in Colorado squawfish larvae. Otolith analysis will be useful for elucidating age, growth, and recruitment patterns of young Colorado squawfish.

Number of daily increments in otoliths, patterns of increment deposition, and otolith growth rates may record life history events of fishes such as timing of hatch, growth rates, periods of physiological stress, life history changes, movements, and changes in water temperature or food abundance (Methot 1983; Campana and Neilson 1985; Penney and Evans 1985; Rice et al. 1985; Eckmann and Rey 1987). Correlation of biological data from otoliths with environmental factors such as water temperature, river discharge, food availability, and predator abundance has elucidated mechanisms that control growth, survival, and recruitment of early life stages of fish (Crecco and Savoy 1985; Houde 1987; Rice et al. 1987).

Application of otolith data in biological investigations was first possible after Pannella (1971) discovered otolith daily growth increments. A subsequent proliferation of research suggests that increment formation and otolith growth rates are influenced by photoperiod, water temperature, diel water temperature fluctuations, food abundance, somatic growth rate, and metabolic rates (Taubert and Coble 1977; Campana and Neilson 1985; Secor and Dean 1989, 1992). Moreover, rhythmic environmental events such as photoperiod and diel temperature shifts, may entrain an endogenous endocrine-driven circadian rhythm that controls daily increment deposition and otolith growth (Campana and Neilson 1985; Secor and Dean 1992). Although otolith and somatic growth are generally positively correlated (Campana and Neilson 1985), the assumption of closely proportional otolith-somatic growth may be flawed because otolith growth is sometimes relatively constant during periods of variable somatic growth (Mosegaard et al. 1988; Reznick et al. 1989; Secor and Dean 1989; Wright et al. 1990; Francis et al. 1993). This "growth rate effect" may invalidate use of length back-calculation techniques that assume

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proportional otolith and somatic growth (Campana 1990). Species-specific validation (estimation of the accuracy of the determination method; Francis 1995) of increment deposition and otolith growth patterns is critical prior to use of otoliths in biological investigations because of the complexity of factors affecting their microstructure.

Here, I report on effects of different environmental conditions on daily increment formation and otolith growth in young Colorado squawfish, *Ptychocheilus lucius* Girard, an endangered cyprinid endemic to the Colorado River basin (Behnke and Benson 1983; Tyus 1991). To determine if otoliths can be used to describe age and growth of early life stages of Colorado squawfish in the wild, I tested the following hypotheses: 1) increment deposition begins at hatch; 2) increment deposition is daily in otoliths of fish reared in different constant and fluctuating temperatures; 3) starvation reduces increment contrast and otolith growth; 4) otolith growth (= increment width) increases when fish are subjected to higher temperature or after first-feeding; and 5) otolith growth rates are directly proportional to somatic growth rates.

Methods

Age validation.--Colorado squawfish embryos were obtained from Dexter National Fish Hatchery and Technology Center, Dexter, New Mexico one day after eggs were fertilized. Groups of approximately 200 1-d-old Colorado squawfish embryos were randomly assigned to treatments (Table 1) to assess timing of otolith formation and effects of different temperature levels and daily temperature fluctuations on otolith microstructure and otolith growth. Fluctuating temperatures mimicked the natural cycle in each treatment aquarium as follows: lowest temperatures occurred from 0100 to 0700

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(e.g., 19.5°C for the 22°C fluctuating treatment); warming occurred at a constant rate from 0700 to 1300 h; temperatures were highest from 1300 to 1900 h (e.g., 24.5°C for the 22°C fluctuating treatment); and cooling occurred at a constant rate from 1900 to 0100 h. Embryos were acclimated to test temperatures at about $2°C \cdot h^{-1}$, and were incubated and reared in 2.0 L aquaria (20 cm x 10 cm x 12.5 cm deep). Flow-through conditions in each aquarium prevented accumulation of wastes. Fish were always sampled between 1200 and 1400 hours and preserved in 100% ethanol prior to and just after hatching (5 d post-fertilization at 22 and 26°C, 6 d at 18°C) and at weekly to monthly intervals thereafter. Timing of initial otolith microstructure formation and otolith size at time of first increment deposition was determined from samples collected before and immediately after hatching.

For age validation, 3-5 individuals were randomly selected from a larger lot of specimens preserved at intervals and were measured (standard [SL] and total length [TL]) to the nearest 0.1 mm). No correction was made for changes in fish length due to preservation because wild fish would be preserved similarly. Otoliths were removed from specimens and mounted on numbered microscope slides in either immersion oil or thermoplastic cement. Otolith maximum diameter and radius were measured with a compound microscope and a calibrated ocular micrometer at 100X or 400X magnification. Otoliths mounted in immersion oil were examined without further preparation, while those in thermoplastic cement were ground on one side with wet-dry sandpaper and lapping film and covered with immersion oil; all otoliths were examined at 400X or 1000X.

Sagittae became irregularly shaped and increments were difficult to distinguish at ages > 30 d, so only the lapillus, which was more consistently oval-shaped, was used in subsequent analyses. Otolith increments for individual fish were counted by the author three times using a blind design where experimental treatment was unknown. The three counts were averaged. Each otolith increment consisted of a relatively wide, light opaque inner band (L-zone) and a relatively narrow and dark outside band (D-zone, terminology after Kalish 1995).

The first increment was one of relatively high contrast (distinct L-zone) that surrounded the otolith core. The outer margin of otoliths examined for age validation was almost always opaque. I interpreted this as representing the L-zone for the day the fish was sampled and counted it as the last increment. A second experienced reader also counted increments for the 22°C constant and fluctuating treatments to determine extent of reader variation or systematic bias.

Least squares regression of increment count (dependent variable) as a function of known age (independent variable) was used to test the hypotheses that: 1) increments were first formed at hatch; and 2) that increments were formed daily and were detected (e.g., that the relationship between increment counts and known age was 1:1). Regression intercepts not significantly different than zero (increment count = 0 at hatch) and regression slopes not significantly different from one were used as evidence to support these hypotheses. Thus, Type II statistical errors were potentially more important in this analysis and a posteriori power of regression relationships was evaluated (Rice 1987). Because age of wild fish will be estimated from counts of daily increments, prediction intervals were calculated for known (= true) age (independent variable) via

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inverse regression (Draper and Smith 1981; Rice 1987) instead of for estimated age (dependent variable).

Analysis of covariance (ANCOVA) of estimated age as a function of true age was used to investigate reader bias, and to detect effects of different temperatures on periodicity of increment formation. The general form of the ANCOVA model was

$$\underline{Y} = \beta_0 + \beta_1\underline{X}_1 + \beta_2\underline{X}_2 + \beta_3\underline{X}_1\underline{X}_2 + \epsilon,$$

where in this instance,

$\underline{Y}$ = estimated age;

β_0 = intercept;

$\underline{X}_1$ = true age in d (covariate);

$\underline{X}_2$ = indicator variable for reader or treatment;

$\underline{X}_1\underline{X}_2$ = interaction;

β_1, β_2, β_3 = coefficients of terms;

ϵ = random error (normally distributed).

For a two group (e.g., reader A and B) model example, substituting $\underline{X}_2 = 0$ and 1, resulted in β_3 as the difference in slope for the two regression lines as follows:

reader A: ($\underline{X}_2 = 0$): $\underline{Y} = \beta_0 + \beta_1\underline{X}_1$;

reader B: ($\underline{X}_2 = 1$): $\underline{Y} = (\beta_0 + \beta_2) + (\beta_1 + \beta_3)\underline{X}_1$.

A test for parallel regression lines was thus H_o: $\beta_3 = 0$ (e.g., a non-significant ANCOVA interaction suggested that slope differences were not significant, Ott 1988). Similarly, a test for intercept differences would be H_o: $\beta_2 = 0$ (a non-significant ANCOVA indicator

variable suggested intercept differences were not significant). In addition to hypothesis tests such as ANCOVA which determines if treatments were statistically different, least squares means (= population marginal means, Searle et al. 1980) were calculated for groups (or treatments) after accounting for the covariate. Comparison of differences in treatment means and confidence intervals about the differences were calculated because these were informative to determine biological significance of treatments, regardless of results of statistical hypothesis tests (Yoccoz 1991, Johnson 1995).

Preserved specimens were used to assess relationships between otolith growth and TL and to calculate slope and intercept parameters for use in length back-calculation of fish at previous ages from otoliths (Carlander 1981). Effects of different temperatures and constant and fluctuating regimes on otolith growth were investigated using ANCOVA, as described above. Otolith diameter was regressed as a function of fish length (TL), and slopes (fish length.group interaction) and intercepts (treatment effect) were compared. Contrasts of means and differences among means were also used to compare treatment effects.

Growth rate effect experiments.--Experiment 1 was to determine if otolith growth rates were directly proportional in fish with different somatic growth rates. Fish from slow and fast-growth treatments were sampled at 3, 22, 28, 56, 82 and 122 d-old and preserved in 100% ethanol. Specimens (3-10 · period^{-1} · treatment^{-1} depending upon availability) were measured (TL) and the left lapillus removed and measured as described above. Analysis of covariance and least squares means were used to compare differences in otolith size between treatments.

Experiment 2 was to determine somatic and otolith growth rates before, during, and after a 6-d starvation period. Healthy and actively feeding Colorado squawfish larvae were lightly anaesthetized (100 mg·l⁻¹ MS-222), measured (TL) under a dissecting microscope fitted with an ocular micrometer, allowed to recover, placed in a 0.24 L cup and assigned to a treatment regime. Fish were measured as described above at the 6-d intervals and then preserved in 100% ethanol. Two fish from each treatment regime died during anaesthesia or were physically damaged so their complete growth histories were not available. The left lapillus was removed from each fish and otolith diameters measured at each time interval at 1000X magnification.

In experiment 3, groups of 200 6-d-old (age at first feeding) Colorado squawfish larvae were randomly assigned to each of five 3.8-L aerated jars representing 0, 5, 10, 15 and 17.5-d long starvation treatments. Up to 10 specimens were sampled from each treatment 0, 5, 10, 15, 20, and 28 d after the experiment began. Mean survival, measured in three separate replicate beakers for each treatment was 87 to 95% for all treatments except 17.5 d, where survival was 57% (Bestgen, in press). Because survival was high in all but one treatment, otolith and somatic growth effects should not be confounded by differential survival or density. Mean otolith size and TL were measured from five specimens per treatment per sampling occasion (procedure as above) to determine if otolith growth rates reflected changes in fish length as influenced by food availability.

Experiment 4 was conducted to determine effects of increased temperature and first feeding on otolith growth in early life stages of Colorado squawfish. Five 6 d-old Colorado squawfish larvae from a stock held at 25°C were placed in each of four 2 L aquaria and maintained without feeding in an 18°C water bath until fish were 14 d-old.

Two aquaria were then randomly assigned to an 18°C or 25°C treatment. One randomly

chosen aquaria in each temperature treatment was fed for the seven day treatment period

while the others were unfed. The 21 d-old fish were sacrificed, measured to nearest 0.1

mm TL and lapillus diameter measured. Differences in fish otolith growth among

treatments were compared with two factor analysis of variance (PROC GLM) which had

main effects temperature (18°C or 25°C) and food (present or absent) and the interaction.

All statistical analyses were conducted with SAS statistical software (SAS

Institute 1988). Plots of data and residuals were used to evaluate normality and

heteroscedastcity and data were transformed where necessary to meet assumptions.

Results

Early otolith microstructure.--Lapillar and sagittal otoliths were present in

Colorado squawfish embryos 1 d before hatch (4 d post-fertilization) at incubation

temperatures of 22 and 26°C, and 1-2 d before hatch (4 d post-fertilization) at 18°C. Four

to eight small, round nuclei (primordia) were the first otolith material formed.

Subsequent material was deposited around fused primordia to form a round to slightly

oval structure. Asterisci (the third otolith) were first found in Colorado squawfish larvae

15 d post-hatch. At hatching, the lapillus and sagitta were similar in size, similarly-sized

at all temperatures, and averaged 37.5 μ (range 32.4-44.3 μ) in diameter. Left and right

otoliths were also similar in size so the left was used exclusively unless it was lost. A

clear, high-contrast otolith increment was deposited the day of hatch in fish reared in all

temperature regimes. Embryos incubated at 18°C hatched about 1 d later than at 22 or

26°C and in some cases had an additional increment inside the high contrast hatch increment.

Age validation.--Increments were deposited daily in otoliths of Colorado squawfish larvae and juveniles. Slopes of regression lines for increment count as a function of known age were not significantly different than 1.0 for constant and fluctuating temperature treatments, for 18, 22, and 26°C fluctuating treatments combined, and for all regime treatments combined (Table 2). A posteriori power to detect a relatively conservative 5% difference (0.05) from the hypothesized slope of 1.0 was $\geq$ 0.99 for all fluctuating treatments and 0.58 for the 22°C constant temperature treatment. Intercepts for all relationships were not significantly different from zero, which was the expected value if daily increments were first deposited at hatching. Increment deposition patterns of the asteriscus were not examined because it was not present at hatch.

Daily increments were relatively clear and more accurately counted in fish from fluctuating than constant temperature which resulted in narrow ($\pm$ 7-8 d) prediction interval widths for known age relative to the $\pm$ 18 d one for fish reared are constant 22°C. Higher frequency of sub-daily increments and lower overall increment contrast made accurate enumeration of daily increments more difficult in otoliths of fish reared at constant 22°C. High r^2 values and residual plots indicated that all relationships were linear. Prediction interval widths for known age calculated for fish < 25-d-old ($\leq$ 12.5 mm TL) were $\pm$ 2.5 d for fish reared in 18, 22, and 26°C fluctuating temperatures, but were $\pm$ 5 d for fish reared in constant 22°C temperature.

The ANCOVA (Table 3) detected no significant difference between readers for slopes (age·reader term) or intercepts (reader term) of regression lines of predicted age as

a function of true age for larvae reared at 22°C. Difference between means for readers 1 (mean age = 41.7 d, SE = 0.845) and 2 (mean age = 40.4 d, SE = 0.840) was 1.3 d ($\pm$ 2.35, 95% CI). Constant and fluctuating temperature treatment data at 22°C were pooled within reader in this analysis because no significant differences were detected between those treatments (reader 1: P = 0.71 for slopes, P = 0.42 for intercepts; reader 2: P = 0.94 for slopes, P = 0.91 for intercepts).

A similar analysis of regression lines for increment count as a function of known age for larvae reared in fluctuating 18, 22, and 26°C temperatures indicated no significant temperature effect on daily increment deposition rates (Table 3). Estimated means were 36.7 d (SE = 0.61, 18°C), 36.3 d (SE = 0.66, 22°C) and 36.1 d (SE = 0.59, 26°C); the difference between means for 18°C and 26°C treatments was 0.6 d ($\pm$ 1.56, 95% CI).

Effects of temperature on otolith growth.--The ANCOVA of otolith diameter as a function of $\log_e$ TL suggested that intercepts and slopes of regression relationships (Table 4) among different temperature treatments were significantly different (Table 5). Mean otolith diameter of fish reared in 18°C was 6.1% less than mean lengths of otoliths from fish reared in other temperature treatments (contrasts of means, P = 0.01). No significant differences were noted among other temperature regimes. The differences between mean otolith diameter of fish reared at 18°C (75.87 µ) and 22°C (79.48 µ, the value nearest that for 18°C) was 3.61 µ ($\pm$ 3.31, 95% CI).

Effects of somatic growth rate on otolith growth.--Three experiments were conducted to determine the effects of slow and fast somatic growth rate (experiment 1) and different starvation periods (experiments 2 and 3) on otolith growth patterns. Experiment 1 verified a growth rate effect, whereby otoliths of slower growing fish

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averaged 19.2% larger (least squares means of lapillus diameter^2 = 222,039 μ, SE = 4,499) than otoliths of faster growing fish (least squares means of lapillus diameter^2 = 186,214 μ, SE = 5,802) of the same length (Fig. 1). The difference of the estimated means between treatments for lapillus diameter^2 was 35,824 μ ($\pm$ 14,475, 95% CI). The ANCOVA for the two relationships indicated that intercepts were not significantly different (df = 1, F = 0.02, P = 0.89), but that slopes were (df = 1, F = 5.77, P = 0.019). When analyzed by ANCOVA squared values for dependent variables resulted in a better fit than $\log_e$TL transformation. The r^2 values for linear equations with squared dependent variables were as high or higher than those achieved by non-linear regression.

Fish in the slow-growth treatment (Fig. 2) averaged 54.7% smaller (least squares means of TL^2 = 472 mm, SE = 20.6) than fish in the fast-growth treatment (TL^2 = 1,041 mm, SE = 27.1). The difference of the estimated means between treatments for TL^2 was 569 ($\pm$ 65.9, 95% CI). The ANCOVA analysis of regression lines for the fast-growth and slow-growth treatments indicated that both intercepts (df = 1, F = 16.29, P < 0.0001) and slopes were significantly different (df = 1, F = 271, P < 0.0001).

Fish in the slow-growth treatment had otoliths that averaged 32.4% smaller (lapillus diameter^2 = 185,314 μ, SE = 5,073) than fish in the fast growth (lapillus diameter^2 = 274,077 mm, SE = 6,788) treatment of the same age (Fig. 3). The difference of means for lapillus diameter^2 between treatments was 88,763 μ ($\pm$ 15,780, 95% CI). The ANCOVA indicated that the intercepts (df = 1, F = 10.27, P = 0.002) and slopes (df = 1, F = 124.09, P < 0.0001) of the two regression lines were highly significantly different, with slope again accounting for most of the observed differences.

In experiment 2, where food was withheld from fish for a 6-d interval between 6-d-long feeding periods, somatic growth of starved fish ceased in both fluctuating and constant 22°C temperature treatments (Fig. 4). However, otolith growth was constant in both temperature treatments. Fish somatic growth was slightly faster in fluctuating (mean = 2.3 mm TL) than constant (mean = 1.9 mm TL) temperatures over the 6 d-period prior to starvation, but was similar in the 6-d period after feeding was resumed (mean = 1.5 mm vs. mean = 1.4 mm TL). Otolith increments were of relatively high contrast before, during, and after the starvation period.

In experiment 3, food was withheld from 6-d old fish for 5, 10, 15 and 17.5 d to determine effects on otolith and fish growth patterns compared to continuously fed control fish. Comparison of trajectories of mean otolith diameter and mean fish length over time suggested similar growth patterns within each treatment (Fig. 5). Otolith and somatic growth rates of larvae in all treatments were similar to continuously fed control fish during the first 5 d (11-d old fish). Fish in the 5-d starvation treatment showed a delayed effect of the starvation period because somatic and otolith growth were reduced in comparison to controls when fish were 16-d old. In 10, 15, and 17.5-d treatments, somatic growth was largely arrested by the time fish were 11-d old but otoliths continued to grow in fish up to 21-d old.

Somatic growth of fish in 10, 15, and 17.5 treatments increased immediately after feeding started but fish did not grow at rates similar to controls until 2.5 to 5 d after feeding. Conversely, otoliths grew only slowly or not at all for 2.5 to 5 d after feeding; after 5 d otolith growth was nearly that of controls.

Width and contrast of otolith growth increments was not reduced during the first 5-d starvation period. However, the space on the otolith corresponding to the starvation periods > 5 d was generally opaque throughout and daily increments were more difficult to distinguish. Daily increments deposited after fish began feeding were dark and of high contrast, usually within 1 to 2 days after the starvation period.

The ANOVA of otolith growth rates in Colorado squawfish exposed to a timed increase in temperature and first feeding (experiment 4) indicated a significant food-temperature interaction (ANOVA, df = 1, F = 12.95, P = 0.0024). Otolith growth was 18% greater at 25°C than at 18°C, when food was available (Fig. 6) but similar at those temperatures when fish were not fed.

Discussion

Early otolith microstructure and age validation.--Otoliths were present in Colorado squawfish embryos prior to hatching in all the 18, 22, and 26°C temperature treatments tested. These temperatures represent the known range during which Colorado squawfish successfully spawn (Nesler et al. 1988; Tyus and Haines 1991; Bestgen and Williams 1994) suggesting that all wild Colorado squawfish larvae should have otoliths prior to hatching.

Daily increment deposition in otoliths of Colorado squawfish was verified for larvae reared in all fluctuating and constant regimes from 18 to 26°C, a range which approximates temperatures expected in the wild. Although slopes and intercepts of regression equations of increment count as a function of known age were not significantly different from 0 and 1 respectively, for fish from all treatments, the r^2 was lower and

prediction interval width for known (= true) age wider for the regression relationship for fish from the constant 22°C treatment. These statistical differences supported the observation that otoliths from fish reared at a constant temperature were more difficult to age accurately than those from fluctuating temperatures. Similarly, Campana (1984) and Neilson and Geen (1985) found that plainfin midshipman *Porichthys notatus* and chinook salmon *Oncorhynchus tshawytscha*, respectively, deposited daily otolith increments in both constant and fluctuating temperature environments, but that increments were of higher contrast in fish reared in fluctuating than constant temperatures.

Only dark, high contrast increments were counted in this study. Occasional presence of lighter, lower contrast sub-daily increments was reduced by ensuring that the microscope focal plane was focused near the middle of the otolith. Dark, high contrast otolith increments should be present in wild Colorado squawfish because water temperature in the wild exhibits regular daily fluctuations, thereby reducing the potential for deposition of non-daily otolith increments and age estimation bias.

Effects of temperature on otolith growth.--The ANCOVA and differences in estimated means and the 95% CI supported the conclusion that otoliths were significantly smaller in fish reared at 18°C than in constant or fluctuating 22°C or fluctuating 26°C suggesting that there may be a positive relationship between temperature and otolith growth independent of somatic growth rate. Temperature may affect otolith growth independent of somatic growth due to temperature-mediated rates of fish metabolism (Mosegaard et al. 1988; Wright 1991; Bradford and Geen 1992) or complex temperature interactions with the endogenous circadian rhythm of otolith calcification and matrix deposition (Secor and Dean 1992). The relatively small 6% reduction in otolith growth

rate at 18°C may affect otolith size-fish size relationships only if Colorado squawfish

occupy that relatively cool temperature for long periods. Incorporation of water

temperature data to explain variation in somatic and otolith growth rates of Colorado

squawfish (sensu Mosegaard et al. 1988; Secor and Dean 1992) would be difficult given

extreme diel and habitat-specific temperature variation in the wild.

Effects of growth rate on otolith growth.-- In experiment 1, differences in

estimated means and the 95% CI and ANCOVA detected a significant difference between

intercepts and slopes of regression lines of lapillus diameter^2 as a function of TL for fast-

and slow-growing treatment fish, indicating that somatic and otolith growth rates were

not proportional. Relationships depicting age as a function of fish TL and otolith

diameter were also significantly different for fast- and slow-growing fish. The

conservative nature of otolith growth compared to somatic growth was illustrated by the

relatively large slope differences in the Colorado squawfish TL^2-age relationships for fast

and slow-growing fish (Fig. 2) compared to smaller slope differences for the lapillus

diameter^2-age relationships for fast and slow-growing fish (Fig. 3). Because growth rate

effects are typically manifest as differences in slopes of the otolith length-fish length

regressions between treatments (Campana 1990), similar intercepts would be expected for

Colorado squawfish hatched under the same pre-treatment conditions. Thus, intercept

differences for TL^2-age and lapillus diameter^2-age regression relationships are likely an

artifact of slope differences.

Non-proportional somatic and otolith growth was also confirmed in experiment 2

because somatic growth nearly ceased but otolith growth was constant during a 6-d

starvation period. The zone represented by the starvation period in the otolith was

accurately determined because fish age was known and because the number of clear daily

increments counted before and after starvation, where increments were slightly less clear,

matched expectations. Measurements of otolith zones representing periods before,

during, and after starvation were within 5.1% for two readers, indicating minimal bias

from measurement error or zone interpretation.

Experiment 3 showed a complex relationship between otolith and somatic growth

when 6-d-old Colorado squawfish larvae that were just beginning to feed were subjected

to 5 to 17.5-d long starvation periods. Somatic and otolith growth were comparable in

control fish and treatment fish starved 5 d (11-d old) probably because residual yolk

sustained fish for at least part of the starvation interval. Delayed somatic and otolith

growth effects measured when 5-d starvation treatment fish were 16-d old were not

expected because fish were feeding. Otoliths of fish from 10, 15, and 17.5-d starvation

treatments continued to grow, albeit slowly, after somatic growth ceased, again

illustrating non-proportional otolith and somatic growth rates. Differences in otolith size

and fish length resulting from starvation periods were then maintained for the duration of

the experiment. As in this study, others (Eckmann and Rey 1987; Maillet and Checkley

1990; Bradford and Geen 1992) found that otolith growth of previously starved fish was

delayed for several days after feeding resumed.

Fish in 10 to 17.5-d treatments showed delayed starvation effects, because

somatic and especially otolith growth was reduced compared to control fish 2.5 to 5 d

after feeding. Fish in starvation treatments were actively feeding within 1 h after food

was offered so delay in feeding can not explain delayed otolith or somatic (TL) growth.

However, the delay period may have been necessary to rebuild metabolic reserves and restore basic physiological processes before otolith and somatic growth could resume.

Otoliths of fish in experiment 3 showed distinct zones representing starvation periods 10 d long or more. The first seven to nine increments, which were obvious in most fish, were probably produced when fish subsisted on yolk. Increments were typically very faint or not present after 10 d in starved fish, but were obvious within 1 to 2 d after feeding began. Thus, effects of starvation depended on duration of the period without food and were manifest as absence of otolith increments, lack of otolith growth, or both. Opaque zones in otoliths of wild fish may indicate periods of low food abundance.

Results of experiments 2 and 3 are in apparent contradiction because otolith growth rates did not change in the former but did in the latter experiment during starvation periods. The smaller (mean TL = 8.2 mm, SE = 0.10) and younger (6 d post-hatch) fish used in experiment 3 were more likely to show effects of longer starvation periods sooner than the relatively larger (mean TL = 11.1 mm, SE = 0.18) and older (16 d post-hatch) fish used in experiment 2 because of relatively smaller energy reserves. Otoliths of larger and older Colorado squawfish larvae would likely exhibit reduced growth if starved longer than 6 d.

Thorrold and Williams (1989) also suggested that otolith growth in smaller, younger larvae may respond more quickly to changes in food abundance than larger and older juveniles, presumably because larvae deplete energy reserves more quickly. In support of this, Maillet and Checkley (1990) and Eckmann and Rey (1987) found that otolith growth rates of relatively small and young larvae (< 10 d old) were reduced almost

immediately after starvation. Reduced increment widths (= otolith growth) were not statistically detectable in larger and older glass fish *Ambassis vachelli* or chinook salmon for at least two weeks after starvation (Molony and Choat 1990; Bradford and Geen 1992). Thus, small-bodied life stages or species that are more vulnerable to starvation (Miller et al. 1988) may more immediately display reduced otolith growth than larger-bodied life stages or species due to differences in energy reserves.

Laboratory conditions in experiment 4 simulated environmental changes experienced by wild Colorado squawfish when larvae are transported downstream of spawning areas in the relatively cool, turbid, and food-poor main channel and then move into relatively warm, low-velocity channel margin habitats (backwaters) with higher food abundance (Haines and Tyus 1990; Tyus and Haines 1991). Increased width of otolith daily increments (result of increased otolith growth) in wild fish may signal timing of arrival and feeding by Colorado squawfish larvae in backwaters, which may allow determination of effects of different flows on transport rates of larvae from upstream spawning areas and ability of larvae to find suitable habitat. Similarly, settlement marks in otoliths of some marine fishes record timing of life history shifts when pelagic larvae switch to a benthic juvenile and adult existence (Victor 1991).

Age of Colorado squawfish larvae is determined by simply counting otolith daily increments. Number and pattern of otolith daily increments will be useful for determining relative importance of cohorts in age-classes, their hatch date distributions. Age determinations made for Colorado squawfish from counting daily otolith increments should incorporate estimates of error, determined by predction intervals for size classes, into analyses. Similarly, growth rate estimates based on fish length at capture and age

(e.g., growth·day^{-1}) should incorporate uncertainty of age estimates into calculations (Rice 1987).

Otolith growth rates in Colorado squawfish depended on water temperature independent of somatic growth rate, which was dependent upon food abundance, water temperature (Bestgen 1996) and many other environmental factors. Otolith growth rates were also dependent upon age- and size-specific responses to starvation, and duration and delayed effects of starvation.

Complex patterns of otolith growth, and the non-synchrony of otolith and somatic growth during periods of variable food abundance, suggest that accurate back-calculation of daily growth for individual fish larvae, and correlation of growth rate changes with time-specific environmental events, may be difficult. This is so because most length back-calculation techniques require a proportional otolith-somatic growth relationship such that a severe reduction (or increase) in growth would be recorded as similarly reduced (or increased) otolith growth. Although length back-calculation may still yield growth rate information for populations rather than individuals, bias of these techniques should be evaluated prior to implementation for Colorado squawfish (Francis 1990, 1995). Delayed otolith growth after starvation periods (e.g., experiment 3) reflected reduced somatic growth in the history of a fish, so it may be possible to calculate reductions in growth for periods of time longer than a day from otolith growth patterns. Changes in otolith microstructure of wild fish that are induced by life history-specific habitat shifts or changes food abundance or temperature may also yield insights into processes that affect growth and survival of early life stages of Colorado squawfish.

Acknowledgements

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Table 1.--Summary of objectives and protocols for experiments. Photoperiod was 14 h light:10 h dark for all experiments, dissolved oxygen was 5-6 mg·L⁻¹, and fish in all experiments were fed at the prescribed rate two times per day.

Experiment	Objective	Treatments	Fish age (d) at initiation	Artemia nauplii per feeding
Age validation. otolith growth	Determine otolith microstructure and growth in specimens up to 165 d old	Constant 22°C, and fluctuating 18, 22, and 26°C ($\pm$ 2.5°C)	1	Ad libitum
Growth effect experiments				
Experiment 1	Determine otolith growth rates in fast- and slow-growing fish up to 122 d old	Slow growth (200 fish·2 L⁻¹) vs. fast growth (100 fish·2 L⁻¹), constant 26°C	1	Slow = 30·fish⁻¹ Fast = 60·fish⁻¹
Experiment 2	Determine effects of starvation on otolith and somatic growth of individual larvae	Starve for 6 d between two 6-d feeding periods, constant and fluctuating 22°C	11	Ad libitum

Table 1 Continued.

Experiment	Objective	Treatments	Fish age (d) at initiation	Artemia nauplii per feeding
Experiment 3	Determine effects of different starvation periods on otolith and somatic growth	Feed larvae after 0 (control), 5, 10, 15, and 17.5-d starvation periods, constant 21°C	6	Ad libitum
Other experiments				
Experiment 4	Determine effect of increased temperature and first feeding on otolith growth	Rear larvae at 18°C and 25°C with and without food, determine 7-d otolith growth differences	14	Ad libitum

Table 2.--Least squares statistics for otolith increment count as a function of days since hatching (known age) for Colorado squawfish incubated and reared for 165 d in fluctuating (± 2.5°C·d^{-1}) 18, 22, and 26°C and constant 22°C temperature treatments. Regressions are increment count $= a + b$(known age), where a is the intercept and b is the slope, P is significance of test of intercept $= 0$ or slope $= 1$. Prediction limits were calculated for mean known age instead of estimated age via inverse regression because age of wild fish will be estimated from counts of daily increments.

Treatment	df	Intercept $a \pm$ SE (P)	Slope $b \pm$ SE (P)	r^2	95% prediction limits for known age of individual larvae ($\pm$ no. of increments)
18°C Fluctuating	46	0.019 $\pm$ 0.6913 (0.978)	1.019 $\pm$ 0.0106 (0.074)	0.995	$\pm$7.8
22°C Fluctuating	41	-0.114 $\pm$ 0.6938 (0.871)	0.992 $\pm$ 0.0111 (0.484)	0.995	$\pm$7.1
22°C Constant	38	1.409 $\pm$ 1.8030 (0.440)	1.013 $\pm$ 0.0282 (0.911)	0.972	$\pm$17.7
26°C Fluctuating	54	-0.123 $\pm$ 0.5690 (0.830)	0.993 $\pm$ 0.0108 (0.497)	0.994	$\pm$7.1
All fluctuating combined	143	-0.130 $\pm$ 0.3768 (0.729)	1.003 $\pm$ 0.0063 (0.585)	0.995	$\pm$7.2

Table 2 Continued.

Treatment	df	Intercept $a \pm SE\,(P)$	Slope $b \pm SE\,(P)$	r^2	95% prediction limits for known age of individual larvae ($\pm$ no. of increments)
All treatments combined	182	$0.178 \pm 0.4751\,(0.708)$	$1.004 \pm 0.0078\,(0.617)$	0.989	± 10.1

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Table 3.--Analysis-of-covariance F-statistics of increment count (estimated age) as a function of true age compared among readers for pooled fluctuating ($\pm 2.5°C \cdot d^{-1}$) and constant 22°C treatment fish (A), and among fluctuating 18, 22, and 26°C temperatures (B). Reader (A) and temperature (B) terms are indicator variables for different readers and temperature treatments, respectively; the F-test probability value is the significance test for differences in intercepts between groups designated by the indicator variable. The age-reader (A) and age-temperature (B) interaction terms test for equivalence of slopes between groups.

Effect	df	SS	F	P	R^2
A. Comparison among					
readers					
Age	1	380250.47	6579.05	0.0001	0.98
Reader	1	1.86	1.86	0.86	
Age-Reader	1	34.92	34.92	0.44	
B. Comparison among					
temperatures					
Age	1	311879.33	16820.11	0.001	0.99
Temperature	2	3.80	0.10	0.90	
Age-Temp.	2	29.53	0.80	0.45	

Table 4.--Least squares statistics for regression of lapillus diameter as a function of $\log_e$ total length of Colorado squawfish incubated and reared for 165 d in fluctuating ($\pm$ 2.5°C·d^{-1}) 18, 22, and 26°C and constant 22°C temperature treatments. Regressions are lapillus diameter $= a + b(\log_e \cdot TL)$, where a is the intercept and b is the slope. Fish < than 2 d old were excluded from this analysis because their otolith and length measurement residuals were positively biased.

Treatment	df	Intercept $a \pm SE$	Slope $b \pm SE$	r^2
18°C Fluctuating	45	-198.11 $\pm$ 5.47	106.73 $\pm$ 2.13	0.983
22°C Fluctuating	41	-220.20 $\pm$ 6.97	116.75 $\pm$ 2.60	0.981
22°C Constant	40	-227.81 $\pm$ 4.62	120.43 $\pm$ 1.76	0.992
26°C Fluctuating	51	-222.37 $\pm$ 5.56	118.43 $\pm$ 2.14	0.984
All treatments combined	180	-216.72 $\pm$ 3.12	115.49 $\pm$ 1.192	0.981

Table 5. Analysis-of-covariance for the relationship lapillus diameter as a function of $\log_e$ TL ($\log_e$TL) for Colorado squawfish incubated and reared in fluctuating ($\pm 2.5°\text{C·d}^{-1}$) 18, 22, and 26°C and constant 22°C temperature regimes. The F-test probability value for the treatment regime indicator variable tests for differences among intercepts. Treatment·lnTL interaction term tests for differences among slopes for different treatment regimes. Least squares means and standard errors for lapillus diameter (μ) are adjusted for the covariate fish length. Contrasts of means indicated that lapillus diameters for fish from the 18°C treatment were significantly ($\underline{P} = 0.01$) smaller than from other treatments.

Effect	df	SS	F	P	R^2
$\log_e$TL	1	604384.38	11087.45	< 0.0001	0.985
Treatment	3	862.70	5.28	0.0017	
$\log_e$TL·Treatment	3	1261.35	7.71	< 0.0001	

Least Squares Means analysis

Temperature and regime	Least squares means of lapillus diameter	SE
18°C Fluctuating	75.87	1.0934
22°C Constant	79.48	1.1494
22°C Fluctuating	81.34	1.1537
26°C Fluctuating	81.65	1.0249

Fig. 1. Regression of (otolith diameter)^2 as a function of Colorado squawfish total length

for fish in slow and fast growth treatments. Slow growth treatment: (lapillus

diameter)^2 = -135,742.1 + 15,658$\cdot$TL; r^2 = 0.97; N = 40; SE of intercept = 11,293,

SE of slope = 485. Fast growth treatment: (lapillus diameter)^2 = -133,328.4 +

13,984.9$\cdot$TL; r^2 = 0.97; N = 24; SE of intercept = 13,847, SE of slope = 490.
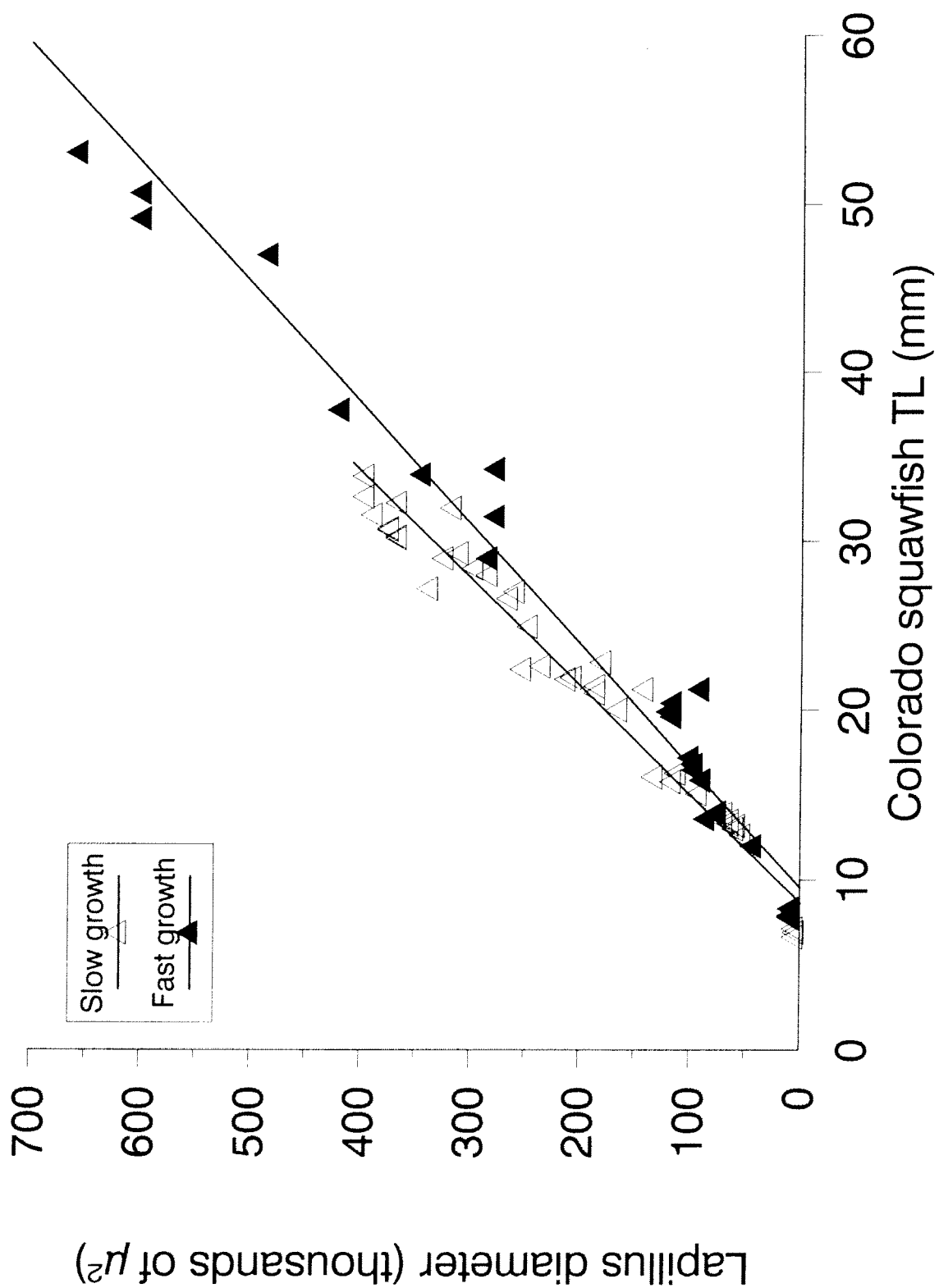
Fig. 2. Regression of Colorado squawfish total length as a function of age (days post-

hatch) for fish in slow- and fast-growth treatments. Slow-growth treatment: $TL^2 = $

35.392 + 8.06·age (days); $r^2 = 0.94$; $N = 40$; SE of intercept = 23.952, SE of slope

= 0.328. Fast-growth treatment: $TL^2 = -186.73 + 22.64·age$ (days); $r^2 = 0.95$; $N = $

24; SE of intercept = 57.003, SE of slope = 1.019.

Figure shows Colorado squawfish TL (thousands of mm²) versus Age (days post-hatch) with Slow growth and Fast growth data.
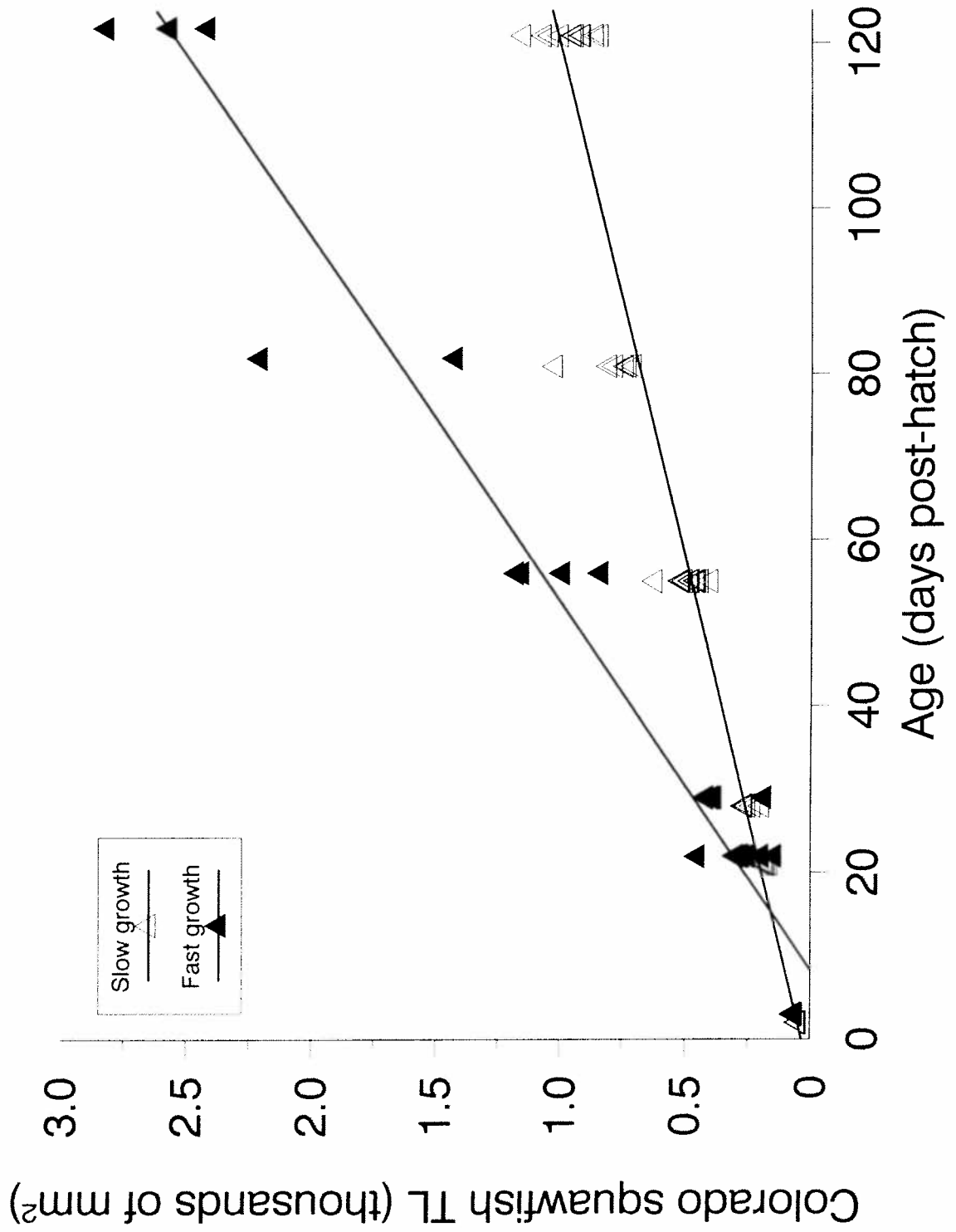
Fig. 3. Regression of otolith diameter as a function of Colorado squawfish age (days post-hatch) for fish in slow- and fast-growth treatments. Slow-growth treatment: lapillus diameter^2 = 17,715 + 3,024.1·age; r^2 = 0.94; N = 40; SE of intercept = 9604, SE of slope = 129. Fast-growth treatment: lapillus diameter^2 = -25,734.3 + 5,409.6·age; r^2 = 0.98; N = 24; SE of intercept = 9228, SE of slope = 162.

Age (days post-hatch)

Lapillus diameter (thousands of μ^2)

Slow growth
Fast growth
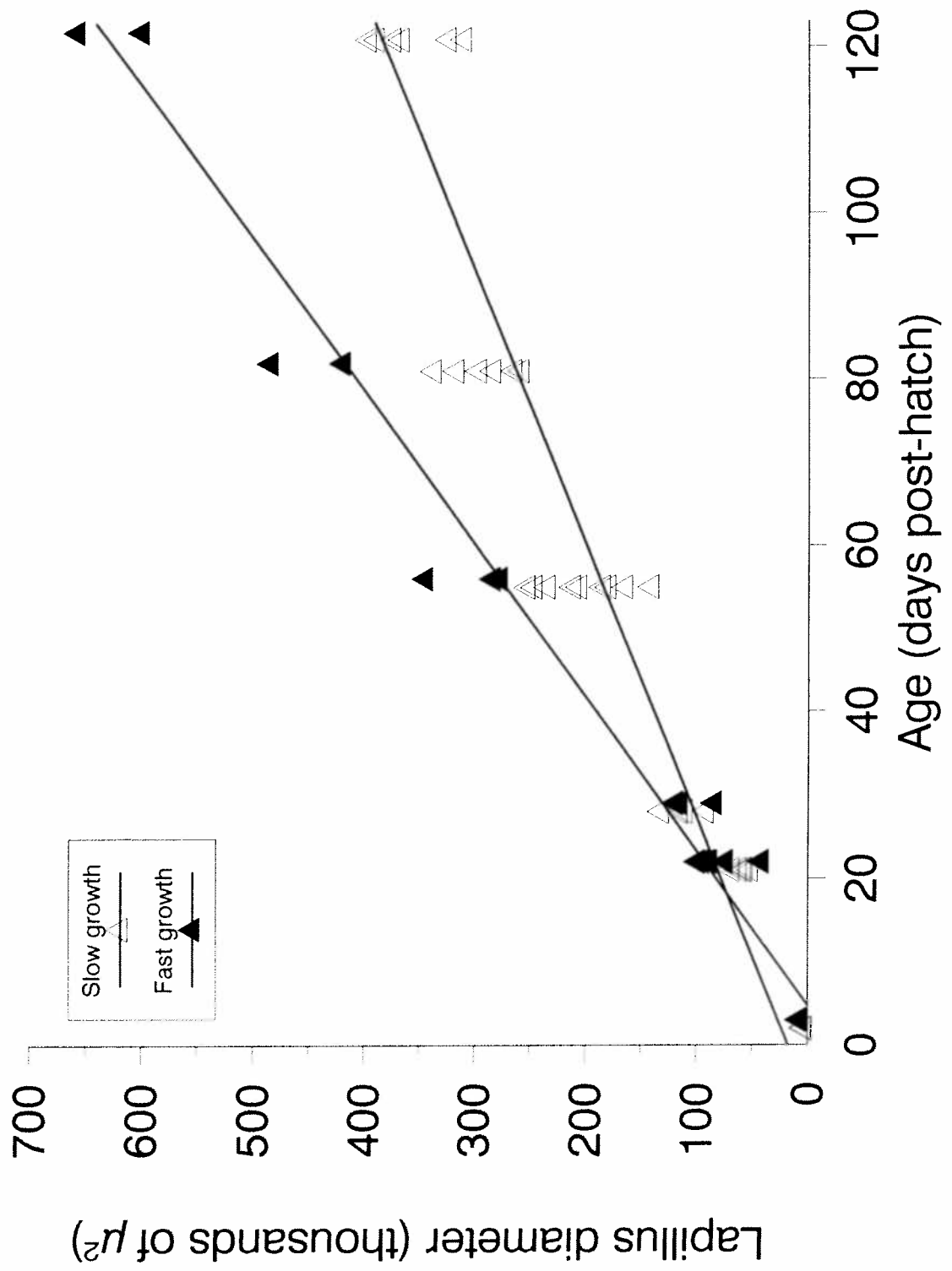
Fig. 4. Somatic and otolith growth patterns of Colorado squawfish larvae in constant (A) and fluctuating (B) 22°C temperature conditions. Fish were 11 d old at the start of the experiment (0 d). Fish were fed for 6 d, starved for 6 d (interval between arrows), fed for 6 d, and preserved. Fish were anaesthetized and measured at the 6-d intervals and otolith diameter at those same intervals later measured. Error bars about means are ± 2 SE (N = 8).

22°C Constant

A

22°C Fluctuating

B
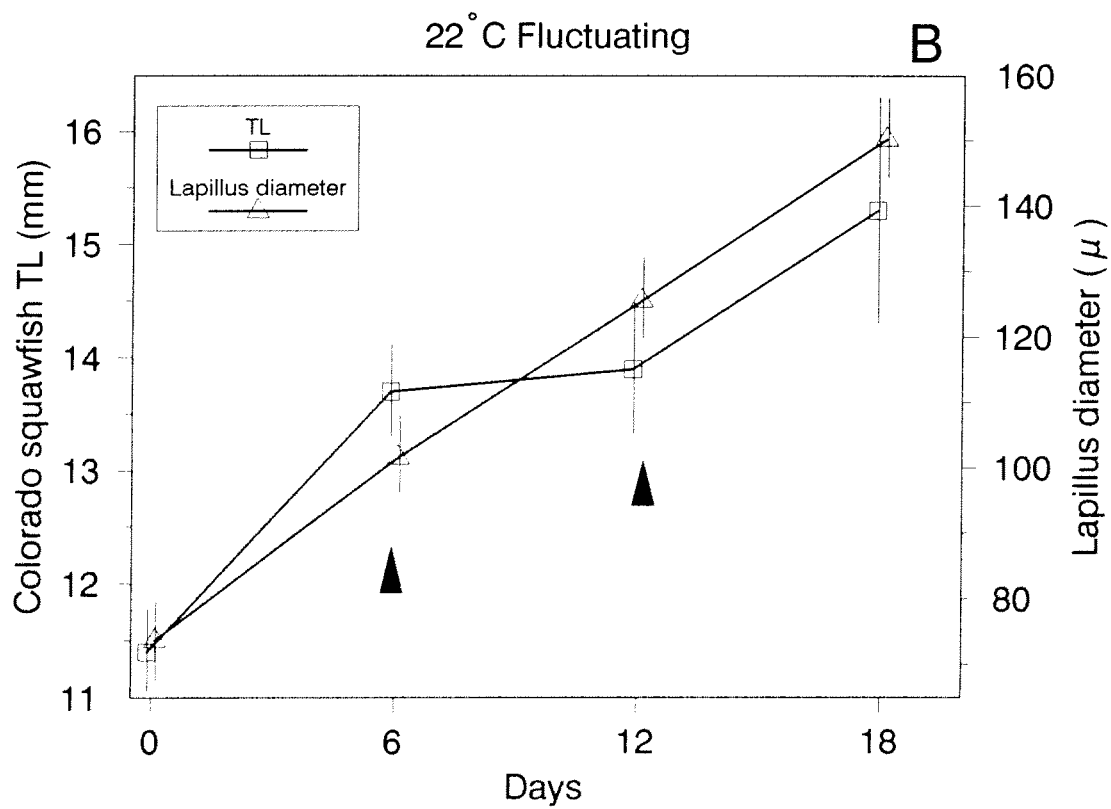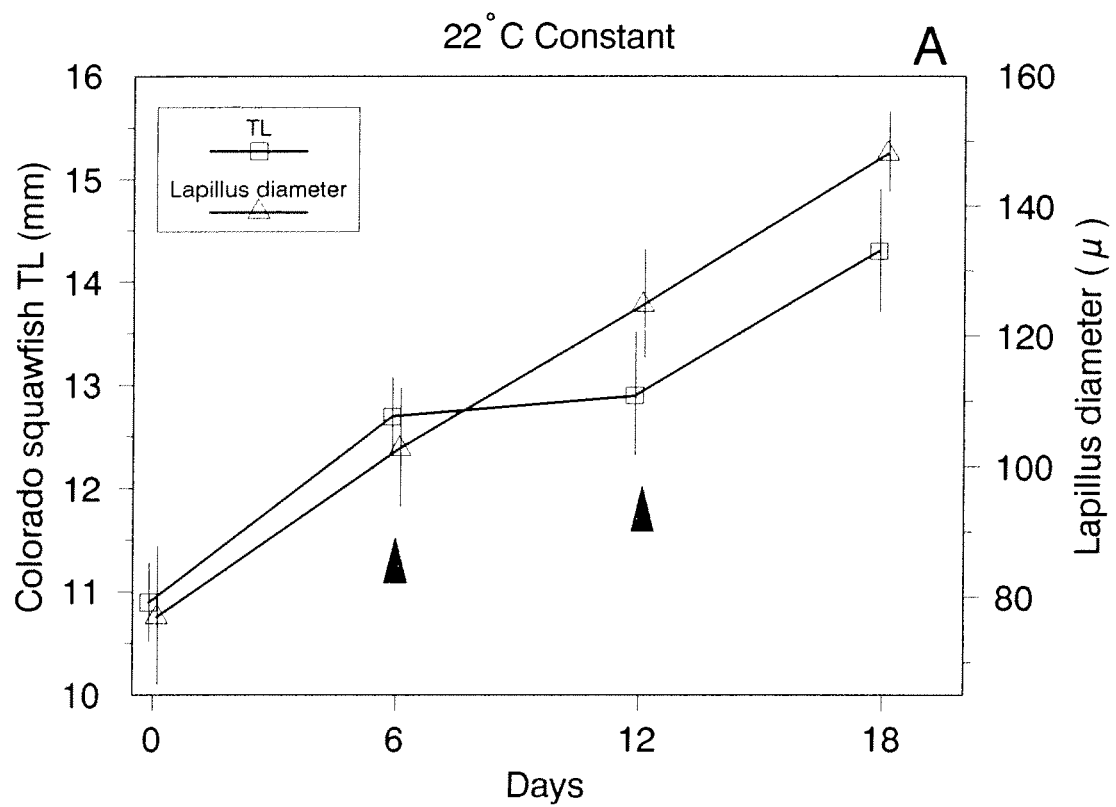
Fig. 5. Somatic (A) and otolith (B) growth patterns of 6-d-old Colorado squawfish larvae

subjected to starvation periods of 5, 10, 15 and 17.5 d, and subsequent growth

after feeding. Error bars about means are $\pm$ 2 SE ($N = 5$).
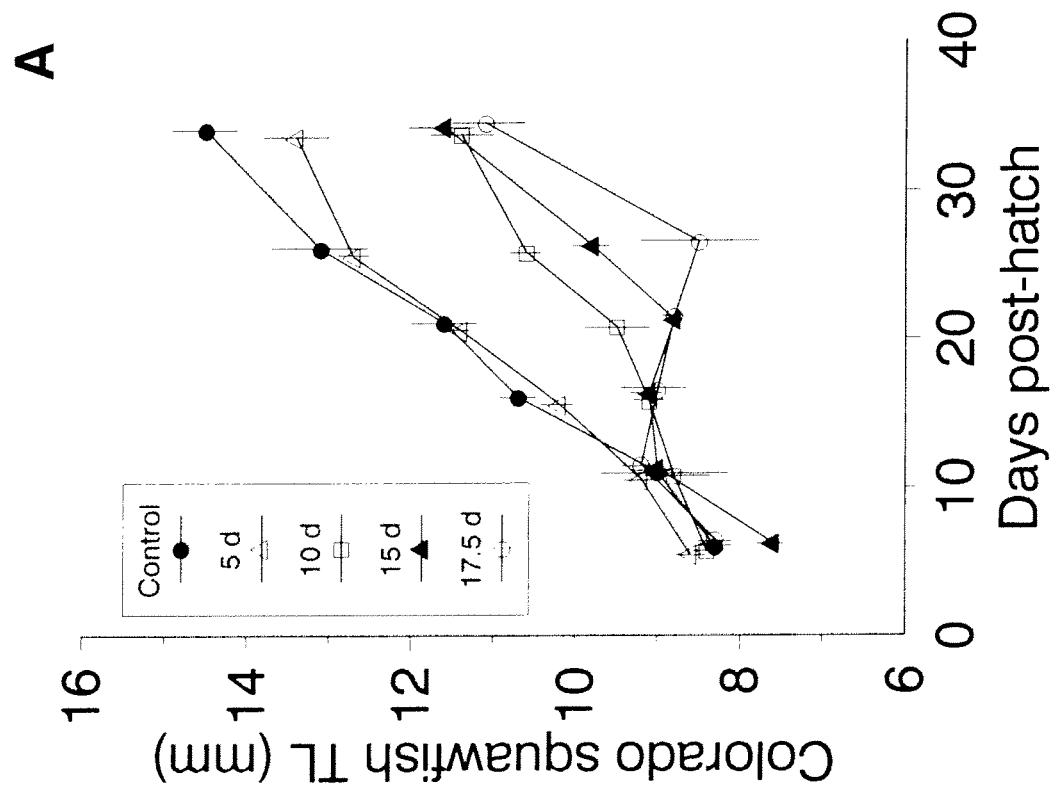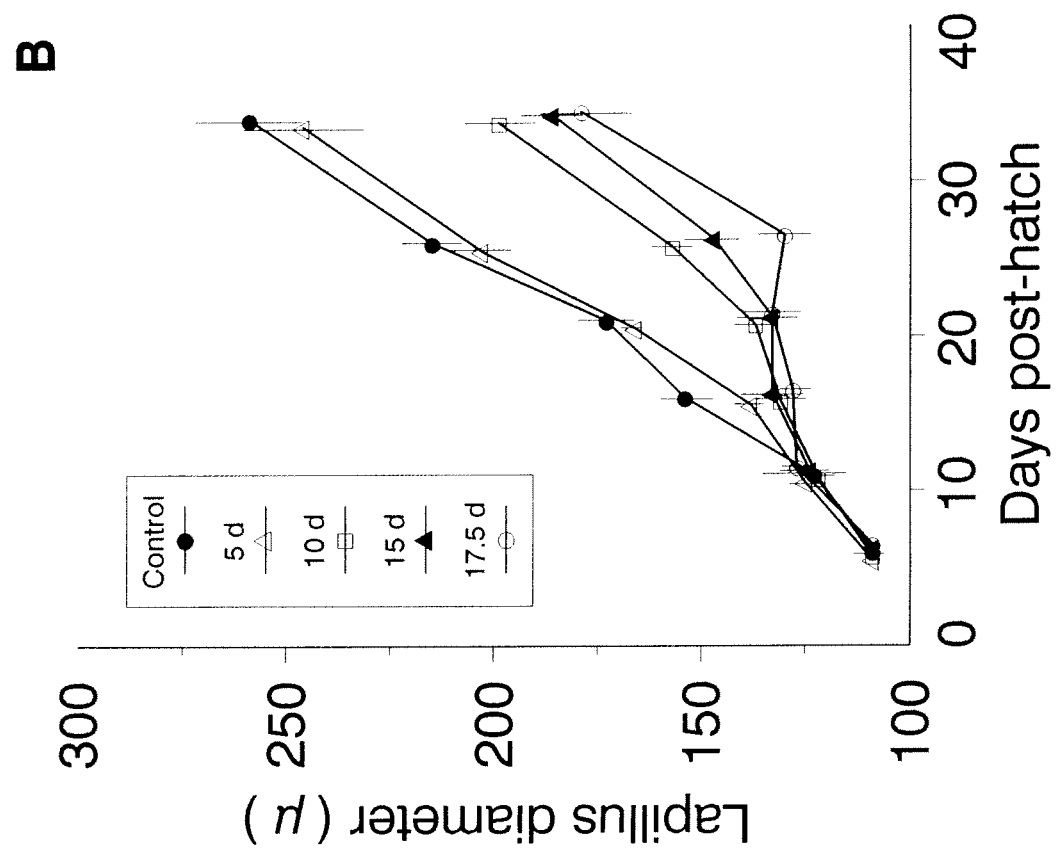
Fig. 6. Mean lapillus diameter ($\pm$ 2 SE, $N = 5$) for four groups of starved 14-d-old

Colorado squawfish larvae held at 18°C and then subjected to experimental

treatments 18°C starved, 18°C fed, 25°C starved, and 25°C fed for 7 d.
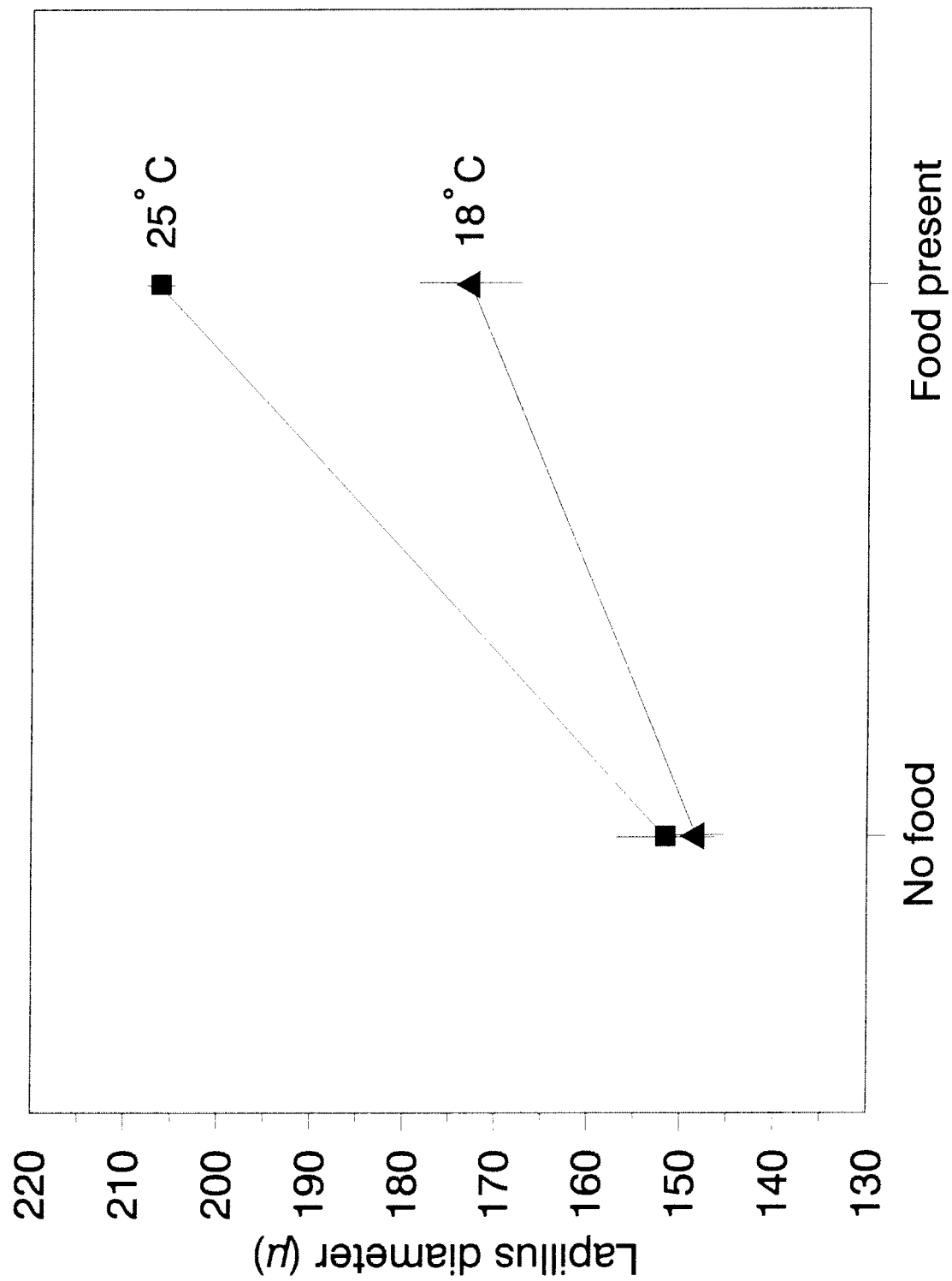
CHAPTER FOUR

INTERACTING EFFECTS OF PHYSICAL AND BIOLOGICAL PROCESSES

ON RECRUITMENT OF COLORADO SQUAWFISH

ABSTRACT

Recruitment is central to population ecology because the abundance of young

individuals often drives dynamics of subsequent life stages. Recruitment variation of

age-0 Colorado squawfish *Ptychocheilus lucius* in the Green River, Colorado River basin,

was related to physical and biological factors that were important at both intra-annual and

annual time scales. Distributions of squawfish hatching dates derived from otolith

increment analyses in 1991 and 1992 indicated that larvae in cohorts that hatched early

survived poorly to fall. Growth rate comparisons suggested that the few early-hatched

fish that survived were a fast-growing subset of the fish present in the same cohort in

summer. I attributed this to a biological factor, size-selective predation mortality by non-

native fishes. In contrast, larvae that hatched late grew relatively slowly but survived at

higher rates due to environmental factors and to declines in abundance of predaceous red

shiners *Cyprinella lutrensis* by mid-summer. An independent individual-based computer

simulation model which had gape-limited red shiners as predators and Colorado

squawfish larvae as prey produced similar size-selective survival patterns. Model

simulations also showed that fish with moderate growth rates survive at twice the rate of

fish with low-growth rates. Growth reductions caused by competition with non-native

fishes or starvation would extend the time that Colorado squawfish were susceptible to

predation but by themselves would not explain the size-selective patterns observed.

Reduced growth rates of Colorado squawfish, which were temporally correlated with a

stochastic physical factor, flooding from summer thunderstorms, may have combined

with size-selective predation to cause very low recruitment in the lower Green River in

1992. Otherwise, recruitment was uncorrelated with discharge and temperature regimes

in the summers of 1991 and 1992. Over a 17-yr record, mean July-August discharge had no effect on annual abundance of Colorado squawfish juveniles in backwaters in the fall except at relatively high discharge. Low abundance of juvenile Colorado squawfish in 1991 and 1992 when size-selective patterns were evident suggested that predation may regulate recruitment in most years. Therefore, discharge management that emphasizes habitat enhancement should be supplemented with strategies to reduce effects of non-native fishes.

Recruitment is central to population ecology because the abundance of young individuals often drives dynamics of subsequent life stages (Hjort 1914, Thorson 1950, Roughgarden 1988). However, understanding mechanisms that regulate recruitment is challenging because numerous life-history processes including timing and success of reproduction, and growth and dispersal of young are involved and systems are often open (Thorson 1950, Connell 1961, Roughgarden et al. 1985, Magnuson 1991). Moreover, most aquatic organisms with larval and adult phases have highly variable recruitment. High fecundity coupled with small variations in regulating processes can cause large differences in survival of larvae (Hjort 1914, Thorson 1950, Fogarty et al. 1991). Understanding recruitment variation of animals like fishes with multi-phase life cycles is particularly difficult because larvae typically disperse, sometimes long distances, away from juveniles and adults and each stage has different environmental limitations.

Recruitment may be regulated by interacting physical and biological processes, which change in relative importance across temporal and spatial scales. In marine systems and freshwater lakes, physical processes such as nearshore currents produced by wind may transport larvae to or from suitable habitats and subsequently influence local or regional recruitment (Frank and Leggett 1981, Roughgarden et al. 1988). In rivers, discharge, temperature, and sediment transport regimes influence timing of reproduction by adults and the quality and quantity of critical habitat for larvae (Welcomme 1979, Petts 1984). Distribution, abundance, and size-structure of reproducing adults determines the supply of larvae upon which other biological processes act (Thorson 1950, Gaines et al. 1985, Underwood and Fairweather 1989). These processes include predation, competition, and starvation, which often interact with environmental

conditions that influence growth to regulate recruitment of larvae (May 1974, Hunter 1981, Houde 1987, Bailey and Houde 1989). Importance of these recruitment-regulating processes may also shift among years (Hjort 1914, Gaines et al. 1985, Connell 1985) and even within years (Methot 1983, Rice et al. 1987, Doherty 1991). Understanding recruitment variation at both scales should clarify the importance of factors regulating structure and dynamics of natural communities and therefore must be the basis for management of recruitment-limited populations.

Most populations of endangered and endemic Colorado squawfish *Ptychocheilus lucius* in the Colorado River basin may be recruitment limited (Tyus 1991). In the largest remaining population in the Green River basin, Utah and Colorado, annual abundance of juveniles in fall (recruits) varies from near zero to 75 fish/100 m^2 of backwater habitat (Tyus and Haines 1991). However, the relative effects of physical and biotic factors on recruitment among and within years is poorly understood. Understanding recruitment patterns of fishes is enhanced if individuals in cohorts with differential survival have unique, recognizable traits. Frequency and pattern of daily increments in otoliths allows estimation of hatching dates, growth rates, and occurrence of periods of stress in individual fish (Pannella 1971, Campana and Neilson 1985, Bestgen and Bundy submitted) and may provide insights into recruitment processes (Crecco and Savoy 1985, Rice et al. 1987, Post and Prankevicius 1987, Cushing 1995). My goal in this research was to use otolith analyses and a long-term annual recruitment data set to evaluate the relative influence of physical and biotic processes that may explain intra-annual and annual recruitment patterns of age-0 Colorado squawfish.

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Colorado squawfish natural history.--Although most recruitment studies on animals with qualitatively different life stages have been in marine systems (Thorson 1950, Roughgarden et al. 1988, Cushing 1995), freshwater animals with analogous life history strategies exist. Potadromous Colorado squawfish, a piscivorous cyprinid endemic to the Colorado River basin, is an example. Adult Colorado squawfish attain a maximum length of 1-1.5 m and may exceed 25 years of age (Tyus 1991). In the Green River basin of Colorado and Utah, adult Colorado squawfish migrate to one of two known high-gradient canyon reaches in early summer for spawning (Tyus 1990). Spawning begins 4-6 wk after peak spring runoff, when water temperatures exceed 16-18°C, and extends up to 6 wk (Nesler et al. 1988, Tyus and Haines 1991). Embryos are deposited over cobble bars and develop in interstitial spaces for 4-6 d at temperatures of 18-26°C (Hamman 1981, Bestgen and Williams 1994). Following the incubation period, larvae hatch and are transported by river currents 40-200 km or more downstream to low-gradient valley reaches where they occupy shallow low-velocity backwaters at the channel margin for the remainder of the summer.

Reduced distribution and abundance of Colorado squawfish throughout the Colorado River basin is likely due to both disruption of physical habitat and negative interactions with non-native fishes (Vanicek and Kramer 1969, Holden 1979, Carlson and Muth 1989, Stevens et al. 1995). Mainstem dams block migration routes, inundate riverine habitat, and reduce maximum discharge and water temperatures in downstream reaches. Over 40 fishes have been introduced in the upper Colorado River basin (Carlson and Muth 1989), many of which may compete with, or prey on, early life stages of Colorado squawfish.

Study Area

The Green River basin drains portions of southern Wyoming, eastern Utah, and northwestern Colorado (Fig. 1) and the Green River is the largest tributary of the Colorado River. Yampa Canyon in the lower Yampa River, Whirlpool and Split Mountain canyons (river kilometer [RK] 555-515) in the upper Green River, and Desolation and Gray canyons (RK 340-211) in the middle Green River have high gradient and mixed cobble, rubble, and sand substrate. A valley reach in the middle Green River (RK 515-340) and Stillwater and Labyrinth canyons in the lower Green River (RK 211-0) have lower gradient and substrate dominated by sand and silt with lesser amounts of cobble. Discharge in the mainstem Green River upstream of the Yampa River has been regulated since 1963 by Flaming Gorge Dam; releases ranged from 22.6 - 130 m^3/s (1964-1996) but were sometimes higher because of spillway releases in high water years (e.g. 1983, Tyus and Haines 1991). Discharge in downstream reaches of the Green River in spring and early summer was high (Fig. 2) and dominated by the Yampa River. In contrast, Green River discharge in late summer, fall, and winter was lower and dominated by releases from Flaming Gorge dam. Discharge in the highly variable and unregulated Yampa River occasionally reached 566 m^3/s in spring but sometimes declined to < 2 m^3/s in late summer (U. S. Geological Survey records, gage 09251000). As a result of an agreement between dam operators and the U. S. Fish and Wildlife Service, releases from Flaming Gorge Dam since 1985 have maintained discharge of the Green River near Jensen at 51 m^3/s $\pm 25\%$ from July through September

to maximize backwater habitat for age-0 Colorado squawfish (Pucherelli et al. 1990). Mean July-August discharge of the middle Green River at Jensen, Utah (gauge # 09261000), and the lower Green River at Green River, Utah (gauge # 09315000), was highly correlated (r = 0.99, 1979-1995) and discharge was higher downstream (mean = 141 m³/s, SD = 108 m³/s) than upstream (mean = 100 m³/s, SD = 73 m³/s) mostly because of added White River discharge.

Methods

Distributions of hatching dates of larvae and juveniles captured in different seasons were determined from otolith analysis and divided into cohorts based on modes of hatch dates for larvae. Relative abundance of cohorts were compared across seasons to determine differential mortality of larvae hatched at different times. Growth rates of juveniles sampled in summer or fall and results of individual-based model (IBM) simulations were used to evaluate whether differences in recruitment could be accounted for by size-selective predation by non-native fishes. Finally, the relationship between fall abundance of juvenile Colorado squawfish and mean July-August discharge was assessed over a 17-yr period to determine whether recruitment differences could be due to discharge variation. The methods to achieve these objectives are described below.

Field collections.--The two sampling sites for Colorado squawfish larvae were the Yampa River just upstream (RK 1) of its confluence with the Green River in Dinosaur National Monument and the Green River about 15 km upstream of Green River, Utah, and each site was sampled in 1991 and 1992. Sampling started late-June to early-July each year, 4-6 wk after peak spring discharge when daytime water temperature exceeded

about 16°C (Nesler et al. 1988, Tyus and Haines 1991). Sampling continued for 4-6 wk after the first squawfish larvae were captured and ended when none were captured for three to five consecutive days, usually by mid-August.

Colorado squawfish larvae were sampled daily at dawn with conical drift nets (0.15 m² mouth diameter, 4 m long, 560 μm mesh) set nearshore in water 30 to 40 cm deep. Three replicate nets were set on each sampling occasion for up to 2 hr, but sampling stopped if debris load exceeded 3.8 L/sample. Water depth at which a white object disappeared from sight was recorded as a measure of water turbidity. To evaluate whether dawn (ca. 0600 hr) nearshore samples represented the abundance, age, and size of larvae transported downstream past sampling sites in 1992, additional diel (26 June and 1, 3, and 4 July) and cross-channel sampling (9, 10, 13, 15, 17, 18, 23, 24, 25, 27, and 29 July) was conducted. Diel sampling consisted of standard dawn nearshore samples supplemented with three replicate samples collected nearshore at noon (1200-1400 hr), dusk (1900-2100 hr) and midnight (0000-0200; 12 samples/d). Cross-channel sampling included nearshore collections during the same four periods and at the surface and bottom in a mid-channel location (3 locations x 4 times x 3 replicate samples = 36 samples/d). Samples could not be collected at all positions during all periods on 15 and 23 July. General Oceanics flow meters (model 2030) suspended in each net mouth measured water velocity throughout sampling periods.

Samples were fixed immediately in 100% ethanol. Fish were removed from debris within 4 hr and preserved in 100% ethanol. Rapid sample processing prevented fish from being stained by debris pigments, which facilitated sorting and identification.

This protocol recovered 100% of a known number of larvae (range 9 - 10) from five experimental samples.

In 1991 and 1992, Colorado squawfish larvae and juveniles (hereafter collectively referred to as juveniles) were captured from backwaters during late July to early August and late September to early October for otolith analyses. Fish were collected in two 20-km long reaches of the Green River, one near Ouray National Wildlife Refuge (RK 407-427, middle Green River), and from near Mineral Bottom in Canyonlands National Recreation Area (RK 69-89, lower Green River). Collections were made using a seine 4.6 m long x 1.3 m deep with 1.6 mm mesh. The relatively deep mouth of the backwater adjacent to the main channel was swept with one seine haul, and one or two more non-overlapping hauls swept portions of the backwater from the mouth to the shallow upstream end. Colorado squawfish retained for otolith analyses and fishes too small to be identified in the field were preserved in 100% ethanol for later identification in the laboratory; other fishes were identified, counted, and released.

Fall abundance estimates of juvenile Colorado squawfish from 1979-1995 were obtained from the Interagency Standardized Monitoring Program (ISMP) database of the Recovery Implementation Program for endangered fishes in the Upper Colorado River basin (pers. comm., C. McAda, U. S. Fish and Wildlife Service, Grand Junction, Colorado). During 1986-1995, the ISMP protocol was to seine the first two backwaters ≥ 30 m^2 in area and ≥ 30 cm deep encountered in each 8-km segment of middle (322-515 RK) and lower (0-193 RK) Green River reaches using the methods described above, except that seine mesh size was 3 mm. Area seined and number and total length (TL) of Colorado squawfish captured were recorded. Before 1986, Tyus and Haines (1991) used

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this same protocol to sample the Green River from RK 35-519. However, only data from their lower Green River strata A and B (RK 35-211) and middle Green River strata E and F (RK 340-515) were used in this study because they corresponded closely to ISMP reaches. Densities of Colorado squawfish were calculated for each backwater by dividing number of squawfish caught by area swept by seines. All squawfish in fall were large enough to be susceptible to sampling gear so that density estimates were not biased.

Laboratory procedures.--Colorado squawfish larvae (< 20 mm TL) captured in drift nets and juveniles captured in summer (9.0-25.4 mm TL) and fall (15.9-66.9 mm TL) seine samples were measured to the nearest 0.1 mm TL. Their ages were estimated by counting daily increments, which were first formed at hatching, in one of the three otoliths, the left sagitta (larvae) or lapillus (larvae and juveniles), and averaging a minimum of two and up to four separate counts (see Bestgen and Bundy (submitted), for details on aging techniques). Otoliths from all but 9 of 2537 fish were readable. The hatching date for each larva was calculated by subtracting its estimated age (d) from the date it was caught in drift nets. In many other studies, ages of larger and older fish were consistently underestimated by otolith techniques (Campana and Neilson 1985). However, laboratory studies showed that ages of Colorado squawfish up to 165 d-old and reared in fluctuating temperatures of 18, 22 and 26°C were estimated without bias (Bestgen and Bundy submitted), so age underestimation of wild fish was discounted.

Distributions of hatching dates.--Distributions of hatching dates of larvae captured in dawn nearshore drift net samples were compared to distributions for juveniles caught in backwaters with seines in summer and fall to evaluate recruitment patterns and differential mortality. Larvae drifting from upstream spawning areas in Yampa Canyon

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downstream to nursery habitat in middle Green River, and similarly from Gray Canyon to the lower Green River, are considered middle and lower Green River populations, respectively. Populations in the middle and lower Green River are thought distinct based on timing of reproduction (Tyus 1990), and age, condition, and size patterns of larvae from 1991-1996 (unpublished data, KRB). Larvae from the middle Green River and lower Green River were each divided into three or four cohorts. Cohorts were defined either by modes in the distributions of hatching dates or by time periods between modes during which relatively few larvae hatched. The number of larvae and fall-caught juveniles in each cohort were tallied.

Under the hypothesis of no differential mortality during the first year of life, the proportion of fish observed in each cohort should remain constant through time. The expected number of juveniles in each cohort during fall was estimated by dividing the number of larvae in each cohort by total larvae caught, and multiplying by the total number of juveniles in each fall sample. A log-likelihood ratio test (G-test) was used to compare observed and expected frequencies of juveniles in cohorts, with degrees of freedom equal the number of cohorts minus one. Relative survival of cohorts within a year, calculated as observed frequency divided by expected frequency of juveniles, were compared to evaluate relative contributions of early, middle, and late cohorts of larvae to recruitment. Relative survival values of 1.0 indicated that juveniles from a cohort occurred in the same proportion as the number of larvae in that cohort, whereas values greater or less than one indicated relative survival different than expected. Shifts in distributions of juveniles in summer and fall relative to larvae provided additional

information about timing of shifts (pre- or post-summer sampling) in recruitment patterns.

Sampling bias may explain differences in distributions of hatching dates for larvae captured in drift nets in summer and juveniles captured with seines in the fall. In order for Colorado squawfish larvae collected in drift nets nearshore at dawn to accurately represent the true distributions of hatching dates, they need only represent the true relative abundance of larvae throughout the season, such that larvae from additional samples collected at other times of the day or other positions in the river would change only the relative heights of modes in frequency distributions. I evaluated this by comparing the number and dates of modes and the temporal extent of cohorts in a distribution derived from larvae in dawn nearshore samples with those from a distribution that included larvae captured during diel and cross-channel sampling (an additional 617 larvae captured on 11 different sampling dates) for the middle Green River, 1992. The resulting relative survival values for cohorts for each of the distributions were also compared. Before this was done, however, it was necessary to increase the number of cohort 1 larvae, for which there were no cross-channel and few diel samples, in same proportion that number of larvae in cohorts 2 and 3 were increased by addition of diel and cross-channel samples. This was accomplished by dividing the number of larvae in dawn nearshore samples in cohorts 2 and 3 (n = 54) into total larvae collected at all times and positions for those same cohorts (n = 671). The resulting factor (12.43) was then multiplied by the number of larvae in cohort 1 dawn nearshore samples. This adjustment allowed equitable comparisons of relative survival values of cohorts calculated from the

distribution of larvae sampled in dawn nearshore nets only and those calculated from the distribution composed of larvae sampled at all times and positions.

Diel and cross-channel abundance of larvae.--Mean abundance of Colorado squawfish larvae in samples collected during diel and cross-channel sampling was analyzed by a general linear model (PROC GENMOD, SAS Institute, Inc. 1993), which calculated maximum likelihood estimates of model parameters. The discrete nature of the data (counts of larvae) and high occurrence of zeros or low capture values suggested a Poisson model. Log transformation of the response variable (i.e., log-link) ensured that the mean number of larvae predicted by the fitted model was positive (SAS Institute, Inc. 1993). The independent variables were sampling date, net position, time, turbidity, and their interactions. Sample dates were classed as turbid or not turbid. The natural logarithm of the volume of water filtered by each net (m^3) was also included as a covariate (offset) in models to account for differences. Volume of water filtered by each net was estimated by multiplying sampling time by flow rate, and by area of the net frame. Model selection was by Akaike's Information Criterion (AIC; Akaike 1981) adjusted for over dispersed data (QAIC; Anderson et al. 1994) with final model goodness-of-fit estimated by the scaled deviance. Abundance of Colorado squawfish larvae in dawn nearshore samples and in samples collected at other times and positions was compared by inspecting the mean and SE of catch rates.

Dawn nearshore samples may detect only a subset of the age- or length-classes of larvae drifting at other times or locations across the channel. Therefore, differences in age (d) and TL (mm) of Colorado squawfish captured in the 1992 diel and cross-channel sampling were analyzed by least-squares GLM (SAS Proc GLM) which had sampling

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time, net position, and their interaction as covariates. Differences in age and TL of larvae on 30 June, and 4, 13, 15, 24, and 27 July when large numbers were captured were evaluated by analysis of variance (ANOVA). Age (d) and TL (mm) response variables were normally distributed. Means and SE's of ages and lengths of larvae in these analyses were also inspected to determine if differences were biologically important.

Growth rates.—Growth rates (mm d^{-1}) of juvenile Colorado squawfish were calculated by subtracting 5.5 mm (mean TL at hatch; Bestgen and Williams 1994) from specimen TL and dividing by age (d) which was estimated from otoliths. The mean and distribution of growth rates of juveniles within a cohort were compared between summer and fall samples to evaluate whether size-selective mortality was occurring between the two sampling dates.

Individual-based model simulations.—The original research thrust was to relate recruitment patterns of age-0 Colorado squawfish to discharge and temperature regimes. Other concurrent research suggested that predation by nonnative fish on larvae, interacting with water temperature and food abundance, affected squawfish growth and survival (Bestgen 1996). Therefore, an individual-based simulation model (IBM) independently developed by Bestgen et al. (1996) was used to explore these relationships and evaluate demographic consequences of size-selective predation patterns revealed by otolith data. This model is described in detail here.

The IBM simulated the growth and survival of a cohort of Colorado squawfish larvae that colonized a backwater with red shiner *Cyprinella lutrensis* predators after drifting downstream from a spawning area. During each day of the simulation, individuals in the cohort were encountered and attacked and captured at rates determined

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by experimentally derived equations that had size and behavior of red shiner predators, size of squawfish larvae, and simulation-specific environmental conditions as predictor variables. Survivors grew each day according to another experimentally determined relationship which had temperature as the predictor variable. Red shiners were used as predators because they co-occur with Colorado squawfish larvae in backwaters of the Green River (Haines and Tyus 1990, Tyus and Haines 1991), and are known to consume fish larvae (Ruppert et al. 1993).

Simulations started on 30 June, a typical date for first appearance of Colorado squawfish in backwaters in most years (KRB, unpublished data), and ended on 30 September, after which recruitment to the juvenile life stage was assumed. The end date was consistent with fall ISMP sampling in late September, which allowed comparison of IBM simulated data to field data. For each simulation, individual larvae of 9 mm TL in a cohort (here N = 10,000) were assigned a baseline growth rate drawn at random from a normal distribution with a mean of either 0.3 (SD = 0.0525), 0.4 (0.07), or 0.5 mm (0.0875) TL/d. Standard deviations of distributions were proportional to the means. These growth rates approximate slow, moderate, and fast-growing juveniles in the wild, and the SD was consistent with that for moderate growth individuals (this study).

Encounter and attack rate (hereafter attack rate) of 38-72 mm TL red shiners on 10-20 mm TL squawfish larvae were predicted from data collected in mesocosm (wading pool) experiments (Bestgen et al. 1996). Prey TL and indicator variables water turbidity, alternative prey, and predator feeding strategy were covariates in a QAIC-selected Poisson GLM of form:

$$Y = -4.75 + 0.147 \cdot x_1 + 0.909 \cdot x_2 + 1.157 \cdot x_3$$
$$- 0.439 \cdot Fs_1 + 0.1036 \cdot Fs_2 - 4.45 \cdot Fs_3 - 1.88 \cdot Fs_4$$

where: Y = log$_e$attack rate·larva^{-1}·6 h^{-1}

x_1 = prey TL,

x_2 = 1 if water is clear; 0 if water is turbid,

x_3 = 1 if alternative prey is absent; 0 if present,

Fs_1 = 1 if simulating the feeding strategy of predator group 1; else = 0,

Fs_2 = 1 if simulating the feeding strategy of predator group 2; else = 0,

Fs_3 = 1 if simulating the feeding strategy of predator group 3; else = 0,

Fs_4 = 1 if simulating the feeding strategy of predator group 4; else = 0.

Standard errors for equation variables are: intercept = 0.557, x_1 = 0.0294, x_2 = 0.266, x_3 = 0.331, Fs_1 = 0.272, Fs_2 = 0.239, Fs_3 = 1.224, and Fs_4 = 0.360.

Groups of red shiners in pools, which remained together throughout trials, exhibited very different predatory behavior. We viewed these feeding strategies (Fs) as representative of differences in predation behavior of groups of wild red shiners. Therefore, feeding strategies were assigned to individual larvae with equal probability by random draws. The estimated attack rates (e^Y) were multiplied by pool area to scale them to a per m^2 basis, by 2.33 to account for predation over a 14 hr rather than a 6-hr period per d (14hr/6hr = 2.33), by predator density (individuals/m^2), and then rounded to the nearest integer. Turbid water reduced attack rate and absence of alternative prey increased it, so those conditions were used in order to yield a moderate attack rate.

When an attack occurred, probability of capture (Pcap) of 9-20-mm TL Colorado squawfish larvae by 35-70-mm TL red shiners was predicted by:

$$\text{logit} Y = -2.2621 - 0.0136 \cdot x_1^2 + 0.0036 \, (x_1 \cdot x_2)$$

where: $Y = \text{Pcap}$

$x_1 = \text{prey TL}$,

$x_2 = \text{predator TL}$.

The logit back-transformation of Y to $\text{Pcap} = e^{\text{logit} Y} \cdot (1 + e^{\text{logit} Y})^{-1}$. Standard errors for equation variables are: intercept $= 0.294$, $x_1^2 = 0.0019$, and $x_1 \cdot x_2 = 0.0007$. The mean and standard deviation of the daily size distribution of predators were interpolated from three samples of red shiners collected from the middle Green River in spring, summer, and early fall 1994. For each attack, a larva was assigned a predator from a TL distribution, which was entered into the probability of capture equation to yield Pcap. Probability of survival on a given day (P_s) was the product of $(1 - \text{Pcap})$ over all attacks. If the number randomly drawn between 0 and 1 that was assigned to a larva was $< P_s$ it was deemed captured. If that larva was assigned a random number that was $\geq P_s$ it survived and continued in the simulation.

Growth rates of larvae under maximum laboratory food rations, which were conservative for Colorado squawfish in the wild (this study), were predicted from daily water temperatures based on an equation in Bestgen (1996). Temperatures were obtained from the middle Green River gauging station at Jensen, Utah in 1994, a year with above-

average water temperature. Red shiner density was set at 3 individuals/m², which is a moderate density based on Haines and Tyus (1990).

Cohort survival, the initial distribution of growth rates, and the distribution for survivors was recorded to determine the magnitude of size-selective mortality induced by red shiner predation in moderate-growth Colorado squawfish larvae. Distributions were calculated from results of five simulations.

Fall recruitment estimates.--The specific goal of this analysis was to evaluate whether regulating summer discharge in the Green River to maximize backwater habitat was indeed beneficial to Colorado squawfish recruitment. This is important because the summer period is a time when releases from Flaming Gorge Dam can significantly affect discharge in the Green River. Mean July-August discharge was used as a predictor variable because that time period generally encompassed the reproductive season of Colorado squawfish and thus, should be the time when recruitment-defining processes are taking place. During periods of higher discharge such as in spring, releases from Flaming Gorge dam, even at full powerplant capacity, are small relative to the Yampa River and would have little effect. Also, unlike the discharge-backwater habitat relationship that has been established for the summer period (Pucherelli et al. 1990), the potential controlling mechanism(s) for a discharge predictor variable in other time periods such as spring is unknown, which would limit interpretations of relationships derived from such an analysis.

Fall abundance of Colorado squawfish measured during 1979-1995 in the lower Green River and middle Green River reaches was regressed as a function of mean July-August discharge for their respective reaches. The goal of this simple linear regression analysis was to compare results with past analyses which were based on data from 1979-1988 (Tyus and Haines 1991). Linear plateau (e.g., segmented) models of Colorado squawfish abundance as a function of mean July-August discharge was estimated with least-squares regression (SAS PROC NLIN) for each of the lower and middle Green River reaches. Assuming that the plateau segment of the model had x values less than the threshold, the model had the general form:

$$y = \beta_0 \text{ for } x \leq x_0 \text{ and}$$

$$y = \beta_0 + \beta_1(x-x_0) \text{ for } x > x_0$$

where x represented discharge level and x_0 was the threshold discharge. A multivariate secant method was used to iteratively fit a line with zero slope through the data that composed the plateau and simultaneously fit another nonzero slope line through the data that exceeded the threshold. The intersection of the two lines, or the join-point, and its confidence interval, was of particular interest because it represented the discharge above which predicted abundance of Colorado squawfish decreased with increased discharge. Intercept, slope, plateau, and the join-point values for initial model iterations were estimated from the linear regressions and plots of the data. Significance and fit of plateau models were evaluated by F-statistics and R^2 values, respectively, calculated from the appropriate sums of squares and degrees of freedom.

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Results

Distributions of hatching dates.--In the middle and lower Green River in 1991,

and in the middle Green River, 1992, larvae captured in drift nets began to hatch between

18 to 22 June (Figs. 3-5), but in the lower Green River, 1992, they hatched as early as 8

June. The vast majority of larvae captured in drift nets were 8-10 mm TL and 6-10 d old,

although larger and older larvae were occasionally caught later in the season.

Comparison of distributions of hatching dates for larvae captured in drift nets and

juveniles captured in summer seine samples in the middle Green River in both years

suggested that few or no Colorado squawfish hatched prior to initiation of drift net

sampling. In the lower Green River, distributions of hatching dates for juveniles caught

in summer seine samples suggested that a few larvae were hatched about a week prior to

beginning drift net sampling.

Distributions of hatching dates for larvae were significantly different from

juveniles captured in fall seine samples. In general, larvae that hatched in the latest

cohorts (3 or 4) had the highest relative survival (Table 1). The exception is in the middle

Green in 1991, where larvae from cohort 2 had the highest survival. The very high

relative survival value for cohort 4 in the lower Green River 1992 was the result of high

survival of the few larvae that hatched late. However, recruitment was very poor because

neither of the two main modes of larvae detected by drift-net sampling, nor the large early

peak of juveniles detected by summer seining were apparent in fall. Rarity of juveniles in

the fall sample from the lower Green River, 1992 was actually underestimated because

the distribution was a composite of eight fish from the regular site which was sampled

with standard effort, plus 28 more fish from seine-sampled backwaters from RK 0 - 211. In no case did larvae from cohort 1 predominate in distributions of hatching dates for juveniles captured in the fall, even when early larvae were exceptionally abundant (e.g., middle Green River 1991).

Sampling bias evaluation showed that adding hatching dates of additional larvae captured during all times and at all positions in the middle Green River, 1992, to the distribution of hatching dates comprised only of larvae captured at dawn nearshore, did not substantially alter the number or date of modes in cohorts 2 and 3. The temporal extent of cohort 2 was increased by a single day because a larva was captured on 13 July. The mode for cohort 3 after addition of larvae shifted from 22 July to 18 July, but the range of dates was unchanged. The rank order of relative survival values for cohorts 1 (relative survival = 0.4), 2 (2.5) and 3 (3.9) was also unchanged by addition of larvae from diel and cross-channel samples compared to those for dawn nearshore samples only (Fig. 5).

Diel and cross-channel patterns of abundance of larvae.--The GLM analysis indicated that day and time variables accounted for most of the variation in mean number of larvae captured (Table 2). However, time·position, and all interactions that included turbidity were also significant in this QAIC-selected model. Position was not a significant main effect ($P = 0.34$), but was included in interactions and all variation due to turbidity was accounted for by interactions so a main effect was not estimated. Higher-order GLM models that included at least one combination of day·position, time·day, and time·position·day variables did not converge because no larvae were captured on some days, times, or positions.

Abundance of Colorado squawfish larvae in dawn nearshore samples (4.2 fish/1000 m³, SD = 4.61, n = 42) was about half that in samples collected at other times and positions (8.7 fish/1000 m³, SD = 12.40, n = 372). In general, mean abundance of Colorado squawfish larvae was moderately high at dawn, highest at noon, and low at dusk and midnight (Fig. 7). Mean abundance of Colorado squawfish larvae in turbid samples (10.0 fish/1000 m³, SD = 29.3, n = 156) was nearly twice that of samples collected in clear water (6.6 fish/1000 m³, SD = 22.1, n = 258) regardless of sampling time or position and was highest at noon in mid-channel surface samples. Highest abundance of Colorado squawfish larvae in clear samples was at noon in mid-channel bottom samples. Mean abundance of Colorado squawfish larvae was highly variable in samples collected at all times and in all channel positions.

Slight over dispersion of the GLM was suggested by a deviance/degrees of freedom value of 1.33; values < 1 indicate under dispersion while values = 1 indicate the data do not contain extra-Poisson variation. Standard errors of model parameter estimates were multiplied by the square root the the deviance (1.153) to correct for over dispersion. The scaled Pearson X^2 for the GLM model indicated slight lack of fit (436.6, 377 df, P = 0.023) which was due mostly to data collected in clear (P = 0.033) rather than turbid (P = 0.13) water conditions.

Slight lack of fit of the data to the GLM model was caused, in part, by the three non-estimable interaction terms. An exploratory GLM model using least squares estimation of model parameters and $\log_e$ transformed (catch + 1) data suggested that all parameters except position and their interactions, including ones not estimated by the maximum likelihood GLM, explained a statistically significant amount of the variation in

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abundance of larvae. This result is not surprising given the number of samples and consequently high power to detect relatively small effects. The GLM analysis corroborated the rank order and magnitude of F-values for time and sampling date produced by the maximum likelihood analysis. The exploratory model also verified the statistically significant, but relatively minor contribution of unestimated interactions.

Position, time, and date effects on age and TL of larvae captured.--Mean ages of Colorado squawfish larvae captured in nearshore, mid-channel surface, and mid-channel bottom nets were within 0.3 d of each other (Table 3). Likewise, mean ages of larvae captured at dawn, noon, dusk, and midnight were similar. These slight differences were not biologically significant. Large samples sizes and high power to detect small differences caused statistically significant differences in age of larvae among drift net positions ($P = 0.01$) and sampling times ($P < 0.0001$) in 1992 middle Green River samples; the position·time interaction was not significant ($P = 0.12$).

An identical analysis with TL as the response variable suggested that Colorado squawfish captured in nearshore, mid-channel surface, and mid-channel bottom nets were of similar size (Table 4) as were mean lengths of larvae captured at dawn, noon, dusk, and midnight. These small differences observed were also not considered biologically significant even though statistically significant effects of position ($P = 0.033$) and time ($P < 0.0001$) were detected; the position·time interaction was not significant ($P = 0.65$).

The ANOVA detected a significant effect of sampling date on age ($P < 0.0001$) and TL ($P < 0.0001$; Table 3). However, inspection of the means and SE's suggested that the only biologically important differences were on the 13 July sample date, when larvae averaged more than a day younger and were nearly 1 mm shorter than in other

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samples. However, that small sample was collected after a thunderstorm when turbidity was very high.

Growth rates.--Within given cohorts, mean growth rates of juvenile Colorado squawfish that survived to fall were generally higher than mean growth of fish sampled in summer. For example, in the middle Green River in 1991, mean growth rates of fish in cohorts 1 and 2 that were sampled in fall were 17.7% and 8.6% greater, respectively, than mean growth rates of fish in the same cohorts sampled in summer (Fig. 8). Mean growth of the cohort 3 fall juveniles, for which there were no summer juveniles for comparison, was the lowest of any cohort.

In the lower Green River in 1991, growth rates of fish in cohorts 1 and 2 were similar between summer and fall samples (Fig. 9). Mean growth rate of fish in cohort 3 sampled in fall was the lowest of the three cohorts. Mean growth rate was especially low (0.328 mm TL/d, n = 7) for individuals that hatched during 31 July - 11 August, coincident with a discharge increase from 76.4 m^3 to 88.3 m^3/sec in the lower Green River which began on 30 July and continued until about 12 August.

In the middle Green River in 1992, mean growth rate of fish in cohort 1 sampled in fall averaged 14.5 % faster than fish in cohort 1 sampled in summer (Fig. 10); growth rates of cohort 2 fish sampled in summer and fall were similar although the former sample was small. Growth rate of cohort 3 sampled in fall was the lowest observed in the middle Green River that year.

Juvenile Colorado squawfish from the summer sample in the lower Green River, 1992, which recruited poorly, had the lowest growth rates observed in the study (Fig. 11). Mean growth rate of fish in cohort 1 that were sampled in fall (n = 2) was 47 % higher

than that of fish in the relatively large (n = 81) summer sample of cohort 1. Mean growth rate of cohort 2 fish sampled in fall was 73 % faster than fish in the corresponding summer cohort. Differences in mean growth rates of fish in the small summer and fall samples for cohort 3 were similar to that for cohorts 1 and 2. Mean growth rate of juvenile squawfish in cohort 4 fish sampled in fall, for which there was no corresponding summer sample, was lowest of all fall cohorts. Hatching dates for the first three cohorts were before an extended period of high discharge due to precipitation in the lower Green River (53.5 - 100.8 m^3/sec), which began on 9 July and did not recede until early August. Most individuals that survived to fall from cohort 4 hatched after discharge subsided (Fig. 6).

Overall, distributions of growth rates for cohorts of Colorado squawfish sampled in summer and again in fall suggested that survivors of early cohorts were usually the fast-growing individuals. In contrast, mean growth rates of later cohorts were lower.

IBM simulations.-- Mean survival of larvae in IBM simulations that had slow (0.3 mm TL/d), moderate (0.4 mm TL/d), and fast (0.5 mm TL/d) mean growth rates was 3.9% (SD = 0.0021), 9.3% (0.0028), and 14.7% (0.0025), respectively. Mean apparent growth rate of Colorado squawfish subjected to predators was 7.2% greater than those in simulations without predators (Fig. 12) which suggested that size-selective predation produces differences in apparent growth rates among recruits similar to those observed based on the otolith data.

Fall recruitment estimates.--Fall abundance of Colorado squawfish juveniles measured during 1979-1995 declined as a function of mean July-August discharge in the lower Green River (log_e *fish abundance* = 3.219 - 0.00597·*discharge*, r^2 = 0.22, *P* = 0.06)

and middle Green River (log_e fish abundance = 2.580 - 0.0113·discharge, r^2 = 0.28, P = 0.03), but relationships were highly variable and in one case only marginally statistically significant. Eliminating 1983 and 1984 data from the analyses, years which may be artifacts of abnormal dam releases, resulted in regression relationships that were not significant (lower Green River log_e fish abundance = 2.939 - 0.00250·discharge; P = 0.63, r^2 = 0.02; middle Green River log_e fish abundance = 1.366 + 0.00572·discharge; P = 0.50, r^2 = 0.04).

Slopes of regressions without 1983 and 1984 data that were near zero justified use of linear plateau-regression to model the relationship of juvenile squawfish abundance as a function of mean July-August discharge. The plateau model for the lower Green River was statistically significant (Fig. 13, P < 0.0001). The join-point discharge estimate was 404 m^3/sec, a value that approaches historical discharge maxima. The plateau model for the middle Green River was also significant (P = 0.0003) and the join-point estimate was 140 m^3/sec. This more reasonable value is likely a reflection of the linear decline in abundance of Colorado squawfish at higher discharge levels rather than the inflated join-point estimate for the lower Green River which had a more scattered distribution of data at higher discharges.

Discussion

Results of otolith studies and the independently developed simulation model are consistent with the hypothesis that predation by non-native fishes has a substantial negative effect on recruitment of Colorado squawfish larvae in the Green River. Distributions of hatching dates showed that the few early-hatched fish that survived were

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among the fastest growing individuals in the cohort. Conversely, individuals that hatched later survived at higher rates but grew relatively slowly. Large, predaceous red shiners that are present in the Green River only during the early part of the reproductive season of Colorado squawfish but absent later was a likely explanation for differential survival and patterns of growth of fish in cohorts.

Physical factors affected recruitment primarily through interactions with biotic factors. This was apparent in the lower Green River in 1992, when increased discharge from thunderstorms was the likely mechanism for reduced growth of juvenile Colorado squawfish, but was not a likely cause of mortality. Instead, growth reductions extended the time that Colorado squawfish were susceptible to predators and that interaction was the likely cause for mortality of Colorado squawfish and low recruitment. The low discharge and relatively high water temperatures present in the lower Green River in 1991 and the middle Green River in 1991 and 1992 should have promoted good growth and high survival of Colorado squawfish. Instead, recruitment was only average in the middle Green River 1991 and below average in other places and years. The importance of biotic rather than physical factors in determining recruitment was further supported by results of linear-plateau regression models, which suggested that there was no relationship between abundance of Colorado squawfish in the fall and mean July-August discharge until relatively high discharge. I will interpret these findings in light of existing theory and past studies, evaluate several alternative hypotheses for distributions of hatching dates, sampling, and growth rates and discuss the implications of these findings for management of the Green River system for Colorado squawfish.

Annual abundance of recruits of early life stages of fishes and invertebrates may depend on timing of reproduction by adults and the abundance of larvae produced, and the interacting effects of food and habitat availability and predator abundance (Cushing 1975, Frank and Leggett 1982, Roughgarden et al. 1988, Cushing 1995). In the few studies conducted on freshwater fishes, hatching date can also affect the differential survival of cohorts of larvae produced within a season. For instance, the relatively few early-hatched larvae of bluegill *Lepomis macrochirus* survived better over winter than abundant, late-hatched larvae that were equally as fast growing because they had more days to grow (Cargnelli and Gross 1996). In contrast, poor early survival of bloater *Coregonus hoyi* eggs was likely a function of low water temperature which extended their development time and susceptibility to predation (Rice et al 1987, Luecke et al. 1990). Similarly, poor recruitment of American shad *Alosa sapidissima* produced early in the year in each of the Connecticut (Crecco and Savoy 1985) and Hudson (Limburg 1996) rivers was associated with high discharge, cold water temperature, and poor growth of larvae.

The common pattern that emerged from studies where success of cohorts was followed from hatching to recruitment, was that relatively successful recruitment of larvae was linked to hatching times or environmental conditions that promoted fast development of eggs and growth of larvae. Fast growth is also important for survival of Colorado squawfish larvae that hatch early in order to avoid predation by red shiners. However, the abundance dynamics of large and predaceous red shiners may be more important than environmental factors in regulating recruitment of Colorado squawfish. This is true because cohorts of Colorado squawfish that contribute most heavily to

recruitment are relatively slow-growing and hatch relatively late in the year after large red shiners die. These conclusions will be discussed below.

Distributions of hatching dates.--Several alternative hypotheses may also explain conclusions about recruitment patterns based on distributions of hatching dates. First, if relative abundance of larvae late in the season was underestimated by sampling, late-hatching cohorts of Colorado squawfish larvae would be expected to have high relative survival similar to that which was observed. However, sampling bias was not a likely hypothesis because differences in the temporal extent of cohorts, dates of cohort modes, and the rank order of relative survival values for cohorts after adding hatching dates of additional larvae to distributions for dawn nearshore samples did not modify the conclusion that late-hatched larvae survived at higher rates than ones hatched earlier.

A second alternative hypothesis is that distributions of hatching dates and relative survival values may be biased if drift-net sampling did not encompass the time that Colorado squawfish larvae were transported downstream. Absence of Colorado squawfish larvae in drift net samples for several days after sampling began, and absence of fall juveniles captured with birth dates earlier than those indicated by drift-net sampling of larvae, supported the claim that drift-net sampling began before significant transport of Colorado squawfish larvae downstream from spawning areas. The presence of juveniles in summer samples that had hatch dates prior to earliest ones for larvae (e.g., lower Green River, 1992) did not affect conclusions because by fall it was clear that early hatched fish had poor survival. Sampling that ended too soon in the season may also have biased results. However, additional intermittent sampling for Colorado squawfish larvae in the middle Green River in 1993 for up to 4 wk, and extended 1991 and 1992

sampling of the lower Green River after the traditional 4-6 wk sampling period (KRB, unpublished data), did not yield additional larvae, which suggested that the sampling season encompassed the spawning and larval transport period.

A third alternative hypothesis is that distributions of hatching dates and relative survival values may be biased by the method used to calculate expected abundance of juveniles. The expected abundance of juveniles in cohorts, which is based on relative abundance of larvae in cohorts, was calculated with the assumption that larvae in cohorts endured an identical rate of mortality until fall recruitment. This may not be true because early hatching larvae may be exposed to mortality factors for a longer time, and thus survive at lower rates than their late-hatching counterparts. Re-calculation of relative survival rates based on the assumption that Colorado squawfish instead suffered an arbitrary 5% mortality per day beginning on the median date of the cohort did not change the conclusion that later cohorts had higher survival than earlier ones (Table 5). Abundance of larvae and expected numbers of juveniles in early-hatched cohorts were reduced in comparison to the scenario where all cohorts of larvae suffered identical mortality rates, because mortality occurred over more days relative to that for cohorts hatched later. However, rank order of relative survival rates re-calculated in this manner was identical in all but one case for each of the middle and lower Green River areas in 1991 and 1992.

Diel and cross-channel patterns of abundance of larvae. -- Previous unpublished research showed that Colorado squawfish larvae were most abundant in dawn drift net samples and were distributed equally across the channel (T. Nesler, Colorado Division of Wildlife, pers. comm.). In contrast, in 1992 mean abundance in dawn nearshore samples

was only about half that for other times and positions. Diel and spatial abundance patterns may differ by year, depending on timing of emergence of larvae, and the frequency of turbidity events that are associated with increased catch rates of Colorado squawfish larvae. Different annual discharge levels will also affect rates of transport of larvae from spawning areas to fixed sampling stations because discharge and water velocity are positively correlated. However, sampling date was the single largest source of variation in the GLM model that predicted mean abundance of Colorado squawfish larvae. Thus, the daily sampling conducted during 1991 and 1992 made it unlikely that any concentrations of larvae were missed as they were transported downstream.

Growth rates.--Growth rates of juveniles in cohorts 1 and 2 in 1991 and 1992 for which samples sizes were adequate for comparisons suggested that fall survivors averaged 9-73% faster growth than the average fish in the corresponding summer cohort. A potential explanation for this pattern is size-dependent predation mortality from a gape-limited predator. Under this hypothesis, larvae that are slow growing should be susceptible to predators for a longer period of time than fast growing fish, so only the fastest growing fish in a cohort survive (Houde 1987, Miller et al. 1988). Growth rate differences among respective summer and fall cohorts were likely underestimates of maximum differences because summer juveniles were exposed only to warm water that would result in the highest mean growth rates (Bestgen 1996). In contrast, fall juveniles were exposed to both warm summer and relatively cooler fall water temperatures (Fig. 2), which likely resulted in lower mean growth rates.

The general pattern of higher mean growth rates for the few survivors remaining by fall in cohorts 1 and 2 compared to fish in those same cohorts sampled in summer

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could also be explained by increased growth rates of juveniles later in the summer. However, this is unlikely because growth rates of cohorts of juveniles hatched progressively later in the season showed an opposite pattern of reduced growth.

Two explanations can be offered for relatively low growth rates of juveniles in cohorts 3 and 4 (the latter only in lower Green River 1992) in fall 1991 and 1992, which survived relatively well, compared to higher growth rates for early and middle cohorts, which survived poorly. First, fish hatched late spent a larger proportion of their life in the slower growth fall period than fish in earlier cohorts, so their average growth rate should have been less. A second reason may be related to the size structure and population dynamics of predators. Length frequency histograms in Farringer et al. (1979) suggested that adult red shiners typically die after spawning in their third summer, which results in a decrease in density of adults and a shift in the size distribution that reflects loss of the largest predators by mid-July to mid-August. This pattern was evident in the Green River in summer 1994 (KRB, unpublished data), when maximum TL of red shiners on 28 June was 72 mm (median TL = 57 mm), but by 26 July had declined to 54 mm (median TL = 43 mm). The same pattern was found in the San Juan River at four New Mexico and Utah sites during 1994 and 1995 (Gido et al. in press, D. L. Propst, New Mexico Department of Game and Fish, pers. comm.). Therefore, reduced predation should result in lower mean apparent growth rates and higher survival for later Colorado squawfish cohorts because the effects of size-selective predation were reduced. Cooler fall water temperatures also likely reduced consumption by predators.

Under-representation of early-hatched larvae in distributions of hatching dates for juveniles sampled in the fall, and the generally faster growth rates of the few early-

hatched survivors, was probably not due to age underestimation that is typical for larger

and older fish (Campana and Neilson 1985). Slopes of regression equations of estimated

age (increment counts) as a function of true age for squawfish larvae reared in the

laboratory for up to 165-d in fluctuating temperatures were not significantly different

from the null hypothesis value of 1, and confidence intervals were small such that

relatively small deviations (e.g., 0.05) from the slope of 1 would be detected with high

probability (Bestgen and Bundy submitted). Moreover, increments in otoliths of wild

fish were much clearer and probably counted more precisely than those from fish reared

in the lab (Bestgen and Bundy submitted). For example, mean within-otolith coefficients

of variation (CV) for ages estimated from increment counts for larvae ≤ 25 d old (13.1%)

and juveniles > 25 d old (9.5%) reared in the lab in fluctuating temperature treatments

were generally higher than for larvae from the middle Green River in 1991 (9.5%) and

1992 (8.4%) and for juveniles from the middle Green River in summer (3.4%) and fall

(3.6%) 1991 and in summer (3.6%) and fall (4.1%) 1992. Otolith increments were

similarly clear for squawfish in the lower Green River. This suggested that relationships

between estimated and true age determined for laboratory-reared fish were representative

for wild fish. Also, confidence intervals about the estimated age of laboratory-reared fish

probably overestimated variation in ages of wild fish determined from counting otolith

increments. Thus, it is unlikely that consistent bias would be encountered when aging

larvae or juveniles caught in the field.

Even though aging bias was unlikely, I assessed robustness of results that

incorporated otolith aging data by assuming that ages of fall-caught juvenile Colorado

squawfish were underestimated by 8 d. I also made the unlikely assumption that ages of

larvae were estimated without error, which increases the likelihood that this test will fail. The 8-d bias value was suggested by inverse 95 % prediction intervals that averaged ± 8 d for the same regression of estimated age as a function of true age described above for laboratory-reared fish. Similar to results presented earlier for the lower Green River 1991 and 1992, and the middle Green River 1992, G-tests showed that distributions of hatching dates for larvae and fall-caught juveniles were significantly different, and distributions and relative survival values indicated higher survival for later rather than earlier cohorts of fish. In the middle Green River 1991, relative survival values were highest for the early rather than the middle cohort. Given the unlikely scenario that ages (and growth rates) of all fish in the middle Green River summer 1991 sample were estimated without error, and that ages of all fish in the corresponding fall sample were underestimated by 8 d, mean growth of fall juveniles (0.43 mm TL/d) would still be a biologically significant 7.5% faster (e.g., Fig. 11) than summer juveniles. Thus, results and inferences based on estimating number of otolith increments appear robust even in the face of considerable aging bias.

IBM simulations.--The IBM simulations suggested that growth reductions of Colorado squawfish larvae of the magnitude simulated were biologically significant because larvae with moderate growth rates were more than twice as likely to survive as slow-growing ones. Higher mean growth rates of survivors exposed to predators in simulations corroborated the hypothesis that size-selective patterns of mortality observed from otolith data were due to predation. It is unlikely that predation by red shiners was solely responsible for patterns or that size-selective mortality factors ceased in fall. However, evidence of predation by red shiners on Colorado squawfish larvae in the

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laboratory (KRB, unpublished experimental results) and field (Ruppert et al. 1993), and

their abundance in backwaters (Haines and Tyus 1990), suggested that predation by this

species may be a major contributor to recruitment variation of Colorado squawfish in the

Green River system. The strong influence that size-selective processes play on

recruitment underscores the importance of understanding the effects of temperature,

discharge pattern, and non-native fishes on growth rates of Colorado squawfish.

Evidence of size-selective predation mortality from otolith studies conducted on

fish captured in 1991 and 1992 may be responsible, in part, for low recruitment levels of

Colorado squawfish in backwaters documented by river-wide ISMP sampling. In the

lower Green River, density of Colorado squawfish juveniles was 6.1 individuals/100 m^2

in 1991 and 2.9/100 m^2 in 1992 which was well below the 17-yr mean density for that

reach (22.7 fish /100 m^2, SE = 6.37). Recruitment of Colorado squawfish juveniles in the

middle Green River was about average in 1991 (10.4 fish /100 m^2) and low (4.3 fish/100

m^2) in 1992 compared to the 17-yr mean density (8.7 fish /100 m^2, SE = 1.90).

Additional research that showed lack of size-selective processes in high recruitment years

would be consistent with the hypothesis that relatively low recruitment in 1991 and 1992

was a result of size-selective predation.

Alternative recruitment hypotheses.--Recruitment patterns of early life stages of

Colorado squawfish in the Green River system revealed by otolith studies and IBM

simulations cannot be explained by other recruitment hypotheses such as starvation,

cannibalism, or competition. Although starvation is primarily a function of body size for

fish larvae (Miller et al. 1988) and Colorado squawfish larvae are only moderate-sized

(5.5 mm TL), 57% of laboratory-reared larvae starved for 17.5 d recovered and survived

when offered food (Bestgen 1996). Predicted time to 50% mortality for a cohort of starved larvae that were 5.5 mm TL at hatch was 15.5 d (equation in Miller et al. 1988), which supported the notion that Colorado squawfish larvae have higher than average resistance to starvation. Thus, this mode of population regulation may be important only during high flow years when larvae likely expend more energy negotiating high-velocity main channels for long periods of time, and are transported farther downstream due to lack of low-velocity habitat.

Cannibalism on age-0 fish may be consistent with size-selective predation, but is unlikely due to low abundance of age 1+ Colorado squawfish in backwaters (unpublished ISMP data). Competition for food between early life stages of Colorado squawfish and other fishes may reduce growth and increase vulnerability to predation for longer time periods. This would only intensify the size-selective pattern of mortality but could not be the sole mechanism for it except in the unlikely case of direct competition-induced mortality.

Flooding, a stochastic physical process, was associated with reduced growth and low recruitment of Colorado squawfish juveniles in the lower Green River in 1992. At discharge levels above 80 m³/s, which is in the range observed in 1992, much of the available backwater habitat was inundated in that mostly canyon-constrained reach (KRB, pers. obs., fall 1991). In the middle Green River, discharge did not increase as much (Fig. 2), and when backwaters were inundated others tended to form because of the relatively broader bankfull channel. Therefore, reductions in habitat or food availability may be responsible for reduced growth of lower Green River fish in 1992. However, it is unlikely that mortality was a direct result of the modest increase in discharge because

Colorado squawfish recruited substantial populations of juveniles in the lower Green River in many other years when mean July-August discharge levels were much higher (Fig. 13). Instead, effects of reduced growth induced by flooding may be manifest by extending the period that early life stages of Colorado squawfish are vulnerable to predation. Similar growth reductions may be induced by hydropower generation. Daily discharge fluctuations also reduce the abundance of nearshore chironomid assemblages (Blinn et al. 1995), which are the primary items in the diets of most young fishes in the Green River (Muth and Snyder 1995). The potentially important relationships between discharge and food availability and fish growth are poorly understood and may be a fruitful avenue for future research.

High summer discharge during some years may transport larvae past nursery areas into unsuitable habitat in some years (Tyus and Haines 1991) resulting in low recruitment. This could not have occurred in 1991 and 1992 because discharge was relatively low throughout the summer. In fact, with the exception of the lower Green River in 1992, the abundance of backwaters available at low discharge (Pucherelli et al. 1990) and warm water temperatures throughout the Green River in summers 1991 and 1992 were ideal for growth and survival of Colorado squawfish (Bestgen 1996). This adds support to the hypothesis that some other factor such as size-selective predation mortality was responsible for the low or average recruitment in those years.

Fall recruitment estimates.--Comparison of simple linear regressions suggested that mean July-August discharge was not as important a predictor of year-class strength during the 17-yr period of record (1979-1995) as reported in a previous study (Tyus and Haines 1991), despite the considerable range of discharge and recruitment levels

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observed. Tyus and Haines (1991) observed strongly negative and significant relationships for the lower (log $fish$ $abundance$ = 4.730 - 0.024·$discharge$, r = 0.86) and middle (log $fish$ $abundance$ = 4.281 - 0.039·$discharge$, r = 0.80) Green River from 1979-1988 (not including 1986). Low recruitment was presumed to be the result of lack of backwater habitat at high discharge levels (Tyus and Haines 1991). The most significant factor causing differences in fall abundance-discharge relationships calculated for the two periods is that effects of the low recruitment associated with high flow events in 1983 (lower and middle Green River mean July-August discharge = 446.2 m³/sec and 319.7 m³/sec, respectively) and 1984 (lower and middle Green River mean July-August discharge = 288.6 m³/sec and 192.3 m³/sec, respectively) were reduced by including more data.

Plateau models suggested that there was no relationship between abundance of Colorado squawfish in the fall and mean July-August discharge until relatively high discharge. These models for the lower and middle Green River also suggest thresholds at which discharge, and presumably lack of backwater habitat, begin to negatively affect recruitment of Colorado squawfish. Management decisions for determining threshold discharge effects should rely more on the middle Green River relationship than that for the lower Green River for two reasons. First, the wide scatter of Colorado squawfish abundance data at higher discharge levels in the lower Green River probably artificially inflated the join-point estimate. It is difficult to imagine that biologically important effects would not occur at much lower discharge levels, especially in the canyon-bound lower Green River, because the threshold discharge indicated by the plateau model approaches historical maxima. Secondly, even if there was reason to suspect that

relatively high discharge levels were beneficial in the lower Green River, such a regime may be at the expenses of recruitment in the upstream middle Green River if it exceeds a critical level, a result that is clearly unacceptable.

Summary.--High intra-annual recruitment variation of Colorado squawfish in both the lower and middle Green River, 1991 and 1992, was a result of poor survival of larvae hatched early in the year and consistently higher survival of larvae hatched later. Evidence of size-selective patterns of growth supported the hypothesis that predation was responsible for low recruitment of cohorts hatched early in the year. Higher recruitment of cohorts of Colorado squawfish hatched later in the year may be a function of lower density and reduced size of older age-classes of red shiners later in the year. Results of an IBM were consistent with recruitment patterns and size-selective mechanisms revealed by otolith data and collectively suggested that low to average riverwide recruitment in 1991 and 1992 may have been due to size-selective predation. Reduced growth of Colorado squawfish concurrent with flooding may be the reason for poor recruitment in the lower Green River in 1992. Seasonal shifts in abundance of predators and the stochastic nature of floods underscores the importance of understanding causes of recruitment variation at intra-annual time scales.

Annual recruitment data may be more useful to elucidate long-term patterns in population status and effects of events that encompass entire reproductive seasons such as extended high discharge. Analyses of long-term annual recruitment data suggested fall abundance of juvenile Colorado squawfish was not well-predicted by mean July-August discharge. a result that was not obvious from past studies. The plateau regression model may better represent the biological reality of the relationship because recruitment was

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high in several years when discharge was also high. Existence of strong biotic and physical interactions that structure recruitment patterns of Colorado squawfish in the Green River illustrated the need for studies at a wide variety of temporal and spatial scales. This analysis also illustrated the necessity of long-term studies to understand recruitment of long-lived animals, like Colorado squawfish, in their natural environment.

Applications.--Recent management to enhance populations of Colorado squawfish in the Green River called for more natural discharge regimes to enhance backwater habitat. Implicit in this recommendation was that habitat and recruitment of Colorado squawfish was limited under the historic dam operation. Based on habitat-discharge relationships measured in a single year, a strategy of annually regulating minimum summer discharge at 51 m^3/sec ± 25% was adopted to maximize backwater habitat (Pucherelli et al. 1990) and recruitment of Colorado squawfish (Tyus and Haines 1991). Even though minimum discharge was not regulated every year due to high and extended summer discharge from tributaries such as the Yampa River, a constant discharge regime is unnatural and undesirable in most riverine ecosystems because of reduced hydrologic variability and potential disruption of linkages between biota and habitat (Power et al. 1988, Ward and Stanford 1989, Wootton et al. 1996). Haines and Tyus (1990) found that red shiner abundance was inversely correlated with summer discharge, which suggests that low discharge regimes may enhance red shiners. Because recruitment of Colorado squawfish was substantial over a wide range of July-August discharge levels and occurred when backwater habitat should have been limited (Pucherelli et al. 1990), and because regulated summer discharge levels were also coincident with some of the lowest

Colorado squawfish recruitment levels measured in the 1979-1995 period, the strategy of providing a single summer discharge regime should be re-evaluated.

Such an assessment would necessarily require a better understanding of dam-induced changes in discharge volume and variability, water temperature, sediment budgets, and effects of non-native vegetation in order to elucidate hydrology-habitat relationships. In particular, understanding the influence of annual, seasonal, and diel discharge patterns on backwater invertebrate communities upon which Colorado squawfish depend (Muth and Snyder 1995), may elucidate discharge management regimes that enhance their growth and survival. Discharge regimes that are not restricted to a single level in summer may also allow managers more operational flexibility in order to fulfill requirements of other endangered species in the Green River like razorback sucker *Xyrauchen texanus*.

Past management strategies de-emphasized importance of effects of introduced fishes because of research emphasis on native fishes, perceived lack of options to control non-native fish abundance, and the poorly understood effects of regulation of the Green River by Flaming Gorge Dam on native fishes. However, my evidence of potentially strong non-native fish effects, coupled with recent evidence that high discharge events may reduce abundance of non-native fishes (Haines and Tyus 1990, Stanford 1994, Gido et al. in press), suggests that hydrology-fish ecology research is deserving of more emphasis. A better understanding of the relative importance of mechanisms regulating recruitment of Colorado squawfish and other native biota may also show that present management emphasis on habitat is inappropriate and should be supplemented or replaced with strategies that reduce effects of non-native fishes.

Acknowledgments

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Table 1.--Mean TL (mm, range) and sampling date for Colorado squawfish juveniles captured in summer and fall from backwaters of the middle and lower Green River in 1991 and 1992. The G-test compared distributions of hatching dates of larvae captured in drift nets to fall juveniles captured with seines (Figs. 3-6) to evaluate differential mortality of cohorts. Relative survival values and the absolute abundance of fall juveniles in cohorts was used to determine which cohort contributed most to recruitment in that reach and year.

Location, year	Mean TL (range) Sample date		G-test, df, P-value	Predominant Cohort
	Summer juveniles	Fall juveniles		
Middle Green River, 1991	17.3 (14.4 - 20.8)	35.9 (17.6 - 53.4)	160.7, 2, <0.0001	2 of 3
	1, 2 August	20 Sept.		
Lower Green River, 1991	21.2 (12.9 - 27.0)	39.2 (22.0 - 65.0)	107.8, 2, <0.0001	3 of 3
	31 July	20 Sept.		
Middle Green River, 1992	20.7 (14.8 - 27.0)	43.2 (21.5 - 66.9)	313.2, 2, <0.0001	3 of 3
	29-31 July	25 Sept.		
Lower Green River, 1992	14.7 (9.0 - 29.9)	33.7 (20.0 - 59.0)	138.8, 3, <0.0001	4 of 4
	28-29 July	19-20 Sept.		

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Table 2.--Type III likelihood ratio F-statistics for significant effects in the Poisson

general linear model analysis of Colorado squawfish abundance in diel and cross-channel

samples collected from the Yampa River in summer 1992.

Effect	Numerator df	Denominator df	F	$P > F$
Time	3	377	41.15	0.0001
Day	13	377	39.46	0.0001
Time x position	6	377	5.07	0.0001
Position x turbidity	2	377	8.44	0.0003
Time x turbidity	3	377	4.11	0.0069
Time x position x turbidity	6	377	2.54	0.0200

Table 3.--Mean age (SE. n) and TL (SE. n) of Colorado squawfish larvae captured in drift net samples collected across the channel at different times in the Yampa River, 1992.

| Response | Position | | | Time | | | |
	Nearshore	Mid-channel surface	Mid-channel bottom	Dawn	Noon	Dusk	Midnight
Age (d)	6.4	6.3	6.1	6.5	6.1	6.3	6.5
	(0.047, 467)	(0.071, 218)	(0.043, 269)	(0.045, 426)	(0.042, 392)	(0.107, 84)	(0.203, 52)
TL (mm TL)	9.0	9.0	8.8	9.0	8.8	9.0	9.1
	(0.030, 463)	(0.032, 218)	(0.025, 267)	(0.026, 425)	(0.023, 387)	(0.060, 82)	(0.140, 54)

Table 4.--Mean age (SE, n) and TL (SE, n) of Colorado squawfish larvae captured in drift net samples collected over the sampling season in the Yampa River, 1992.

Response	Collection date					
	30 June	4 July	13 July	15 July	24 July	27 July
Age (d)	6.8	6.4	4.7	6.3	6.4	5.9
	(0.067, 106)	(0.093, 47)	(0.010, 49)	(0.060, 155)	(0.056, 141)	(0.040, 120)
TL (mm TL)	9.0	9.1	8.2	8.9	8.9	8.9
	(0.044, 106)	(0.060, 44)	(0.064, 49)	(0.029, 154)	(0.050, 140)	(0.034, 120)

Table 5.--Sensitivity analysis of relative survival values. Cohort relative survival values were recalculated using a daily 5% rate of mortality for larvae beginning on the median day of the cohort (see Figs. 3-6 for comparison).

Reach and Year	Cohort relative survival values			
	1	2	3	4
Middle Green River 1991	0.8	4.5	0.5	
Lower Green River 1991	0.6	0.7	2.6	
Middle Green River 1992	0.5	1.1	2.4	
Lower Green River 1992	2.0	0.5	0.1	12.5

Fig. 1. The Green River study area. Primary spawning areas for Colorado squawfish are

in the lower Yampa River in Yampa Canyon and in Gray Canyon downstream of

RK 251. Primary nursery habitat for age-0 squawfish was the middle (RK 515-

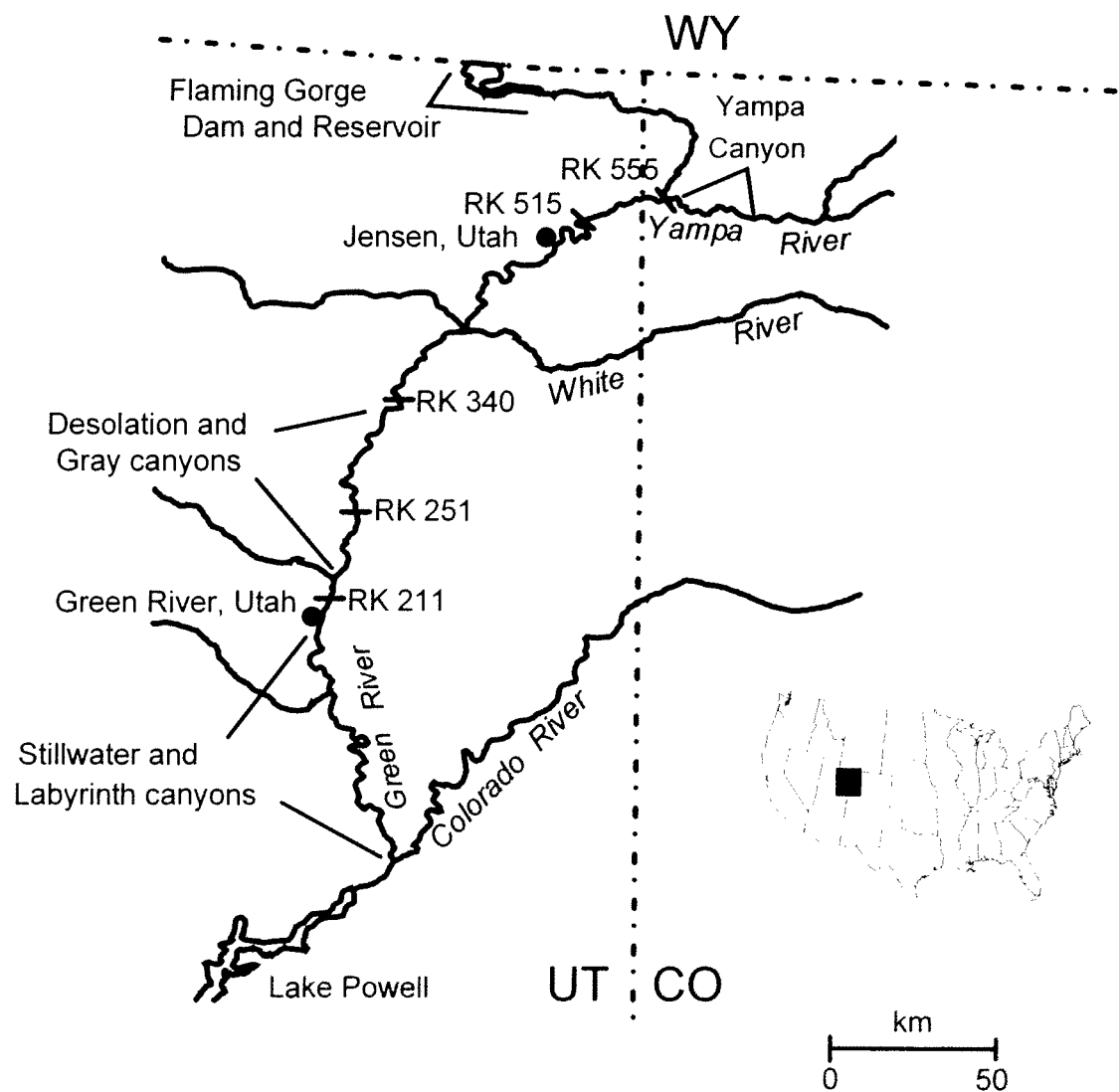340) and lower (RK 211-RK 0) Green River. Filled circles depict towns.

Flaming Gorge
Dam and Reservoir

WY

Yampa
Canyon

RK 555

RK 515
Jensen, Utah

Yampa River

River

RK 340

White

Desolation and
Gray canyons

RK 251

Green River, Utah — RK 211

Green River

Colorado River

Stillwater and
Labyrinth canyons

Lake Powell

UT ; CO

km

0   50

Fig. 2. Discharge and temperature regimes for the middle Green River, near Jensen, Utah

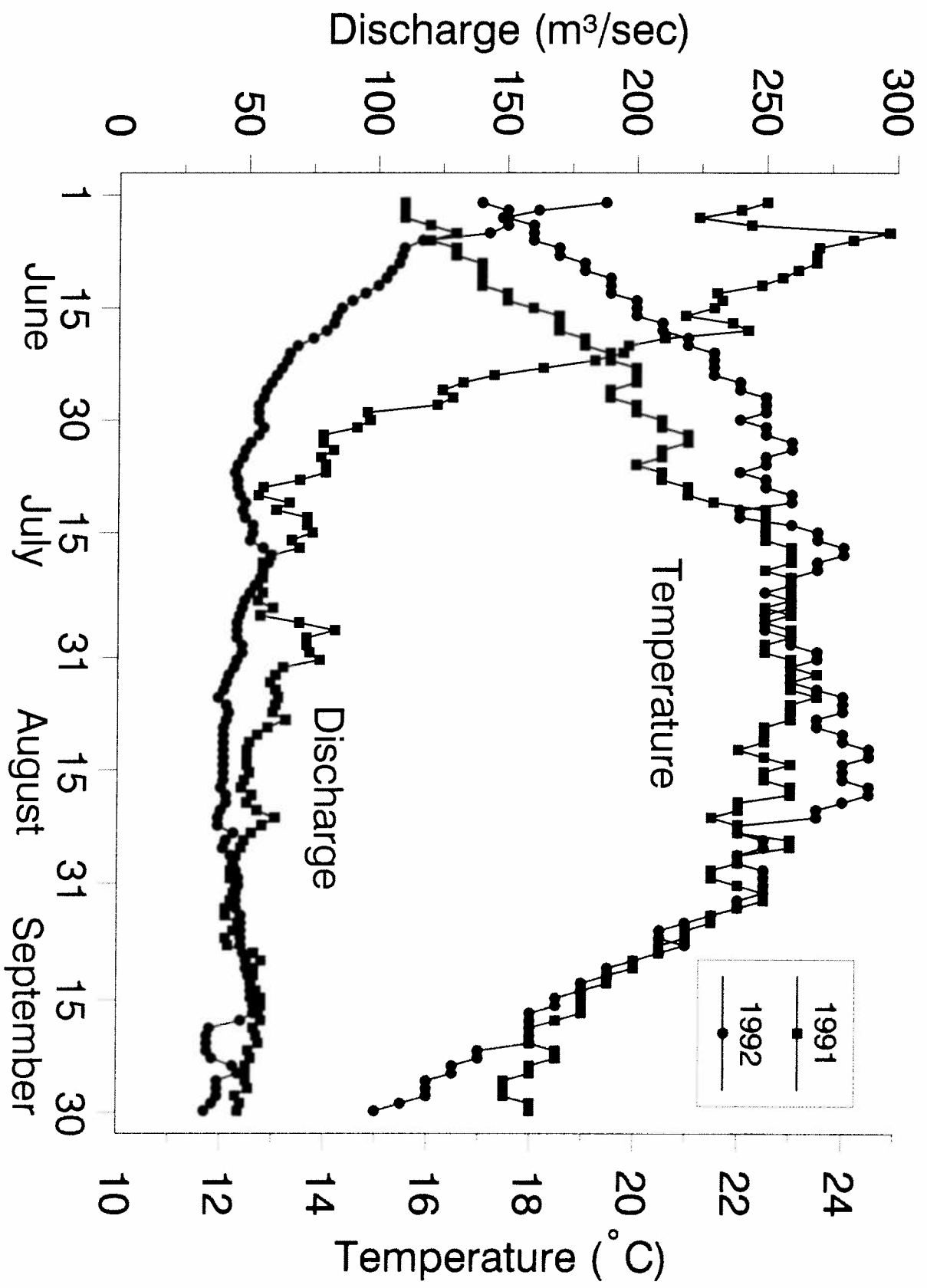(gauge # 09261000), 1991 and 1992.   Symbols represent daily values.

Fig. 3. Distributions of hatching dates of drift-net caught Colorado squawfish larvae and

seine-sampled juveniles caught in summer and fall in the middle Green River,

1991. Cohort 1 fish are represented by the left-hand most set of black-filled bars

in the histogram, cohort 2 fish by the empty bars, and cohort 3 by the farthest

right-hand set of black bars. The arrow intersecting the hatching date axis in the

distribution for larvae represents the last date samples were collected.
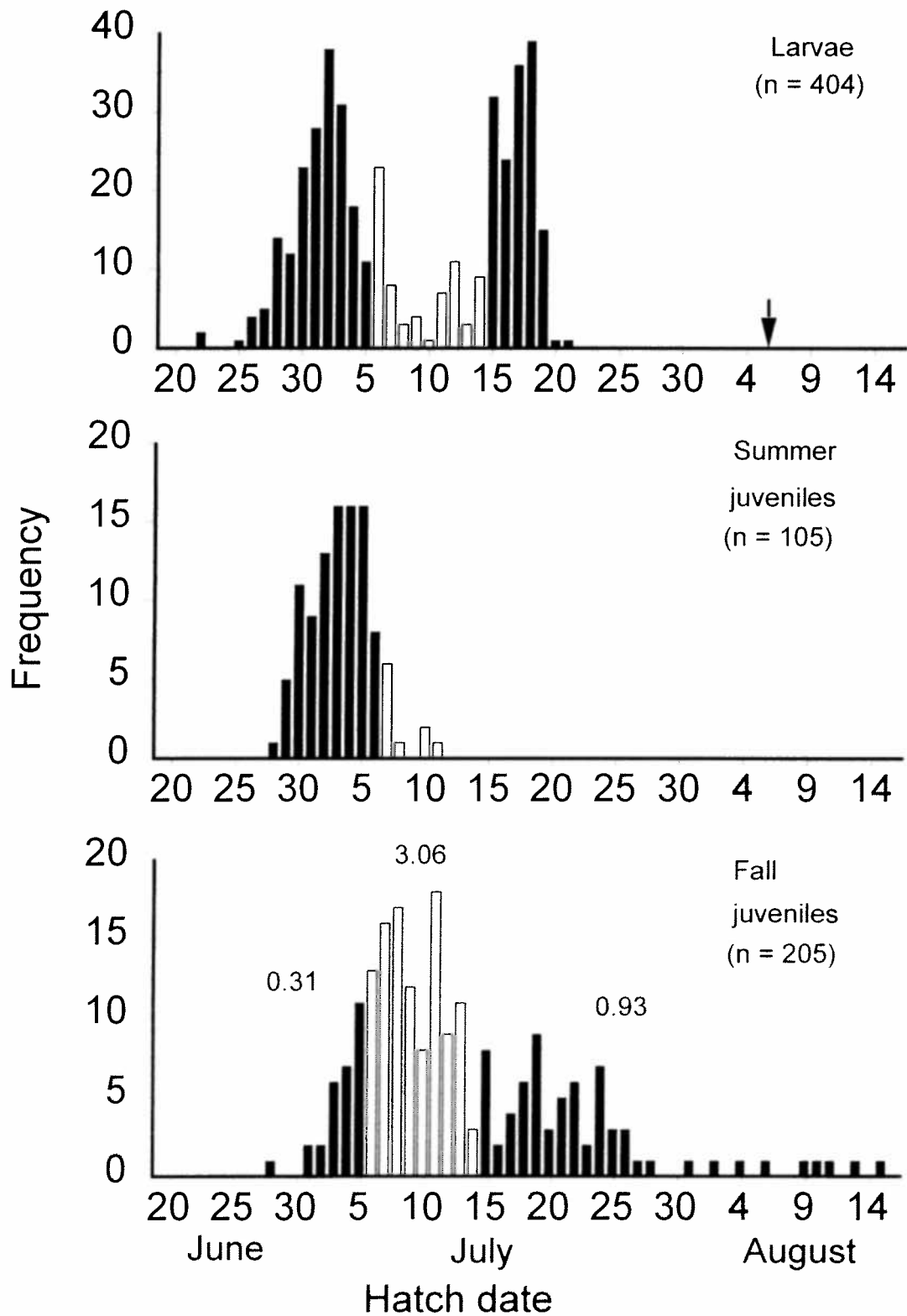
Middle Green River, 1991

Fig. 4. Distributions of hatching dates of drift-net caught Colorado squawfish larvae and

seine-sampled juveniles caught in summer and fall in the lower Green River,

1991. Cohort 1 fish are represented by the left-hand most set of black-filled bars

in the histogram, cohort 2 fish by the empty bars, and cohort 3 by the farthest

right-hand set of black bars. The arrow intersecting the hatching date axis in the

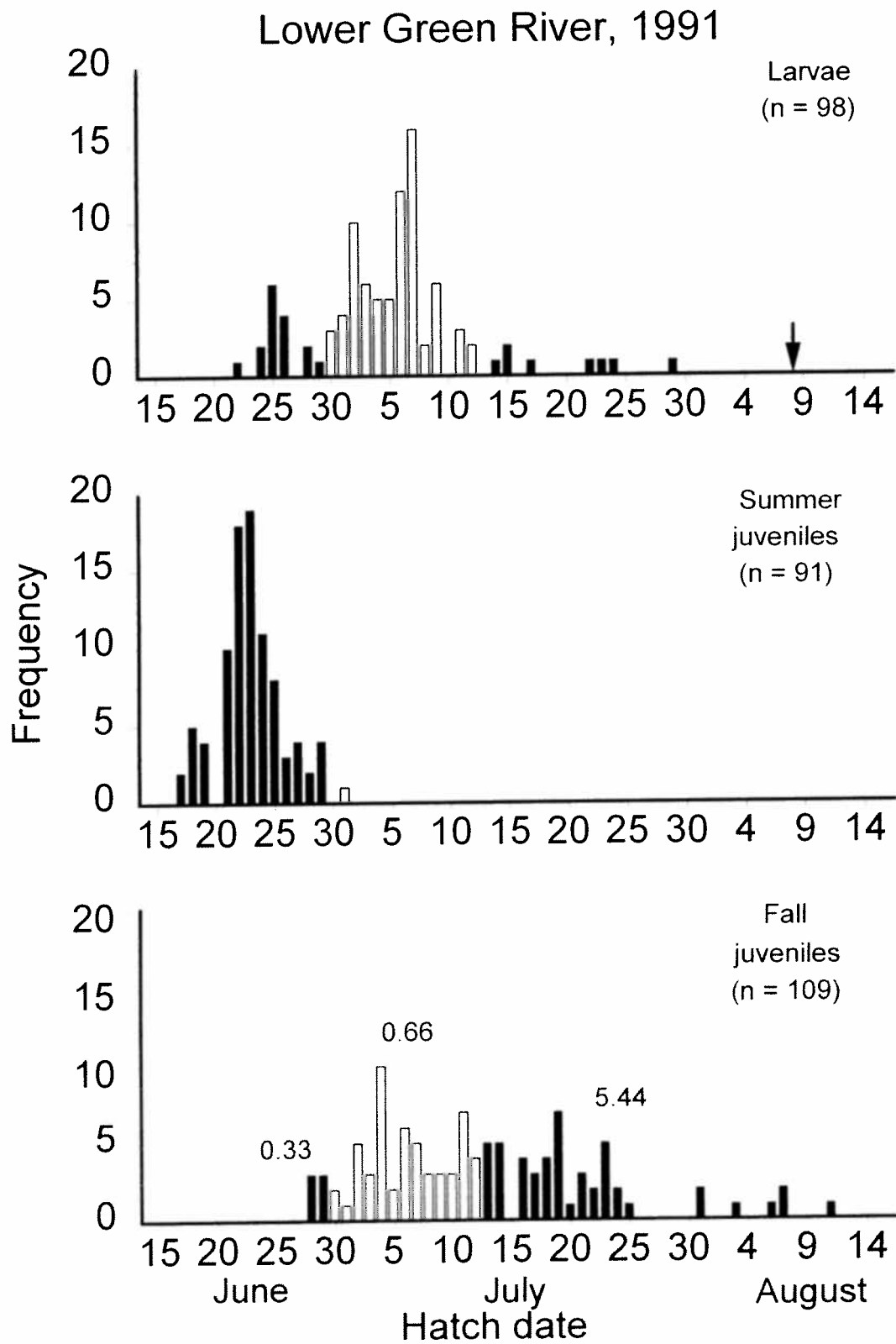distribution for larvae represents the last date samples were collected.

Lower Green River, 1991

Larvae
(n = 98)

Summer
juveniles
(n = 91)

Fall
juveniles
(n = 109)

0.33

0.66

5.44

Frequency

Hatch date

June   July   August

Fig. 5. Distributions of hatching dates of drift-net caught Colorado squawfish larvae and

seine-sampled juveniles caught in summer and fall in the middle Green River,

1992. Cohort 1 fish are represented by the left-hand most set of black-filled bars

in the histogram, cohort 2 fish by the empty bars, and cohort 3 by the farthest

right-hand set of black bars. The arrow intersecting the hatching date axis in the

distribution for larvae represents the last date samples were collected.
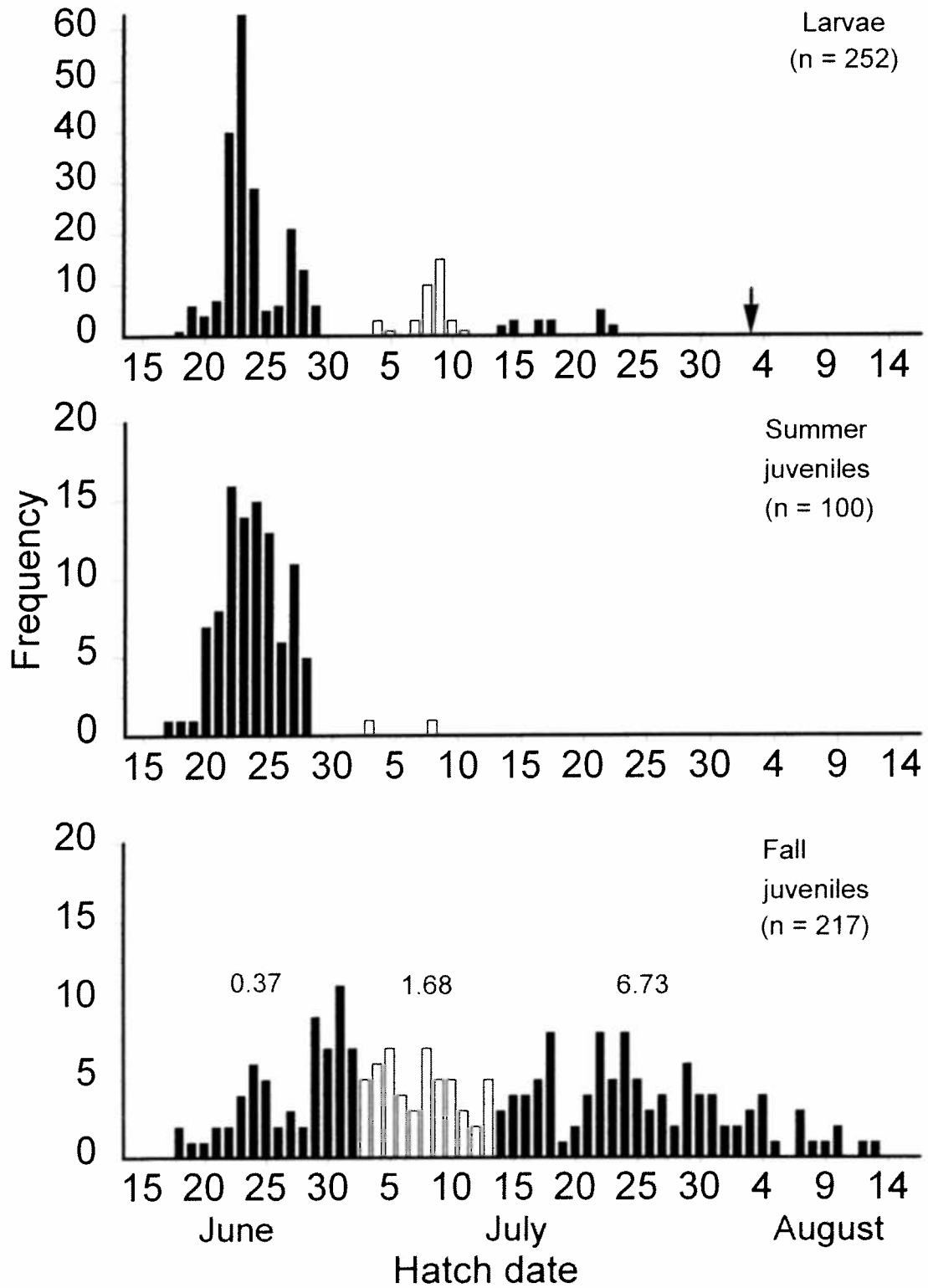
Middle Green River, 1992

Larvae
(n = 252)

Summer
juveniles
(n = 100)

Fall
juveniles
(n = 217)

0.37   1.68   6.73

Frequency

June   July   August

Hatch date

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Fig. 6. Distributions of hatching dates of drift-net caught Colorado squawfish larvae and

seine-sampled juveniles caught in summer and fall in the lower Green River,

1992. Cohort 1 fish are represented by the left-hand most set of black-filled bars

in the histogram, cohort 2 fish by left-hand most set of empty bars, cohort 3 fish

are the furthest right-hand set of black bars, and cohort 4 by the furthest right-

hand set of empty bars. The arrow intersecting the hatching date axis in the

distribution for larvae represents the last date samples were collected.

Lower Green River, 1992

Larvae
(n = 179)

Summer
juveniles
(n = 124)

Fall
juveniles
(n = 36)

Frequency

1.0   0.38   0.18   25.0

5 10 15 20 25 30   5 10 15 20 25 30   4   9
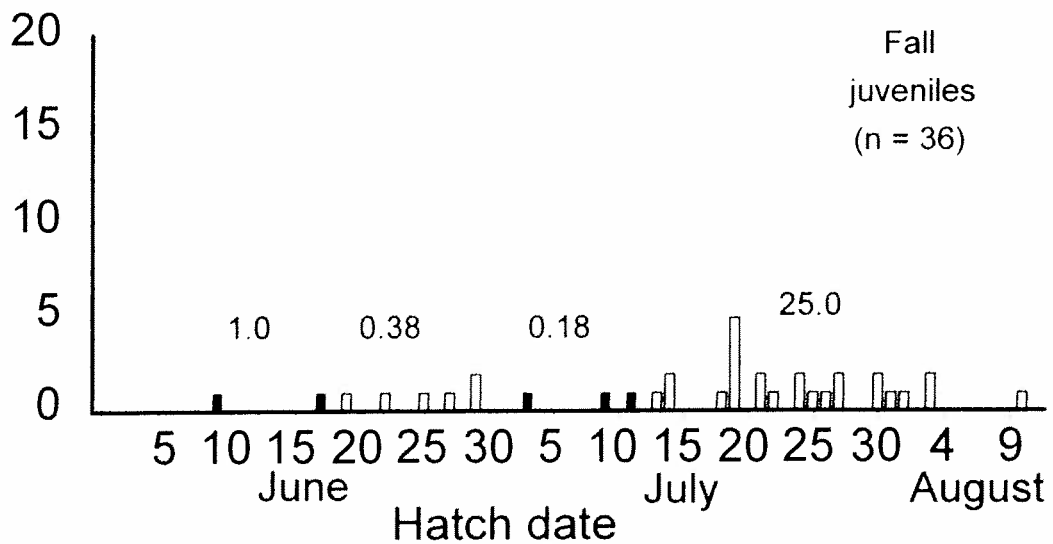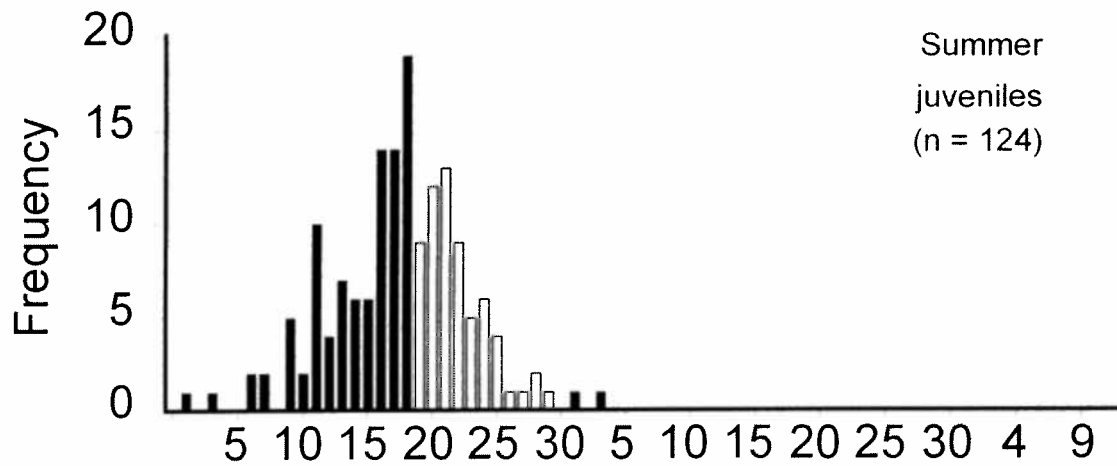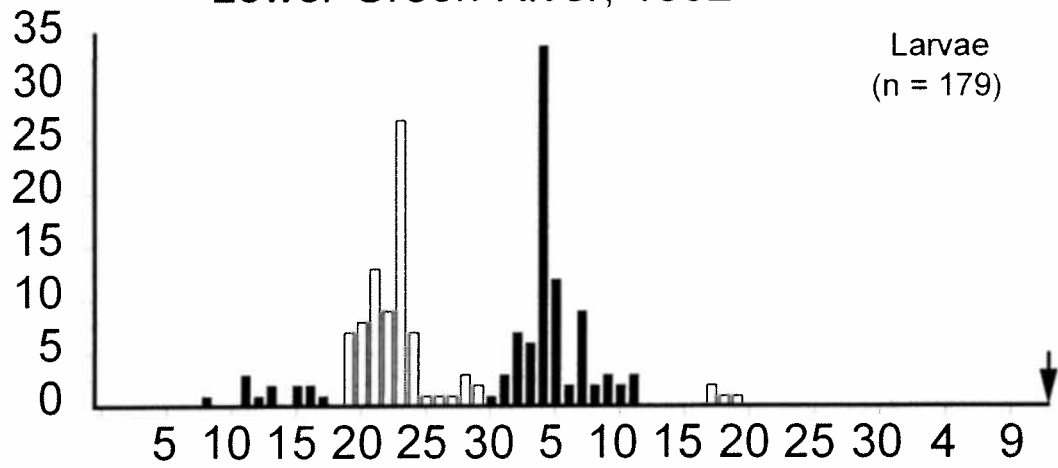June   July   August

Hatch date

Fig. 7. Mean abundance of Colorado squawfish larvae collected in drift net samples at four times and in three different channel positions in the middle Green River in summer 1992 as predicted by a Poisson general linear model. Vertical lines through symbols represent $\pm$ 1 SD.
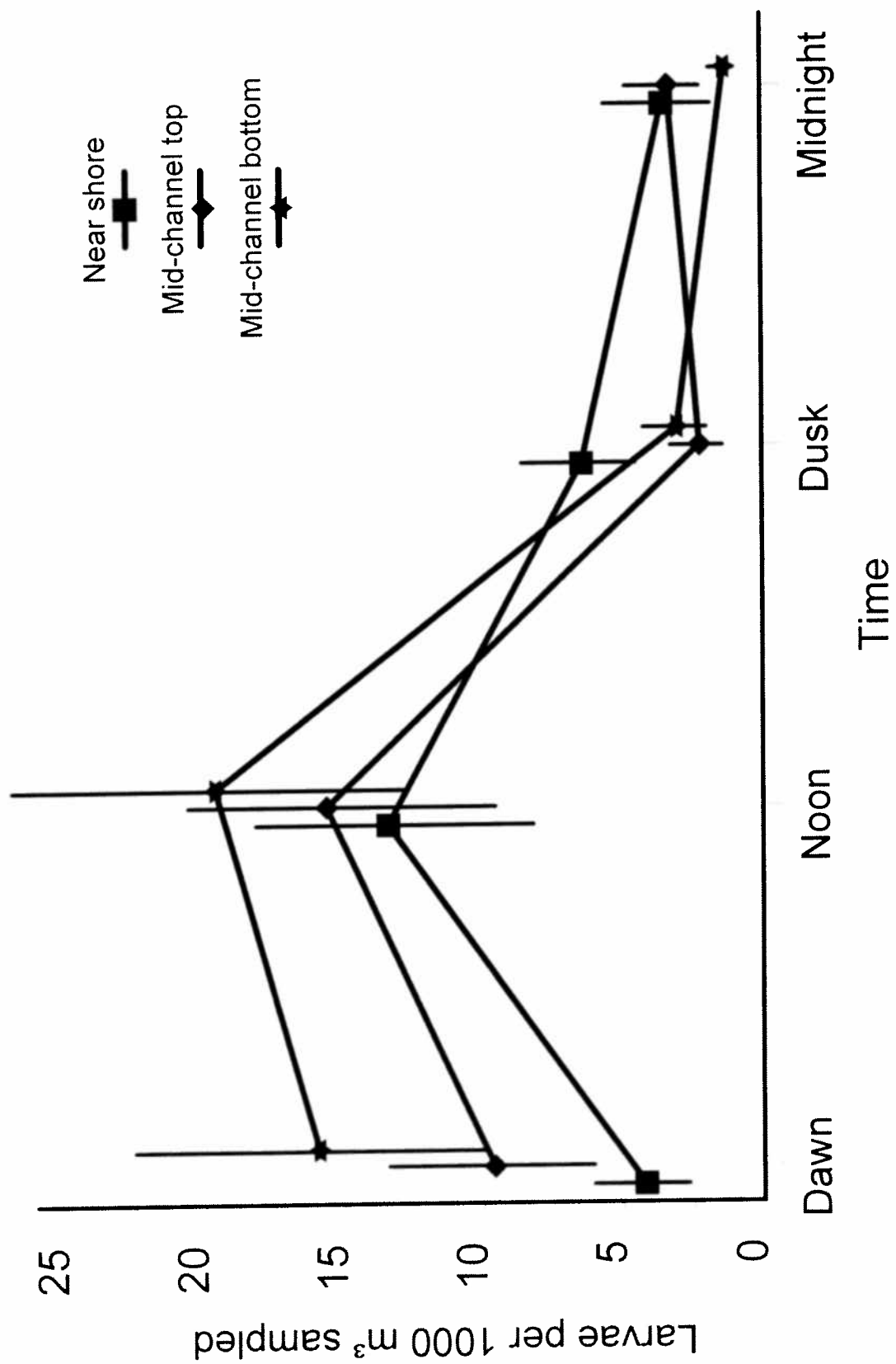
Fig. 8. Mean (black symbol, vertical bar is $\pm$ 1 SD) and distribution of individual growth

rates of juvenile Colorado squawfish in cohorts sampled in summer and again in

fall in the middle Green River, 1991. Means are centered within cohorts and do

not represent the median hatch date of individuals. The single summer sample

was collected before fish in cohort 3 hatched so there is no corresponding summer
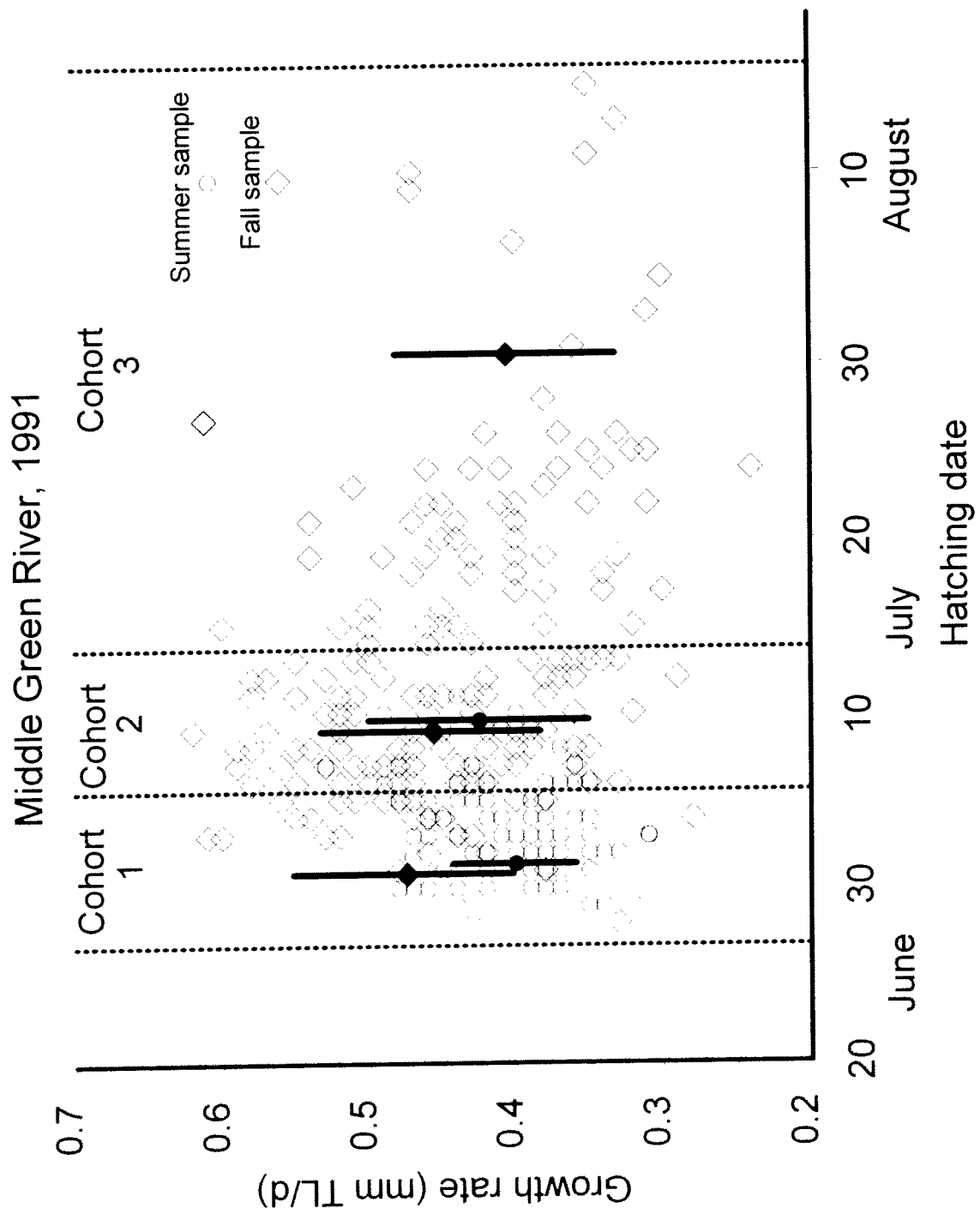
sample for fall cohort 3.

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Middle Green River, 1991

Cohort 1 · Cohort 2 · Cohort 3

Summer sample

Fall sample

Growth rate (mm TL/d)

Hatching date

June 20 · 30 · July 10 · 20 · August 10

Fig. 9. Mean (black symbol, vertical bar is $\pm$ 1 SD) and distribution of individual growth

rates of juvenile Colorado squawfish in cohorts sampled in summer and again in

fall in the lower Green River, 1991. Means are centered within cohorts and do not

represent the median hatch date of individuals. The single summer sample was

collected before fish in cohort 3 hatched so there is no corresponding summer
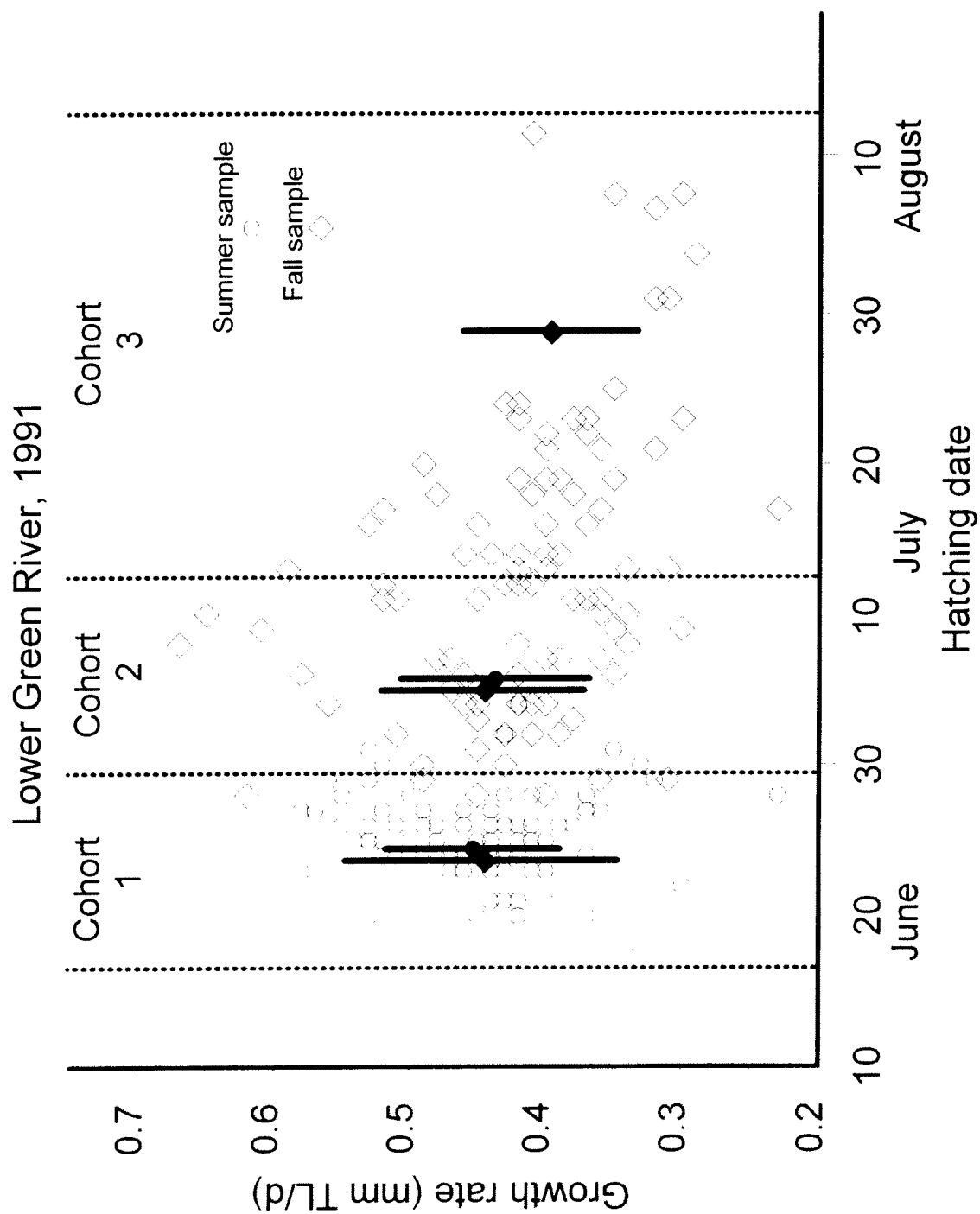
sample for fall cohort 3.

Lower Green River, 1991

Cohort 1   Cohort 2   Cohort 3

Summer sample

Fall sample

Growth rate (mm TL/d)

Hatching date

June   July   August

Fig. 10.   Mean (black symbol, vertical bar is $\pm$ 1 SD) and distribution of individual

growth rates of juvenile Colorado squawfish in cohorts sampled in

summer and again in fall in the middle Green River, 1992. Means are

centered within cohorts and do not represent the median hatch date of

individuals. The single summer sample was collected before fish in cohort

3 hatched so there is no corresponding summer sample for fall cohort 3.
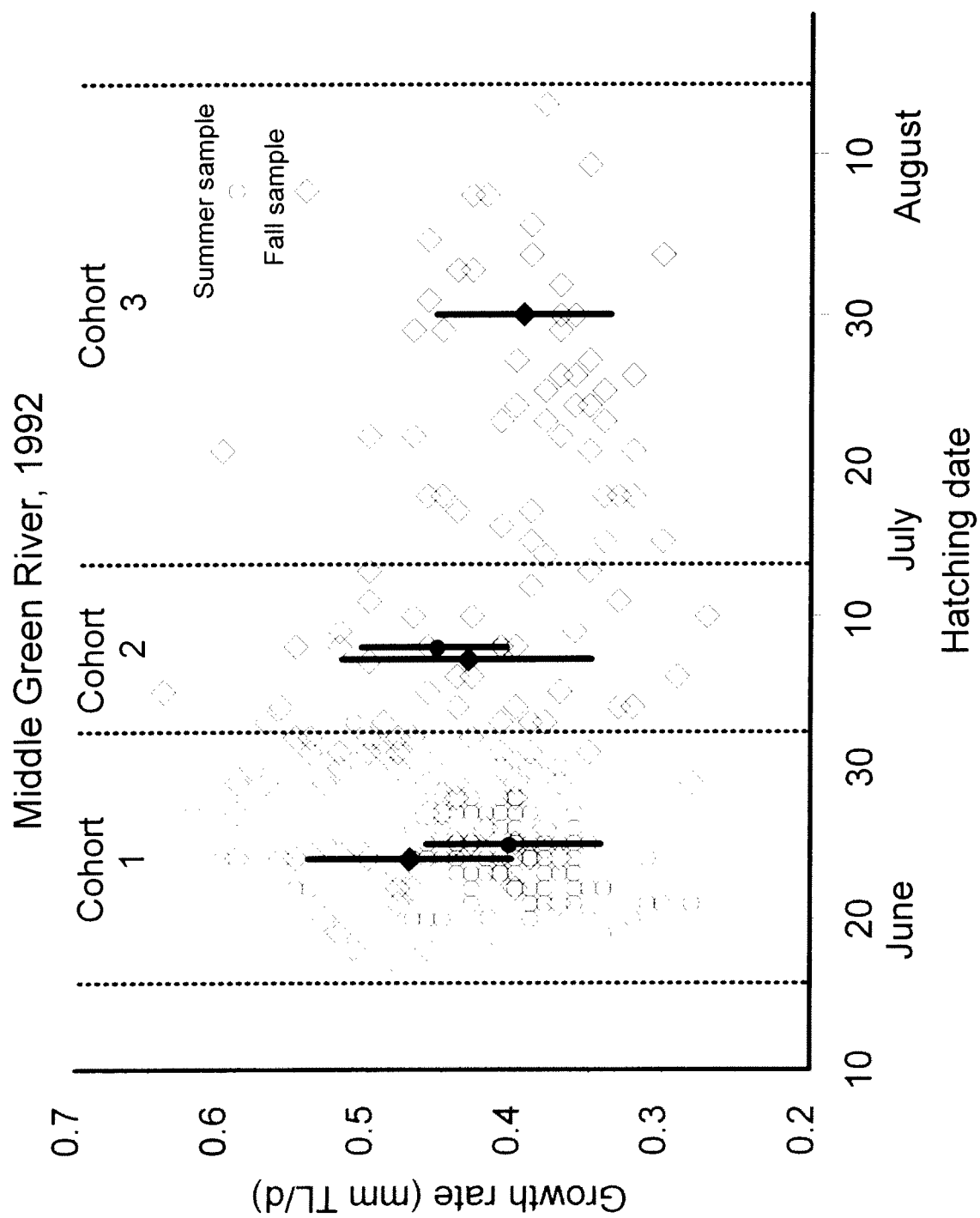
Middle Green River, 1992

Cohort 1   Cohort 2   Cohort 3

Summer sample

Fall sample

Growth rate (mm TL/d)

Hatching date

June   July   August

Fig. 11.   Mean (black symbol, vertical bar is $\pm$ 1 SD) and distribution of individual

growth rates of juvenile Colorado squawfish in cohorts sampled in

summer and again in fall in the lower Green River, 1992. Means are

centered within cohorts and do not represent the median hatch date of

individuals. The single summer sample was collected before fish in cohort

3 hatched so there is no corresponding summer sample for fall cohort 3.
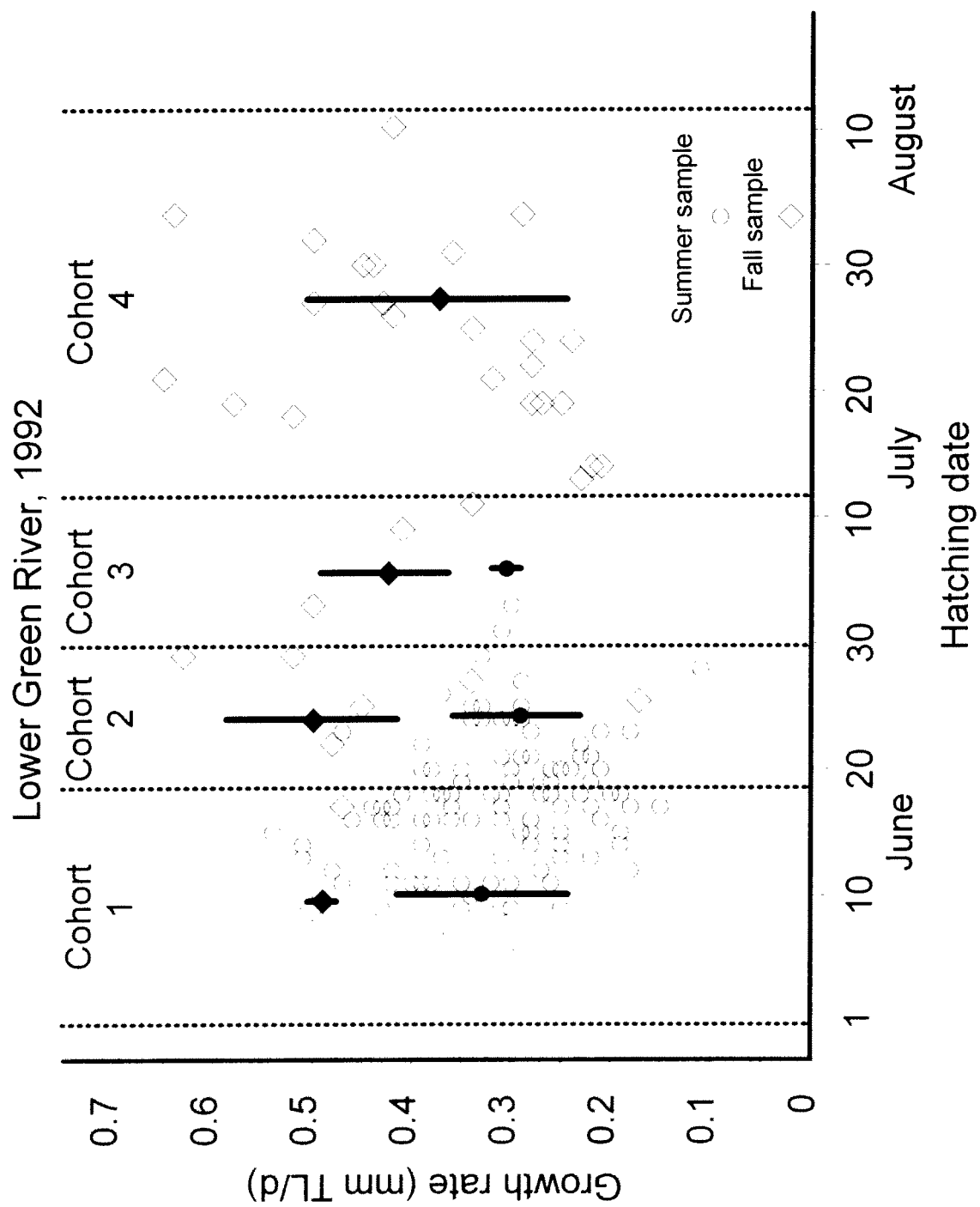
Lower Green River, 1992

Fig. 12.   Frequency distributions (mean of 5 simulations) of growth rates of

Colorado squawfish larvae with and without size-dependent predation by

red shiners in an individual-based recruitment model. Individuals in both

cohorts were assigned a growth rate by random draw from an initial

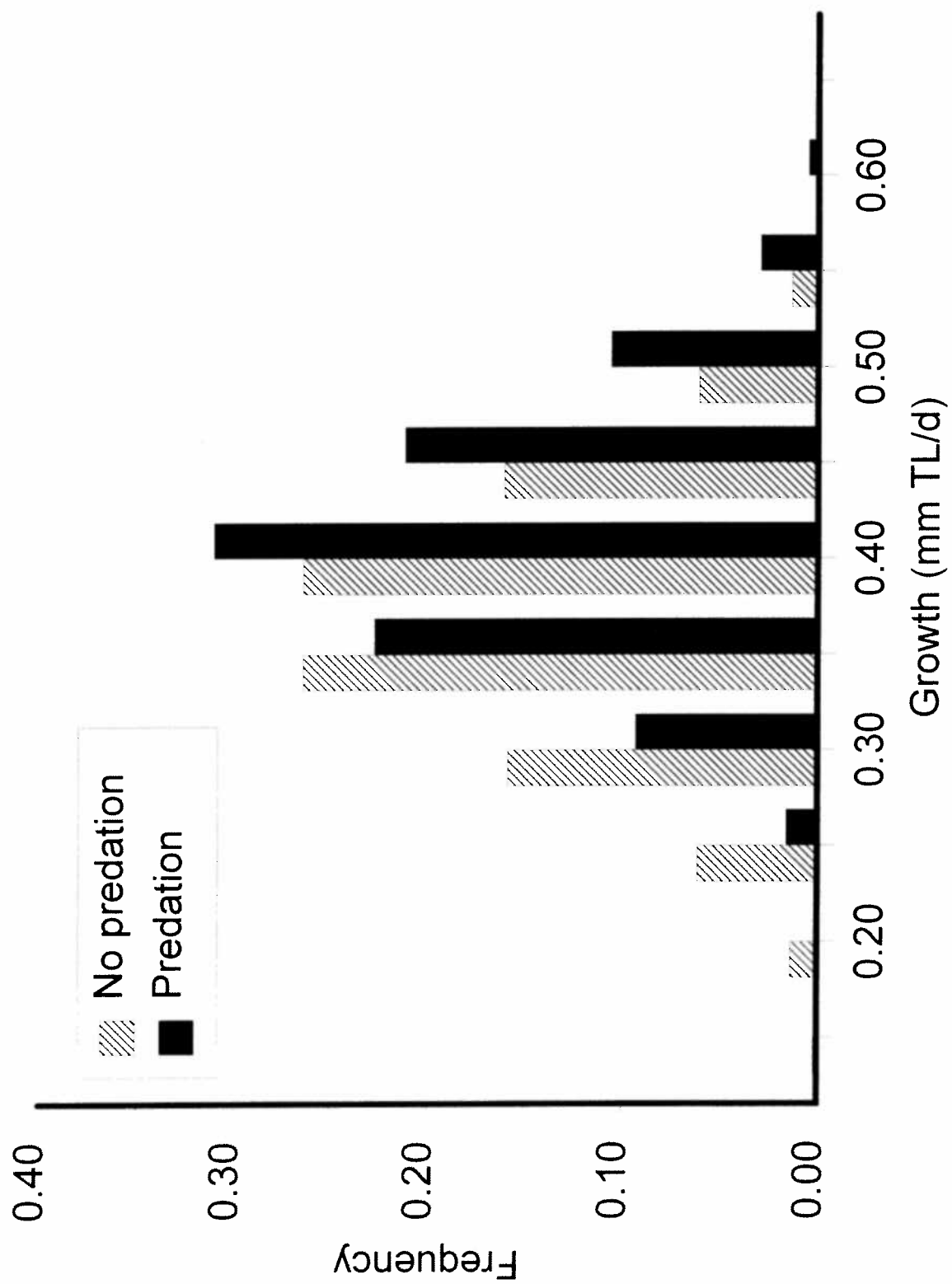distribution of growth rates that had a mean of 0.4 mm TL/d (SD = 0.07

mm TL/d).

Fig. 13.   Relationships of abundance of juvenile Colorado squawfish captured in

backwaters (per 100 m^2 of habitat) as a function of mean July-August

discharge (m^3/s) for the period 1979-1995 in the lower (A) and middle (B)

Green River, Utah, estimated by a linear plateau regression model. The

lower Green River model ($F_{2,15}$ = 25.6, P < 0.0001; r^2 = 0.77) parameter

estimates (SE) are: intercept = 2.46 log$_e$ *Colorado squawfish* (0.346),

slope = - 0.0336 (0.0), and join-point = 404 m^3/sec (34.3). The slope of

the regression line for the lower Green River was calculated without error

as it comprised the join point and the single remaining point, thus there is

no standard error. Middle Green River model ($F_{3,14}$ = 12.1, P = 0.0003; r^2

= 0.72). parameter estimates (SE) are: intercept = 1.84 log$_e$ *Colorado*

squawfish (0.339), slope = - 0.024 (0.0093), and join-point = 141 m^3/sec

(37.0). The vertical bar associated with the join point is the 95%

confidence interval of the intercept of the plateau. The horizontal line just

above the join point is the 95% confidence interval of the threshold

discharge. The two digit numbers near each point denote the year (e.g., 95

= 1995).
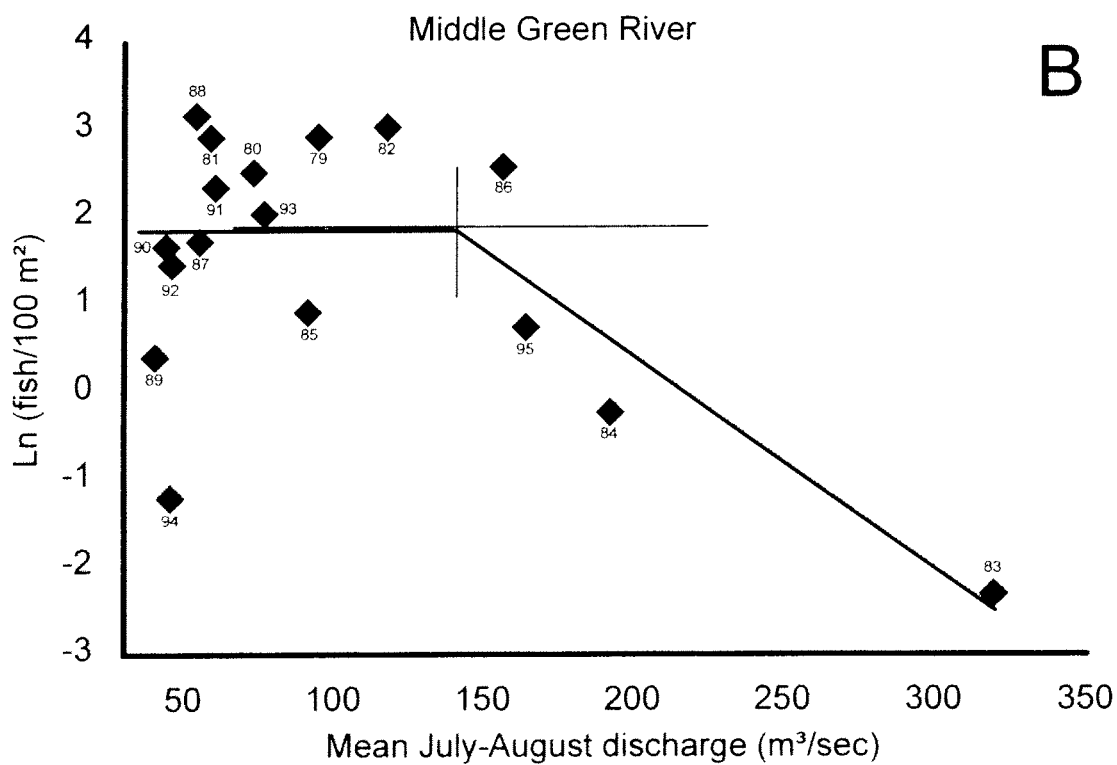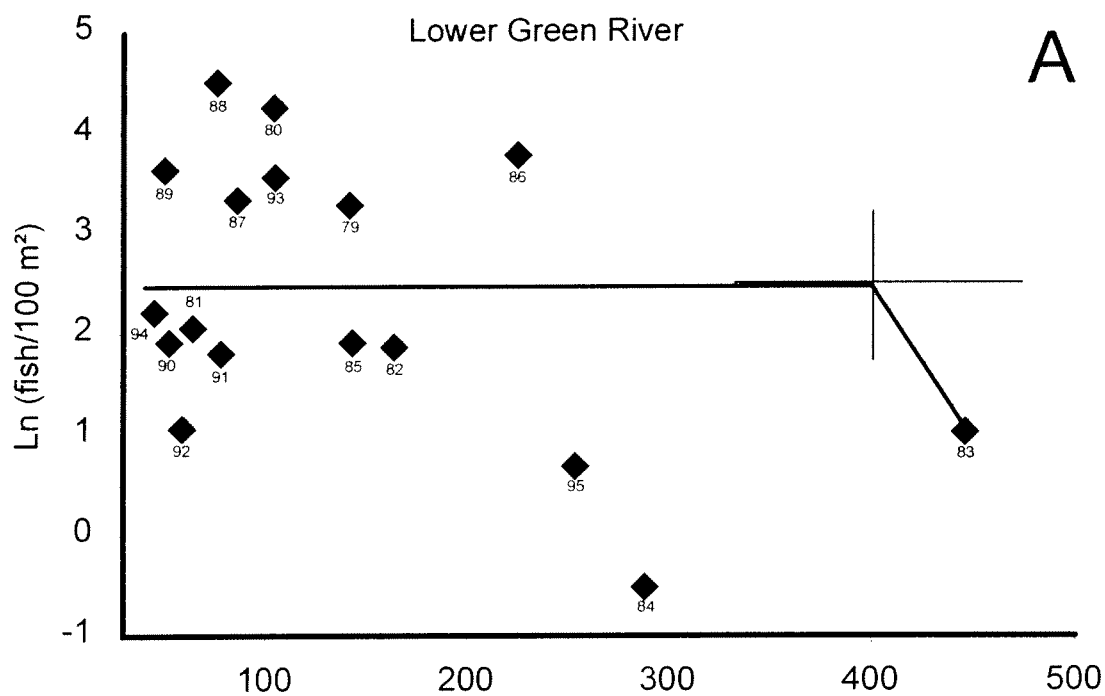
CONCLUSIONS

Both physical and biological processes may regulate recruitment of age-0

Colorado squawfish in riverine ecosystems (Tyus 1991). However, efforts to recover

Colorado squawfish in particular, and most endangered fishes in the Colorado River basin

in general, have focused on physical factors such as discharge and its relationship with

habitat. This emphasis is likely the result of the intuitive appeal of providing habitat, and

also because of the practical need to protect scarce and declining streamflows in the

Colorado River basin. Effects of biological factors such as predation by non-native

fishes, while acknowledged as potentially important, remain ambiguous. This is likely a

result of lack research emphasis and lack of demonstrated direct effects (e.g., endangered

fishes in stomachs of non-native predators), evidence of which is difficult to obtain.

However, perceptions about the relative importance of physical and biological factors

impeding recovery of endangered fishes may be in error because of lack of rigorous

evaluations to support conclusions.

In the research described here, results of laboratory experiments and a field study

were used to evaluate the role of several important physical and biological factors on

recruitment of age-0 Colorado squawfish. Laboratory studies showed that early life

stages of Colorado squawfish can hatch, grow, and survive under a variety of water

temperatures and food abundance levels that emulate conditions in rivers of the Colorado

River Basin. Low hatching success of Colorado squawfish embryos at high temperatures (30°C) may not be important because these temperatures rarely occur in main channels of the upper Colorado River basin. Similarly, survival of Colorado squawfish larvae denied food for up to 15 d after they could eat (at 6 days old) was high and nearly equivalent to continuously fed controls in laboratory trials. This suggested that starvation was not likely to regulate recruitment of age-0 Colorado squawfish in the wild. Linear plateau regression models showed that fall abundance of juvenile Colorado squawfish in backwaters was unaffected by mean July-August discharge in nursery habitat reaches, except at very high discharge. Results of these laboratory and field investigations suggested that age-0 Colorado squawfish were adapted to a wide variety of water temperature and discharge conditions. Therefore, physical factors may regulate recruitment of age-0 Colorado squawfish only in relatively rare instances.

In contrast, several lines of evidence from laboratory and field studies indicated that biological processes may interact with physical variables to regulate recruitment. Slower growth of larvae in the laboratory at water temperatures < 22°C may be important because growth rate defines the amount of time that larvae are susceptible to small-bodied, predaceous non-native fishes which are abundant in backwaters where age-0 Colorado squawfish reside (Haines and Tyus 1990). Distributions of hatching dates derived from otolith analysis of Colorado squawfish captured in the lower and middle Green River in 1991 and 1992 suggested that early-hatching cohorts of larvae survived relatively poorly to fall, whereas late-hatching cohorts survived relatively well. Moreover, growth rate comparisons suggested that the few early hatched fish that

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survived to fall were a fast-growing subset of the fish present in the same cohort in summer. In contrast, late-hatching larvae grew relatively slowly.

I attributed this to size-selective to predation mortality by introduced fishes. Slow growth rates and high survival for late-hatched fish, a pattern that is incongruous with that for early-hatched larvae, was probably due to environmental factors and to natural spawning mortality of large predaceous red shiners *Cyprinella lutrensis* in mid- to late-summer. An independent individual-based computer simulation model, which had a gape-limited red shiners as predators and Colorado squawfish larvae, corroborated size-selective patterns revealed by comparing summer and fall growth rates of wild juveniles because fish that survived in simulations were the fastest growing individuals. Additional IBM simulations also showed that Colorado squawfish larvae that had moderate growth were more than twice as likely to survive as larvae with slow growth. These results suggested that the importance of physical factors such as habitat availability or floods that influence growth rates of larvae may be expressed through interactions with biological factors such as predation to affect recruitment of age-0 Colorado squawfish.

Alternative hypotheses such as competition or physical habitat could not explain differential survival of cohorts of Colorado squawfish larvae with different hatching dates nor the size-selective mortality patterns. Competition for scarce resources in backwaters may reduce growth of larvae and increase the amount of time they are susceptible to gape-limited predators but is an unlikely source of direct mortality. Warm water temperatures and abundant backwaters during the summer in 1991 and 1992 favored high growth and survival of larvae so physical habitat was unlikely to influence recruitment patterns.

Management to achieve recovery of endangered Colorado squawfish should focus on reducing effects of factors that most limit populations. Results presented here suggest that physical processes may be relatively less important compared to effects of biological ones in regulating recruitment of age-0 Colorado squawfish. Past management strategies de-emphasized effects of introduced fishes because of research emphasis on native fishes, perceived lack of options to control non-native fish abundance, and the poorly understood effects of regulation of the Green River by Flaming Gorge Dam on native fishes. However, evidence of potentially strong non-native fish effects (this study), coupled with recent evidence that high discharge events may reduce abundance of non-native fishes (Haines and Tyus 1990, Stanford 1994, Gido et al. in press), suggests that research on these issues is deserving of more emphasis. A better understanding of the relative importance of mechanisms regulating recruitment of Colorado squawfish and other native biota may also show that present management emphasis on habitat is inappropriate and should be supplemented or replaced with strategies that reduce effects of non-native fishes.

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