
The Role of Hybrid Vigor in the Replacement of Pecos Pupfish by Its Hybrids with Sheepshead Minnow

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Abstract: *Many species are jeopardized by hybridization and genetic introgression with closely related species. Unfortunately, the mechanisms that promote or retard gene flow between divergent populations are little studied and poorly understood. Like many imperiled fish species, the Pecos pupfish (*Cyprinodon pecosensis*) is threatened with replacement by its hybrids with a close congener. We examined swimming performance and growth rate of hybrid pupfish to determine the role of hybrid vigor in the genetic homogenization of *C. pecosensis* by its hybrids with sheepshead minnow (*C. variegatus*). The F1 hybrids, backcross hybrids, and purebred *C. variegatus* displayed greater swimming endurance than purebred *C. pecosensis*. In addition, F1 hybrids and *C. variegatus* grew more rapidly than *C. pecosensis*. The ecological superiority of hybrids probably promoted their rapid spread through and beyond the historic range of *C. pecosensis*. These results indicate that eradication of hybrids and restoration of *C. pecosensis* to its native range is unlikely. Extinction of unique species via genetic homogenization can result from human activities that increase gene flow between historically fragmented populations; conservation managers must weigh the potential for such a catastrophe against the presumed benefits of increased interpopulation gene flow. This example illustrates how, after hybridization has occurred, conflict may arise between formerly complementary conservation goals.*

Key Words: conservation objectives, *Cyprinodon*, extinction, fish, gene flow, hybridization, Pecos River, swimming endurance

El Papel de Vigor Híbrido en el Reemplazo de *Cyprinodon pecosensis* por Sus Híbridos con *C. variegatus*

Resumen: *Muchas especies están en peligro por hibridación e introgresión genética con especies cercanamente relacionadas. Desafortunadamente, los mecanismos que promueven o retardan el flujo génico entre poblaciones divergentes son poco estudiados y comprendidos. Como muchas especies de peces en peligro, *Cyprinodon pecosensis* está amenazada con reemplazo por sus híbridos con un congénere cercano. Examinamos el desempeño natatorio y la tasa de crecimiento de peces híbridos para determinar el papel del vigor híbrido en la homogenización genética de *C. pecosensis* por sus híbridos con *C. variegatus*. Los híbridos F1, los híbridos de retrocruza y *C. variegatus* puros mostraron mayor resistencia natatoria que *C. pecosensis* puros. Adicionalmente, los híbridos F1 y *C. variegatus* crecieron más rápidamente que *C. pecosensis*. La superioridad ecológica de híbridos probablemente promovió su rápida dispersión dentro y más allá del rango histórico de *C. pecosensis*. Estos resultados indican que la erradicación de híbridos y la restauración de *C. pecosensis* en su rango nativo es poco probable. La extinción de especies únicas por vía de homogenización genética puede ser resultado de actividades humanas que incrementan el flujo génico entre poblaciones históricamente fragmentadas; los gestores de conservación deben sopesar el potencial de tal catástrofe con los supuestos beneficios*

del incremento de flujo génico entre poblaciones. Este ejemplo ilustra como, después de que ha ocurrido la hibridación, puede surgir un conflicto entre metas de conservación complementarias en el pasado.

Palabras Clave: *Cyprinodon*, extinction, flujo génico, hibridación, objetivos de conservación, peces, resistencia de nado, Río Pecos

Introduction

Hybridization and genetic introgression (mixing of gene pools; Anderson 1949) of narrowly distributed native species with introduced species is an important force driving the loss of global biological diversity (Levin et al. 1996; Rhymer & Simberloff 1996). Breeding with introduced species may threaten native species by (1) limiting the reproductive success of the native (if hybrids are less fit than purebreds) or (2) assimilating endemic genomes (if hybrids are viable and successful; Huxel 1999). Genetic homogenization is a problem caused by outbreeding of formerly isolated lineages; thus, it differs from other conservation concerns that are often related to a lack of gene flow among population units. Also, when hybrids are reproductively and ecologically viable, their taxonomic status and that of their parental species becomes controversial, as does their conservation status (O'Brien & Mayr 1991; Smith et al. 1995; Rosenfield et al. 2000).

Hybridization and genetic introgression are particularly prevalent threats to the diversity of freshwater fish (Epifanio & Nielsen 2000; Perry et al. 2002). Of threatened, endangered, and recently extinct freshwater fish species, approximately 40% became imperiled, at least in part, because of hybridization or genetic introgression (Miller et al. 1989; Williams et al. 1989). Despite the conservation implications, little attention has been paid to the behavioral and ecological mechanisms that promote hybridization and introgression (but see Grant & Grant 1997; Seehausen et al. 1997; Wirtz 1999; Good et al. 2000; Lexler et al. 2003).

Several assumptions about hybridization and genetic introgression contribute to the paucity of mechanism-oriented research on genetic homogenization of rare species. First, hybrids are often assumed to be less viable than their parent species (e.g., Epifanio & Philipp 2001). The forces that reduce hybrid fitness have been studied in certain cases (e.g., Howard et al. 1998; Burton et al. 1999; Hatfield & Schluter 1999), but studies that demonstrate hybrid superiority to at least one of their parental species are increasingly common (e.g., Arnold 1997; Good et al. 2000; Welch & Rieseberg 2002). Second, when hybrid populations are persistent, it is often assumed that they result from the constant migration of parental types into the hybrid zone and would otherwise not be self-sustaining (Barton & Hewitt 1985), or that they can exist only in marginal or disturbed environments, where competition from parental types is diminished or absent (e.g., Ander-

son 1948, 1949). However, hybrid fitness may equal or exceed that of parental types over a wide range of environments, although this possibility is often overlooked. Because conservation biologists usually emphasize protection of monophyletic lineages (often referred to as "genetically pure" organisms) and undisturbed or "pristine" environments, organisms carrying introgressed alleles and areas that have been severely affected by human activities may receive less attention from conservation researchers. Studies of the behavioral and ecological performance of hybrids can provide valuable information to those interested in the genesis and loss of biological diversity (Scribner 1993; Grant & Grant 1997; Wolf et al. 2001).

We analyzed the ecological viability of hybrids between an endemic fish species, the Pecos pupfish (*Cyprinodon pecosensis*), and an introduced congener, the sheepshead minnow (*C. variegatus*). Like several species in this genus, *C. pecosensis* is jeopardized by hybridization with introduced *C. variegatus* (Garrett et al. 2002). In this case, genetic introgression has been geographically extensive and extremely rapid (Echelle & Connor 1989; Echelle et al. 1997), suggesting that hybrids experience a selective advantage over the native species. We therefore tested the hypothesis that hybrid pupfish display ecological attributes that provide a proximal mechanism for a selective advantage over *C. pecosensis*.

Previous research demonstrated that sexual selection accelerated the formation and spread of the hybrid swarm (Rosenfield & Kodric-Brown 2003). Both *C. variegatus* and F1 hybrids outcompete male *C. pecosensis* for territories and breeding access to females. Also, female *C. pecosensis* express a visual preference for male *C. variegatus*, even in the presence of male conspecifics. These behavioral asymmetries promote production of F1 and backcross hybrid progeny. But, unless hybrid offspring were ecologically viable, the hybrid swarm would not have formed (and certainly would not have spread so rapidly) from what is believed to be a small number of introduced *C. variegatus* (Childs et al. 1996).

We studied growth rates and swimming performance of F1 hybrids relative to their parental species under controlled laboratory conditions. Growth rates are an important ecological variable because, within fish species, size is negatively correlated with risk of predation and age of maturation. Rosenfield and Kodric-Brown (2003) demonstrated that male F1 hybrids of both reciprocal crosses were vigorous competitors for breeding territories and

females. However, in other cases of hybridization, hybrid inviability often develops in later generations (e.g., Edmands 1999). For this reason, we compared swimming performance of both F1 and backcross hybrids to swimming performance of parental individuals. Swimming performance is a good measure of ecological viability because it is negatively correlated with predation risk and positively correlated with success in food acquisition and dispersal (Webb 1988; Videler 1993). Among pupfish, swimming endurance is also correlated with the ability to gain and hold breeding territories (Kodric-Brown & Nicoletto 1993).

Background

C. pecosensis is endemic to the Pecos River of southern New Mexico and western Texas (Echelle & Echelle 1978). *C. variegatus* is the most widespread member of its genus with a geographic range extending along most of the Atlantic and Gulf coasts of the United States and Mexico (Lee et al. 1980). Both species are omnivorous and feed throughout the water column, although benthic materials (diatoms and algae) are emphasized in the diet (Ross 2001). They have similar tolerances for extreme salinity and temperatures (Bennett & Beitinger 1997; Haney 1999; New Mexico Department of Game and Fish 1999). The two species are closely related but are not sister taxa (Echelle & Echelle 1978, 1992). Although they have similar ecological tolerances and are trophic generalists, these two species differ diagnostically in morphology, scale patterns, pigmentation, mating colors, and allozyme and mitochondrial DNA (mtDNA) markers (Echelle & Echelle 1978, 1992; Childs et al. 1996). In addition, the two species and their F1 hybrids differ behaviorally (Rosenfield & Kodric Brown 2003).

Following introduction of *C. variegatus* into the Pecos River of Texas in the early 1980s, backcross hybrids between this species and *C. pecosensis* completely replaced the latter throughout more than half of its native range in <5 years (Echelle & Connor 1989). Since its formation, the hybrid swarm has expanded to a stretch of the Pecos River beyond the historical range of *C. pecosensis* (Wilde & Echelle 1992). Studies of genetic patterns in the hybrid swarm have not detected any selection against particular hybrid genotypes (Echelle & Connor 1989; Childs et al. 1996). In addition, these studies suggest that the introduction of *C. variegatus* occurred only once, at a single locality, and consisted of a very small number of individuals. Currently, *C. pecosensis* populations are limited to two sets of sinkhole habitats (Bitter Lake National Wildlife Refuge and Bottomless Lakes State Park, Chavez County, NM) and associated waters in the Pecos River's historical floodplain.

Methods

Study Organisms

Between 1996 and 1999, we collected *C. pecosensis* from Figure 8 Lake in Bottomless Lakes State Park and one sinkhole in Bitter Lake National Wildlife Refuge. We purchased *C. variegatus* from a commercial breeder (Aquatic Biosystems, Fort Collins, CO) and collected them from Lake Balmorhea, Texas (a naturalized population and the putative source of *C. variegatus* introduced to the Pecos River). In addition, *C. variegatus* were obtained from Sea Rim State Park, Texas. We used these fish and their offspring as subjects in swimming experiments and to produce offspring for growth experiments. Fish collected in the field were used to produce all of the *C. variegatus* male \times *C. pecosensis* female ($C_v \times C_p$) and *C. pecosensis* male \times *C. variegatus* female ($C_p \times C_v$) F1 hybrid pupfish used in swimming experiments. When juvenile purebreds were produced for the growth experiments, we used male and female parents from different source populations to maximize outbreeding.

In June 2002, we collected backcross hybrid pupfish from Salt Creek, Reeves County, Texas, for use in our swimming experiment. Salt Creek is an intermittent creek with a barely detectable current during most months (it can flow rapidly during wet years or following heavy rain events). Recently, backcross hybrids have replaced Salt Creek's native *C. pecosensis* (only pure *C. pecosensis* were detected in 1998; A. Echelle, unpublished data). Each fish we collected had morphological characteristics that identified it as a backcross hybrid. Molecular analysis of the pupfish at this site indicates that the entire population consists of backcross hybrids with approximately 40% of alleles attributable to *C. variegatus* (A. Echelle, unpublished data).

Mass-Length Relationships

Mass is an important variable in behavioral and ecological studies, but length is much easier to measure on living fish. Therefore, we determined the mass-length relationship for each of the species in our study. *C. pecosensis*, *C. variegatus*, and their hybrids have different morphologies (e.g., *C. variegatus* have deeper bodies; Wilde & Echelle 1997). We measured the mass (to the nearest 0.01 g) and standard length (to the nearest 0.1 mm) of formalin-preserved adult specimens of *C. pecosensis* ($n = 112$), *C. variegatus* ($n = 106$), $C_v \times C_p$ ($n = 36$), and $C_p \times C_v$ ($n = 52$) to determine whether different, species-specific mass-length relationships existed. The sex of each specimen was also identified. All specimens were either voucher specimens from field collections or offspring produced in our laboratory. We used analysis of covariance (ANCOVA) to determine whether the mass-length relationship was the same for both sexes and across species.

Swimming Performance

We measured critical swimming velocity (U_{crit}) in a Brett-type apparatus (Brett 1964), consisting of a transparent Plexiglas tube (2.6 cm in diameter) attached to a flow meter attached to a water pump. The pump was placed in a large carboy filled with saltwater (8 ppt, 21–23° C). Water exiting the Plexiglas tube flowed back into the carboy, forming a closed system. A pupfish was placed into the tube at an initial flow rate of 10.99 cm/second. Once an individual began to swim actively, we activated a digital timer. After 180 seconds at the initial flow, we increased the flow rate each 180 seconds by 1.57 cm/second until the fish was no longer able to hold its position in the flow. A net was placed at the mouth of the swimming chamber to catch the fish when it ceased to swim in the current. We stopped the timer when the fish fell into this net. To distinguish between fish that could not swim at a higher flow rate and those that simply lacked the motivation to continue swimming, fish that landed in the net were encouraged to swim by placing them back into the flow. This process was repeated until the fish landed in the net for a third time (Young & Cech 1996). At this point, the final flow rate and amount of time spent swimming at that rate were recorded and used to calculate U_{crit} following Beamish (1978).

After the swimming trial, each fish's length was measured. Only data for fish 27–36 mm in length were analyzed. This size range approximates that typical of breeding adult *C. pecosensis*. Species and sex were also recorded. We conducted this experiment on 34 pure *C. variegatus* (17 male:17 female); 51 pure *C. pecosensis* (24:27); 37 *Cv* × *Cp* (19:18); 40 *Cp* × *Cv* (24:16); and 28 backcross pupfish (14:14) collected from Salt Creek and reared under our animal care protocols for >3 weeks. Swimming performance typically varies with fish length (Stahlberg & Peckmann 1987). We used ANCOVA to determine whether the relationship between U_{crit} and length was the same for each species and sex.

Growth

Juveniles of each species were produced outdoors in stock tanks (1.2 m in diameter). Each purebred species was produced in two stock tanks, and each of the F1 hybrid crosses was produced in a single separate stock tank. We placed 4 males in each stock tank with 6–10 females for 96 hours. Adult fish were then removed from the stock tank to prevent additional spawning and to limit egg predation.

After 15 days incubation, we harvested juveniles (5.5–6.5 mm long) from the stock tanks and moved them into 37-L aquaria in a greenhouse. Every effort was made to select juveniles of the same size for use in a particular aquarium. Eight juvenile pupfish, four from each of two competing species, were placed in each aquarium and al-

lowed to grow for 42 days. Three combinations of species pairs were studied: *C. pecosensis* and *C. variegatus* (9 replicates), *C. pecosensis* and F1 hybrid (8 replicates), and *C. variegatus* and F1 hybrid (5 replicates). For the first 21 days of a trial, all fish were fed approximately 0.65 g of a well-mixed and ground combination of equal masses of three different commercial fish foods (flake food and sinking granules, Tetra, Blacksburg, VA, and freeze-dried brine shrimp, San Francisco Bay Brand, Newark, CA). The ration was doubled for the last 21 days of a trial. We used this mixture to distribute food throughout the water column to minimize any inadvertent advantage provided to one species of fish by any one type of food. After the growth period ended, we euthanized fish with an overdose of MS-222 (methane tricaine sulfonate) and froze specimens until their length and mass could be measured. Presence of mature males (as indicated by breeding coloration) was documented.

All aquaria contained dechlorinated water mixed to 8 ppt with commercial, aquarium-grade sea salt. Aquaria were not internally heated, so temperature varied within and across days and across seasons. Aquaria were exposed to natural light and the photoperiod also varied across days and seasons. We conducted trials of this experiment in the summer (July–August) and fall (September–November) of 1998 and 1999. Water temperatures peaked at approximately 28–29° C during summer trials and 21–22° C during fall trials. Different aquaria in the same trial experienced the same temperature and light regimes because they were located in the same area of the same greenhouse. Temperature and light levels were also considered roughly equivalent for all fish in trials conducted during the same season of different years. Aquaria were fitted with undergravel filters. Aquaria were cleaned once during the 6-week growth period to eliminate excess food, waste products, and algae.

We calculated average length and mass of the four individuals of each species in each aquarium and used these means in analyses. Average length and average mass were analyzed separately with three-way analyses of variance (ANOVA). Species, combination of species in an aquarium ("combo"), and season were treated as main effects. The combo term allowed us to detect the effect of different competitors on the growth of each species. Variability between replicates (separate aquaria with the same combo) was accounted for by nesting replicates within season and combo. Planned comparisons were then used to determine the exact nature of significant effects.

Results

Mass-Length Relationships

ANCOVA on mass (log-transformed) with sex and species (*C. pecosensis*, *C. variegatus*, *Cv* × *Cp*, or *Cp* × *Cv*) as

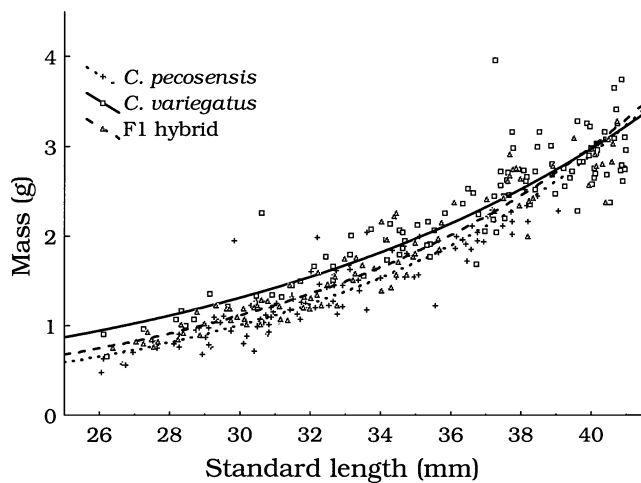


Figure 1. Mass-length relationships for *C. pecosensis*, *C. variegatus*, and their F1 hybrid offspring.

independent variables and length (log-transformed) as a covariate revealed that the $\log(\text{mass}):\log(\text{length})$ relationship was the same for both F1 hybrid crosses ($F_{1,290} = 2.09$, $p = 0.149$). There was no significant effect of sex on the $\log(\text{mass}):\log(\text{length})$ relationship ($F_{1,290} = 1.24$, $p = 0.266$), nor was sex a factor in any significant higher order interactions. Therefore, we combined the two hybrid crosses into one group (“F1 hybrid”) and combined male and female pupfish within species in subsequent analyses.

Different species had $\log(\text{mass}):\log(\text{length})$ relationships with significantly different slopes ($F_{2,300} = 9.037$, $p = 0.0002$; Fig. 1). Because of the significant interaction term, the main effects of the ANCOVA were not interpretable. Thus, we calculated linear $\log(\text{mass}):\log(\text{length})$ relationships for each species separately and analyzed the differences between these regressions using a Tukey test (Zar 1999) for each integer value of length from 26–41 mm (the range of lengths we measured for *C. pecosensis*). Over this range, *C. pecosensis* always weighed less, at any given length, than F1 hybrids ($q_{2,196} > 3.01$, $p < 0.05$; Fig. 1). Similarly, *C. pecosensis* that were 26–40 mm weighed less than *C. variegatus* ($q_{2,214} > 3.52$, $p < 0.05$); at approximately 41 mm in length, the weights of *C. pecosensis* and *C. variegatus* were statistically indistinguishable ($q = 2.64$, $p > 0.05$). For lengths between 26–38 mm, *C. variegatus* weighed more than F1 hybrids of equal length ($q_{2,190} > 2.94$, $p < 0.05$); for lengths between 39–41 mm, the weights of these two species were indistinguishable ($q_{2,190} < 1.97$, $p > 0.05$). For lengths between 27–36 mm (a typical size range for mature *C. pecosensis* in the field and the range used in our swimming endurance trials), *C. variegatus* weighed approximately 0.27–0.28 g (14–39%) more than *C. pecosensis* (Fig. 1). Over the same length range, F1 hybrids weighed approximately 0.09–0.14 g (7–12%) more

than *C. pecosensis* of the same length and 0.14–0.18 g (6–19%) less than *C. variegatus* of the same length.

Swimming Performance

The ANCOVA on U_{crit} with fish grouped into 1 of 10 sex-species combinations (e.g., male *C. variegatus*, female *C. variegatus*, male *Cv* × *Cp* hybrid, female backcross hybrid) and length as a covariate revealed significant differences among the groups ($F_{9,179} = 5.9347$; $p < 0.0001$). The slope of the relationship between length and U_{crit} was the same for all groups ($F_{9,170} = 0.70$, $p = 0.710$). No difference in U_{crit} was detected between the two hybrid crosses (ANCOVA planned comparison: $F_{1,179} = 2.04$, $p = 0.150$). Estimated mass (calculated using the species-specific mass-length relationships described previously) was substituted for length in the ANCOVA and also revealed significant differences in swimming ability across species-sex groups. We used length (which was measured directly) instead of mass (which was not) in subsequent swimming analyses. As a result of these findings, a simpler analysis was employed: (1) U_{crit} was divided by length to create a new variable (length-corrected U_{crit} ; Stahlberg & Peckmann 1987) that allowed comparison of swimming ability among fish of different sizes and (2) the two F1 hybrid crosses were combined into one group.

The resulting two-way ANOVA revealed that both sex and species affected length-corrected U_{crit} , and the sex*species interaction was not significant ($F_{1,3} = 0.15$, $p = 0.932$). On average, U_{crit} was 1.7 cm/second greater for males than for females (planned comparison: $F_{1,182} = 8.19$, $p = 0.005$; Fig. 2). Length-corrected U_{crit} was higher among *C. variegatus* (mean $U_{\text{crit}} = 26.9 \pm 3.45$ cm/second) than *C. pecosensis* (24.3 ± 4.02 cm/second; $F_{1,182} = 3.99$, $p = 0.047$; Fig. 2). No significant difference in swimming endurance was detected between F1 hybrids (mean $U_{\text{crit}} = 27.1 \pm 3.44$ cm/second) and backcross hybrids (29.3 ± 3.29 cm/second; $F_{1,182} = 2.26$, $p = 0.134$), so their scores were combined in subsequent analyses. Hybrids had greater length-corrected U_{crit} than *C. pecosensis* ($F_{1,182} = 19.01$, $p < 0.0001$; Fig. 2). Hybrids displayed higher length-corrected U_{crit} than *C. variegatus* but these differences were not significant (planned comparison: $F_{1,182} = 2.71$, $p = 0.101$). The data adhered well to the assumptions of equal variances among groups (Levene’s test: $F_{7,182} = 0.51$, $p = 0.827$) and normality of residuals.

Growth

We analyzed growth data with a three-way ANOVA with species, season, and combo as main effects. Variability between replicates (different aquaria with the same combination of species) was accounted for by nesting aquaria within season and combo. This analysis was conducted separately with mean length and mean body mass (log-transformed) as the dependent variable.

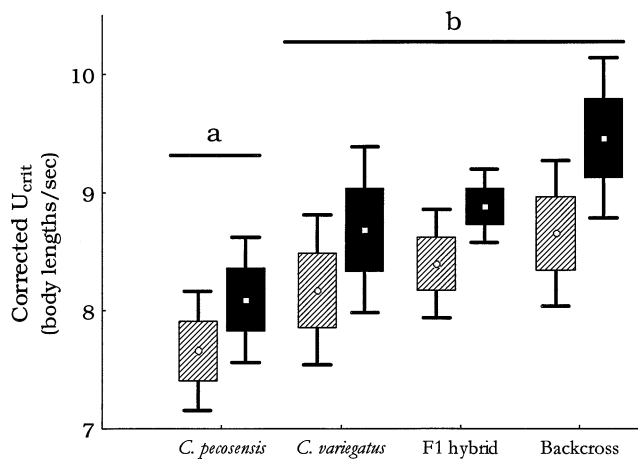


Figure 2. Corrected critical swimming speed (U_{crit} centimeters/second/standard length) of males (\square , black bars) and females (\circ , hatched bars) of *C. pecosensis*, *C. variegatus*, F1 hybrids, and backcross hybrids (approximately 40% *C. variegatus* alleles). No significant differences in swimming performance were detected between *C. variegatus* and both classes of hybrids (“b”); but, each of these groups had greater swimming endurance than *C. pecosensis* (“a”). Symbols represent mean corrected U_{crit} (boxes, ± 1 SE; whiskers, ± 2 SE).

For the overall analysis, species and season had significant effects on growth (Table 1). The species*season interaction was marginally significant. This interaction term reflected a greater response of *C. variegatus* and F1 hybrids to increased summer temperatures than the response displayed by *C. pecosensis* (Fig. 3). The interaction did not change the qualitative nature of the growth asymmetry; thus, the simple main effects were interpretable.

Table 1. Results of a three-way analysis of variance for differences in growth (increase in standard length) during trials involving pairs of pupfish species.*

Effect	df	MS	F	p
Intercept	1	12714.17	5264.18	<0.0001
Season	1	1365.48	50.95	<0.0001
Combo	2	10.15	0.38	0.691
Season*combo	2	4.68	0.18	0.841
Error ₁ = replicate (season combo)	16	26.80		
Species	2	76.29	31.59	<0.0001
Species*season	2	8.50	3.52	0.054
Species*combo	1	0.43	0.18	0.679
Species*season*combo	1	0.02	0.01	0.9274
Error ₂	16	2.42		

*Separate trials were conducted with each of three combinations (“combo”) of species: *C. pecosensis* and *C. variegatus*; *C. pecosensis* and F1 hybrids; and *C. variegatus* and F1 hybrids.

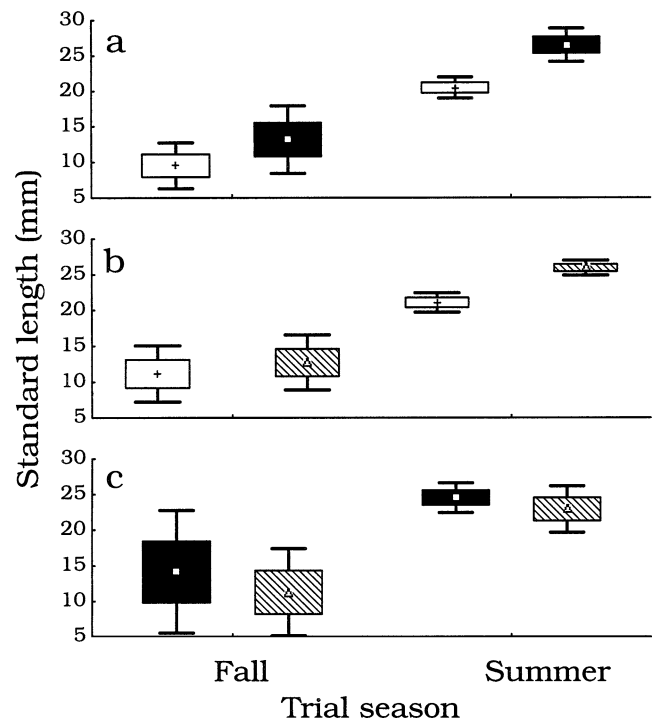


Figure 3. Differences in pupfish size after approximately 42 days in dyadic, competitive-growth trials conducted during two seasons: (a) *C. variegatus* (\square , solid boxes) and *C. pecosensis* (+, unshaded boxes) raised together; (b) *C. pecosensis* raised together with F1 hybrids (Δ , hatched boxes); (c) *C. variegatus* raised with F1 hybrids. Trials began with juvenile fish approximately 5.5 mm in length. Symbols represent mean lengths (boxes, ± 1 SE; whiskers, ± 2 SE).

C. variegatus juveniles grew significantly faster than *C. pecosensis* juveniles (planned contrast: $F_{1,16} = 42.80, p < 0.0001$; Fig. 3). Juvenile F1 hybrids also grew significantly faster than *C. pecosensis* juveniles ($F_{1,16} = 16.06, p = 0.001$, Fig. 3). Juvenile *C. variegatus* grew faster than F1 hybrid juveniles ($F_{1,16} = 4.94, p = 0.041$). Each species grew faster in summer trials than they did in fall trials ($F_{1,16} = 50.95, p < 0.0001$; Table 1, Fig. 3).

Our analysis revealed no interaction between a species’ growth rate and the species with which it was reared (Table 1). Therefore, our experiment uncovered no interspecific competitive interactions; however, by controlling for environmental variability, our experiment did reveal genetically based differences in growth rates. Stronger results were obtained when this statistical analysis was conducted with mass as the dependent variable, but the log-transformed mass data did not conform to the assumption of homogenous variances.

Fifteen male pupfish displayed breeding coloration (an indication of sexual maturity) before the end of the growth trial (age since fertilization, approximately 59

days). Of these, 11 males (7 *C. variegatus* and 4 F1 hybrids) matured in aquaria containing *C. pecosensis* and 4 males (3 *C. variegatus* and 1 F1 hybrid) matured in aquaria containing both *C. variegatus* and F1 hybrids. No mature *C. pecosensis* males were observed during growth trials. All mature males were observed in summer trials.

Discussion

Our analyses suggest that the extremely rapid genetic introgression between the two parent species was accelerated because hybrids were ecologically superior to the endemic, *C. pecosensis*. At equal length or equal mass, F1 hybrids, backcross hybrids, and *C. variegatus* each had greater swimming endurance than *C. pecosensis*. Results of previous studies have shown a correlation between U_{crit} and the ability of male Pecos pupfish to maintain desirable breeding territories (Kodric-Brown & Nicoletto 1993) and that male F1 hybrids and *C. variegatus* outcompete male *C. pecosensis* for such territories (Rosenfield & Kodric-Brown 2003).

The lack of decreased swimming performance among F1 or backcross hybrids suggests no incompatibility between the *C. variegatus* and *C. pecosensis* genomes. Indeed, there is evidence of synergy between the two (Rosenfield & Kodric-Brown 2003; Kodric-Brown & Rosenfield 2004; current study).

The rapid growth of F1 hybrids and *C. variegatus* relative to *C. pecosensis* probably amplified their ecological and behavioral advantages. At equal lengths, *C. variegatus* and F1 hybrids displayed greater swimming ability than *C. pecosensis*, and, among juveniles that hatched at the same time, *C. variegatus* and F1 hybrids were consistently longer than *C. pecosensis*. Thus, the discrepancy in swimming ability between *C. pecosensis* and the other pupfish is expected to increase with age. Also, at equal mass, male F1 hybrids and *C. variegatus* outcompeted male *C. pecosensis* for territories (Rosenfield & Kodric-Brown 2003). Our results reveal that these findings were conservative because, all else being equal, mature male *C. pecosensis* will be shorter and weigh less than male F1 hybrids or *C. variegatus*.

Swimming and growth advantages probably translate into a life-history advantage for *C. variegatus* and hybrids. Both size and swimming ability are positively correlated with the ability of male pupfish to establish and maintain breeding territories (Kodric-Brown & Nicoletto 1993). Body size (mass) is also usually important in determining when female fish mature (e.g., Healey 2001). Therefore, both F1 hybrid and *C. variegatus* should reach maturity faster than *C. pecosensis*. We saw evidence of this in our growth study, when several male F1 hybrid and *C. variegatus* reached maturity during the 6-week growth period but no mature male *C. pecosensis* were detected. Age at

first maturity can markedly influence overall population growth rates (Cole 1954).

Conservation Implications

Our results and results of previous studies of this hybridization system do not bode well for conservation or restoration of *C. pecosensis* in the wild. Pecos pupfish are less vigorous in terms of growth and swimming performance than sheepshead minnow or hybrids between the two species. In addition, female Pecos pupfish appear to prefer male *C. variegatus* over conspecifics, and male Pecos pupfish are inferior competitors for territories and mates compared with male *C. variegatus* and F1 hybrids (Rosenfield & Kodric-Brown 2003). Even though some populations of *C. pecosensis* may be more vigorous than the two populations studied here (Kodric-Brown & Rosenfield 2004), the lack of strong pre- or postmating isolating mechanisms between these two species and the ecological and behavioral attributes of hybrids mean that the remaining populations of *C. pecosensis* are at risk.

In two other cases where rare pupfish species have hybridized with *C. variegatus*, management activities have successfully prevented eradication of the native species. For example, hybridization and introgression between *C. variegatus* and *C. bovinus* (another pupfish endemic to the Pecos River watershed and a close relative of *C. pecosensis*) have been detected on two separate occasions. Both times, conservation biologists were able to save pure *C. bovinus* with a combination of selective trapping, application of piscicides, and reintroduction of the native species (Echelle & Echelle 1997); the success of this approach was facilitated by the limited geographical extent of the hybrid swarm. Hybridization between *C. variegatus* and *C. elegans* (the third pupfish species endemic to the Pecos River drainage) has continued in a limited fashion for >30 years, without significant introgression (Echelle & Echelle 1994). *C. variegatus* and *C. elegans* are distantly related (Echelle & Echelle 1992, 1994) and some mechanism(s) limits gene flow between the two species. In contrast, the introgression between *C. variegatus* and *C. pecosensis* is unlikely to be halted or reversed because (1) hybrids have such high fitness relative to their parental species; and (2) the hybrid swarm is geographically widespread, inhabiting an area surrounding over 500 km of the Pecos River and its tributaries in Texas and New Mexico (Childs et al. 1996; Echelle et al. 1997). Successful eradication of the hybrid swarm over such a large area and subsequent restocking with *C. pecosensis* seem logistically impractical.

Hybridization is often associated with environments that have been severely modified by human activities, leading to the common inference that the success of hybrid organisms depends on the existence of marginal habitats (Anderson 1948, 1949; Hubbs 1955). The Pecos River

is a highly disturbed environment that bears little resemblance to the river in which *C. pecosensis* evolved (Hall 2002). In a less-disturbed context, *C. variegatus* genes may have spread through the *C. pecosensis* genome more slowly (Kodric-Brown & Rosenfield 2004). But, our experiments (Rosenfield & Kodric-Brown 2003; current study) and the proliferation of hybrids in the wild (Childs et al. 1996; Echelle et al. 1997) occurred over a range of environmental conditions, and hybrids were superior to *C. pecosensis* in every fitness measure studied. Hybrids have even spread beyond the historically documented range of *C. pecosensis*. In this instance, hybrid superiority is an intrinsic phenomenon; modification of the Pecos River ecosystem was not the sole force behind hybrid success. Still, restoration of historical ecological conditions (principally a natural flow regime and riparian vegetation) might favor unique aspects of the *C. pecosensis* genome within the hybrid swarm.

Although the genetic introgression between these two species was unusually rapid, lessons learned from this event may inform efforts to protect other insular species. For example, this case illustrates the potential danger of efforts to increase gene flow between habitat "islands." The remaining populations of *C. pecosensis* are restricted to sinkholes and wetlands off the main channel of the Pecos River. This precarious conservation status is typical of many freshwater fishes because these species are usually isolated on relatively small freshwater islands (Rosenfield 2002) and, among island taxa, reproductive isolation is often only a by-product of geographical isolation (e.g., Grant & Grant 1996). Efforts to conserve freshwater organisms must balance the potentially catastrophic results of increased interspecific gene flow (e.g., via immigration corridors or intentional and unintentional translocations) with the possible benefits of interpopulation genetic exchange.

This and other cases of genetic introgression require conservation biologists to clarify, and perhaps reformulate, their conservation goals (Allendorf et al. 2001). Common reasons for protecting rare species include maintaining (1) genetic diversity and evolutionary potential; (2) heritable morphological and behavioral diversity; and (3) ecosystem functions (Nielsen 1995; Meffe & Carroll 1997). These goals are usually parallel, benefiting from the same conservation actions. But, after species' gene pools merge, conservation activities that promote one goal may be at odds with other goals. For example, because hybrids look and behave differently than either of their parents, the unique Pecos pupfish phenotype is jeopardized by hybridization. The close proximity of introduced *C. variegatus* genes threatens the remaining populations of *C. pecosensis*. Protection of the unique morphology and behavior of Pecos pupfish calls for efforts to eradicate hybrids from the Pecos River. On the other hand, hybrid pupfish carry the genetic markers that they inherited from *C. pecosensis* (Childs et al. 1996; Echelle et

al. 1997). Hybrids represent what remains of the unique genetic diversity that was found in the Pecos River's *C. pecosensis* population. Similarly, despite the ecological and behavioral differences between hybrids and Pecos pupfish, a Pecos River ecosystem with pupfish (even hybrid pupfish) is more like the historical Pecos River than one without pupfish. Now that the hybrid swarm has replaced *C. pecosensis* throughout much of the Pecos River drainage, eradication of the hybrid swarm conflicts with the goal of maintaining ecosystem function. Hybridization and genetic introgression are always best avoided, but, after introgression has occurred, the management response to a hybrid swarm depends on one's conservation goal. In this particular case, the success of hybrids between Pecos pupfish and sheepshead minnow and the spatial extent of the hybrid swarm appears to have foreclosed certain options.

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