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Survival of Intergroup Percid Hybrids

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Abstract 264 Intertribial percid hybrid tests were made among the Percini, Luciopercini and Etheostomatini. Only those between the Luciopercini and Etheostomatini (both reciprocals) fed, indicating a close phylogenetic relationship. A series of morphologic characters also support separating the Percini from the other two tribes of North American Percidae.

Fish eggs can be fertilized by sperm of related species and most of the putative natural hybrids are between relatively closely related species (Hubbs, 1955). The paternal and maternal chromatin of relatively distantly related taxa may interact in the environment of the egg cytoplasm to initiate development and the amount of successful ontogeny is reasonably closely associated with the accepted phylogenetic relationship (Hubbs, 1967a). The relationship of hybrid survival to morphologically based phylogeny has been shown for cobitids (Suzuki, 1957), cyprinids (Suzuki, 1968), centrarchids (Hester, 1970), cyprinodonts (Hubbs and Drewry, 1959), salmonids (Simon and Noble, 1968), and many others. The overall concordance of hybrid experiments and classification (Nikoljukin, 1952; Kryzhanovsky, 1968) permits application of hybrid survival to help in determining phylogenetic relationships when morphological data are conflicting.

Many percid hybrids have been produced artificially. Hybrids between *Stizostedion vitreum* and *S. canadense* have been reared by Nelson (1968), hybrids between *Perca fluviatalis* and *Gymnocephalus* (=*Acerina*) *cernua* have been reported by Kammerer (1907), and a series of crosses among darters reported by Hubbs (1967b) who also reported less success in interfamilial crosses. Despite all of the tests on percid hybrids, none have been done between darters and other percids and laboratory tests between *Perca* and *Stizostedion* had severely deformed larvae (Kammerer, 1907; Balon, 1956) in striking contrast to the putative "normal" natural hybrid reported by Peippo (1962).

Collette (1963) discussed the various methods of arranging the percid genera and the following summary is based on his tribal groupings. The darters (Etheostomatini) are a nearctic compact group of small benthic fishes comprising approximately 100 species in three or more genera, *Percina, Ammocrypta*, and *Etheostoma*. The walleyes (Luciopercini) are a group of 5 closely related large piscivores usually placed in one genus (*Stizostedion*). The perches (Percini) are a group of 7 species in 3 genera, *Perca, Gymnocephalus*, and *Percarina*. The fourth group is an eurasian group (Romanichthyini) of four species in two genera, *Zingel* and *Romanichthys*.

This paper reports on the survival of hybrids between species representing the first three tribes and of representatives of those percid tribes with a variety of available species. The three groups have been arranged with the following relationships: perches and walleyes in one group contrasted with darters (Jordan, 1923); perches and darters, in one group different from but related to walleyes (Collette, 1963); and the three groups of equal taxonomic rank (Hubbs and Lagler, 1958). The hybridization results support the last interpretation but are in best accord with a grouping of darters and walleyes contrasted with the perch and its allies.

Materials and Methods

The gametes were removed from the parents and the eggs and embryos incubated following techniques developed by Strawn and Hubbs (1956) as modified by Hubbs (1971). Yellow perch eggs adhere to each other in large one-layered mats and strings. After fertilization the eggs were divided into smaller units by cutting with scissors. The ripe adults were maintained in sexual isolation to reduce spontaneous mating. *Stizostedion* females retained their eggs until the eggs were stale but egg strands extruding from *Perca* females were used successfully in hybridization tests. *Stizostedion* males could be used successfully on the day following depletion of sperm supply by stripping but *Perca* males could not be reused once the sperm had been depleted.

The first series of tests were done April 1, 1969. The crosses were made at the portable laboratory of the Texas Parks and Wildlife Commission on the shores of Lake Meridith, near Fritch, Hutchinson County, Texas. Hybridization was done at lake water ambient temperatures (ca 10°C) and the eggs returned to Austin in petri plates in styrofoam coolers. The temperatures were 15°C or less upon arrival in Austin where the eggs were placed in enamel rearing trays at 15°C. Because of instrumentation problems, the room temperature soon reached 20°C and remained there for the rest of the test interval.

The stocks used came from the following Texas localities: *Stizostedion vitreum*, Lake Meridith at Fritch, Hutchinson County; *Percina caprodes, Etheostoma lepidum, E. spectabile, Notropis venustus, Dionda episcopa*, and *Lepomis auritus*, Llano River at Junction, Kimble County; *Esox lucius*, stock tank near Lake Meridith, and *Morone chrysops*, Lake Nasworthy 5 km southwest of San Angelo, Tom Green County.

The second series of tests were done in April, 1970. A few darter eggs were stripped in Ann Arbor and held 1–2 days at 10°C and returned to Austin in styrofoam containers as were all the 1969 tests. The rest were done in Austin where the incubation temperatures available were 9.5°C, 12.5°C, and 16.5°C. Eggs were placed in enamel rearing pans at the start of each test. Each lot of *Perca* and *Stizostedion* eggs exceeded 100 and frequently a lot was split as development progressed and rearing space became available. Often some of the eggs were moved from a cold to a warm incubation temperature.

The stocks used came from the following sources: *Perca flavescens*, Saline hatchery, Michigan Department of Conservation; *Stizostedion vitreum*, mouth of Thames River, Tilbury, Ontario, Canada; *Percina sciera*, Old Town on San Marcos River, Hays County, Texas; *Etheostoma spectabile*, Old Town on San Marcos River, and Big Maries River at Westphalia, Missouri; *Etheostoma caerulum*, *E. blennioides, E. tetrazonum*, and *E. zonale*, Big Maries River; *Gambusia affinis*, Clear Creek, 16 kilometers west of Menard, Menard County, Texas; *Fundulus chrysotus*, Martin Dies State Park, Jasper County, Texas; *Platypoecilius helleri*, aquarium stock; *Lepomis macrochirus*, San Marcos River in San Marcos, Hays County, Texas; *L. microlophus*, Martin Dies State Park; *Chaenobryttus coronarius*, farm pond 6 kilometers northeast of Manor, Texas; *Lepomis punctatus*, San Marcos River in San Marcos, Hays County, Texas; *L. cyanellus*, San Marcos River in San Marcos; *Haplochromis*, aquarium stock; *Cichlasoma cyanoguttatum*, San Marcos River in San Marcos.

Two different types of indices may be used to indicate hybrid success (1) duration of hybrid survival and (2) development stage achieved by the hybrids. Usually the two factors are similar but when discordant the former has recently been discounted. Slight differences in experimental conditions (typically temperature) can cause vastly different developmental rates in percids (Hubbs, *et al.*, 1969) and survival times if the hybrids and/or controls were to die at a specific ontogenetic stage but be reared at different temperatures.

Even without this distortion a time emphasis is hazardous. As early as 1910 Moenkhaus reported that hybrids often had reduced developmental rates and Hubbs and Drewry (1959) showed that the delays were often associated with reduced survival. Finally, Blair (personal communication, 1970) and Pierce (personal communication, 1970) have reported bufonid and hylid hybrids that remained in larval stages for more than 1 and 2 years respectively after the controls metamorphosed. Retarded developmental rates would be expected of animals which had contrasting (and conflicting?) genetic messages; or hybrids would be predicted to slow development when their parental genomes were distinctly different. Therefore, I have used the developmental stage achieved as an index of hybrid success even though percid hybrids and controls had comparable developmental rates at the same temperature.

Results

Three groups of eggs were used in the 1969 tests, one series (*Esox lucius*) provided no useful data because all experiments failed to gastrulate. This may be due to the extraordinarily high temperatures (15–20°C) used for incubation or because the female used may have been egg bound. The other eggs were all percids and results were in accord with current classifications.

Stizostedion vitreum eggs were exposed to visible quantities of *Dionda episcopa* sperm (3 tests with 3 males) and both mashed testes from a *Lepomis auritus* and in none of the 5 tests was any gastrulation noted. Walleye eggs were also exposed to *Morone chrysops* sperm in 3 separate tests with 3 males. All 3 had gastrulated eggs on day 3, abnormal embryos on day 4, and pigmented eyes (2,5, and 11) on day 5. Only one young with a stubby tail hatched. Its heart was beating but it did not swim off of the bottom. Two others broke the egg shell but died with their heads still in the shells. The others had sunken heads and died without advanced embryonic development. All 3 tests died on day 9 or 10. Walleye eggs were exposed to *Esox lucius* sperm in 10 tests. Abnormal embryos were noted in 7 tests on the fourth day, and in 4 of these tests none of the 2,3, 5 and 8 embryos cephalized and all were dead by the 8th day. The other 3 tests had one (from 4 embryos), 2 (from 12 embryos), and 4 (from 45 embryos) cephalized embryos on the fifth or sixth days. The 4 in the last test developed pigmented eyes on a head sunk into the yolk mass. No heart was observed and all embryos were dead by the ninth day.

Tests of *Stizostedion vitreum* eggs with percid sperm were more successful. All 3 control tests had embryos on the third day, had tail free embryos on the fourth day and pigmented eyes (2, 15, and 12) on the fifth day. Hatching (87%) occurred on the seventh through ninth days and the last young died on the 12th to 13th days, apparently from starvation. The 3 tests with *Etheostoma spectabile* sperm all gastrulated on the third day, had tail free embryos on the fourth day, and had pigmented eyes (29, 62, and 152) on the fifth. The first 2 listed tests had only one and 2 (respectively) normal embryos hatched but the last had 107 mostly abnormal young. The test with 2 embryos was ended on the 13th day but the other 2 lasted until the 28th. The 3 tests with *E. lepidum* sperm all gastrulated on the third day, had embryos on the fourth, and had pigmented eyes (18, 16, and 22) on the fourth or fifth days. Only 3 embryos hatched, 2 abnormal from the second and one normal in the last. The abnormal young were dead on the 11th day but the other lived 28 days. The 3 tests with with *Percina caprodes* sperm gastrulated the third day, had embryos the fourth day and pigmented eyes (22, 22, and 94) the sixth day. The first had none hatch, the second 17 mostly abnormal hatch the last of which died on the 25th day and the last had 10 normal young that lived until the 28th day. All darter-walleye combinations had some feed-

ing larvae.

The reciprocal tests were parallel. Controls of *E. spectabile* were similar to those reported by Hubbs (1967b), but are listed here because of the unusual thermal stresses involved. They gastrulated the third day, had embryos the fourth, had pigmented eyes the sixth and 90%+ hatched on the eighth and ninth days. Five of the 6 tests of *E. spectabile* eggs exposed to *S. vitreum* sperm developed (the other had non-spherical=immature? eggs). They gastrulated the third day, had embryos some with their tails free on the fourth day and pigmented eyes (14, 21, 16, 15, and 29) the fifth day. 95% hatched and most seemed normal. The first had all dead on the tenth day and the last survived until the 21st day while the other 3 were gone on the 17th but had fed. The 3 tests with *E. lepidum* eggs gastrulated the third day, had embryos, some with tails free, the fourth day, and had pigmented eyes (83, 17, and 50) on the fifth or sixth days. All hatched and the last young died on the 13th, 16th, and 17th days respectively, the last two tests included young that fed. Only 3 of the 6 tests with *P. caprodes* eggs developed (the other 3 used eggs from the same female). The 3 successful tests gastrulated the third day, had tails free the fourth day, and had pigmented eyes (22, 33, and 14) the fifth day. 93% Hatched and the last embryos died the 13th, 14th, and 15th days respectively.

The *Stizostedion vitreum* control young swam toward the surface upon hatching and were often free swimming the second day. The *Etheostoma spectabile* control young remained on the bottom for one week after hatching. Both reciprocals of the hybrids might be free swimming but each hybrid would settle to the bottom for prolonged intervals—the *S. vitreum* controls only did this when dying.

In 1970, the *Stizostedion vitreum* controls were started at 9.5°C (4 times) and 16.5°C (4 times). Those exposed to the cold incubation temperatures were much more successful than those placed in hot temperatures. Only one egg hatched but it lived until its yolk sac was absorbed as did those maintained at 9.5°C. The developmental rate at 16.5°C was notably faster than that at 9.5°C,—*i.e.* the rate for embryo formation was 2/3rds and for later stages about 1/2 (Table 1). Obviously the effects of temperature on developmental rate are greater just before hatching than just after fertilization. Because only one test survived to the end of the experimental interval, the duration of survival may provide the best index for determining optimal conditions. Use of absolute time would ignore thermal effects so a rate calculation was used that had one day at 16.5°C equivalent to two days at 9.5°C and 1.5 days at 12.5°C. Most transfers do no better than those held at constant temperature. The few that did were too scattered to provide a distinct pattern but 12.5°C seems to be a better temperature than either 9.5 or 16.5°C.

The survival of the *Stizostedion* controls shows that there is a racial difference in maximum developmental temperature tolerance. Eggs from Lake Meridith, Texas (introduced from Iowa) develop at 20°C whereas eggs from the Thames River, Ontario, have trouble at 16.5°C.

The walleye eggs incubated but not exposed to sperm did not gastrulate.

Walleye females were crossed with yellow perch males each with copious supplies of milt 9 times, 4 at 9.5°C and 5 at 16.5°C. Two of the 9.5°C samples had subsamples transfered to 16.5°C. None of the 1000+ eggs gastrulated.

Walleye eggs were exposed to darter sperm 8 times. The 2 incubated at 16.5°C did not gastrulate but all of those at 9.5 did. The 2 tests with *E. blennioides* sperm gastrulated the sixth day, had tails free on the 10th and 12th days, had eyes pigmented the 14th and 15th days and hatched the 21st through 23rd days (5 of 9 with pigmented eyes). The young lived until the 35th to 37th days. Both series had been split and transfered to 12.5°C on the 9th day. Five of 6 eggs with pigmented

Table 1. Survival of young *Stizostedion* maintained at hatching temperatures.

Days at 9.5°C	Days to achieve various ontogenetic stages						
	0	1	2	3	All 9.5°C (4 tests)	9	9
		Transferred to 16.5°C				Transferred to 12.5°C	
Embryo	4	3	2	1	6	—	—
Tail free	?	6	5	4	8–9	—	—
Pig eye	7	7	6	6	14	3	3
Hatch	10	—	9	7	23(21–25)	8	9
Dead	16	10	19	12	46–47	28	36
Index days	32	—	40	27	46–47	51	63
							survived ←

Survival of young transferred to warmer temperatures after hatching		
Transferred 9.5–12.5°C	9.5 to 16.5°C	9.5– 12.5– 16.5°C
25+12*=43	21+24**=69	9+10*+ 3**=30
30+ 7*=40	21+24**=69	9+10*+ 7**=38
	22+ 2**=28	9+11*+ 2**=30
	22+ 4**=30	9+11*+11**=48
	23+ 3**=29	9+12*+ 6**=39
	23+ 7**=37	9+12*+11**=49
	25+ 3**=31	9+13*+10**=48
	28+ 7**=42	9+14*+18**=66
	28+11**=50	9+15*+21**=74
	32+ 1**=34	
	32+ 4**=40	
	32+15**=62	

* 1 day at 12.5°C is considered to equal 1.5 days at 9.5°C.
** 1 day at 16.5°C is considered to equal 2 days at 9.5°C.

eyes hatched and one survived the experimental period. Three were observed to have large quantities of brine shrimp in their guts and to have grown in length and bulk. Walleye eggs were exposed to *E. caeruleum* sperm 3 times. At 9.5°C the eggs gastrulated the sixth or seventh days and had tails free the tenth day, had eye pigmentation the 13th day and hatched the 17th day. The survival was low (only one of 5 eggs with pigmented eyes hatched) but the hatched young lived 30 days. One lot of walleye eggs was exposed to *E. spectabile* sperm. The eggs gastrulated the sixth day, had tails free the ninth day, pigmented eyes the 12th day, and hatched the 22nd day. The hybrid lived until the 32nd day. This lot was also split and the sublot incubated at 12.5°C. Two eggs hatched the 16th day and both fed on brine shrimp but died the 47th day.

All of the young walleye darter hydrids were recorded as being on the bottom about half of the time and free swimming the other half of the time.

Walleye eggs were also exposed to sperm of the following species: *Fundulus chrysotus* (3 times), *Gambusia affinis* (2), *Platypoecilius helleri*, *Cichlasoma cyanoguttatum* (2), *Haplochromis*, and *Lepomis microlophus* (2). None of the eggs gastrulated.

The egg complements of 7 darters were exposed to walleye sperm. All were incubated at 16.5°C. Only 3 had gastrulation. The test with *E. blennioides* eggs gastrulated the third day, had pigmented eyes the seventh day but did not hatch. The 2 tests with *P. sciera* eggs gastrulated the third day, had tails free the fifth day, had eyes pigmented the sixth day and hatched the twelfth day. The only young that hatched lived 20 days.

Controls of *Perca flavescens* were done at 9.5, 12.5, 16.5°C and all possible increasing temperature combinations. It soon became obvious that developmental rate was inversely correlated with egg number in the incubation chamber, and that development was virtually nil at 16.5°C (only 2 of 6 tests gastrulated and no eggs lived longer than 5 days incubation). The developmental rate at 12.5°C was more rapid than that at 9.5°C as shown in parentheses, gastrulation occured after 4 (5 to 6) days, eyes formed in 5 to 6 (6 to 8) days and pigmented in 11 to 12 (16) days, and the tail was free in 7 (10) days, straight in 11 or 12 (16) days. Three of the 7 samples at 12.5°C fed as did one of 9 at 9.5°C. Several samples were transferred to 16.5°C and those that had gastrulated usually developed pigmented eyes and had straight tails. Two sets of eggs transferred from 9.5 to 12.5°C fed as did one each of those transferred from 9.5 to 16.5°C and 12.5 to 16.5°C at about gastrulation.

Yellow perch eggs are imbedded in a large jellatinous mass and may break out at about the time the eyes form or wait until after they become pigmented. Early (premature?) young do not move and seldom survive to swim free in the water. Successful young free swim at about the time the eyes pigment.

Nine lots of *Perca flavescens* eggs were placed in incubation chambers without being exposed to sperm. Four contained eggs in which gastrulation occurred. The first lot was suspected of being contaminated due to sloppy technique but the others were done with care—before any males were stripped that day. Three of the tests were at 9.5°C and the other at 12.5°C. The developmental rates for 9.5°C (12.5°C in parentheses) were gastrulation in 8(7) days, eyes in 10 days, but never pigmented, and tail free in 11 to 13 days. None of the young were observed to have a heart beat and all had the tail curled down over the yolk mass. All of the embryos developed at rates significantly slower than those of the "fertilized" controls and are interpreted to have uniparental origin.

Yellow perch eggs exposed to copious supplies of walleye milt were incubated at 9.5°C (13), 12.5°C (9), and 16.5°C (3). None of the eggs in the 16.5°C tests gastrulated. The rates for 9.5°C are slower than those at 12.5°C (in parentheses): gastrulation occurred after 4 or 5 (3 or 4) days; eyes formed in 8 to 10 (5 to 6) days and pigmented in 17 (12) days, tails were free in 7 to 11 (6 to 7) days and straight in 17 days. None of the young at 12.5°C developed straight tails and none of those in either temperature were free swimming as also occurred in the 20 other tests in which incubation temperatures were changed. Likewise none of the young were observed to feed and all but one young died with large yolk sacs.

Yellow perch eggs were exposed to *E. spectabile* sperm numerous times. None of those incubated at 16.5°C gastrulated. Those at 9.5°C developed slower than those at 12.5°C (in parentheses): the eggs gastrulated in 4 or 5 (3 or 4) days, the eyes developed in 7 (5) days and pigmented in 15 or 16 (10 or 11) days, the tail was free in 7 (6) days and straight in 12 (10) days. Most young hatched normally; however all young incubated at 9.5°C (21 pans), 12.5°C (18 pans) and at increasing temperatures (63 pans) failed to feed. Yellow perch eggs were also exposed to *P. sciera* sperm 4 times. At 9.5°C the eggs gastrulated after 5 days (3 or 4 at 12.5°C); the eyes developed after 7 (5 at 12.5°C) days and pigmented after 14 (10 at 12.5°C) days, the tail was free after 8 (7 at 12.5°C) days and straight after 13 (10 at 12.5°C) days. Most young hatched normally but none were observed to have fed in the 18 incubation pans. Yellow perch eggs were exposed to *E. blennioides* sperm 5 times. The one incubated at 16.5°C did not gastrulate but those at 9.5°C gastrulated in 4–5 days, developed eyes before the tenth day and had eye pigmentation by the 16th day, and the tail was free in 11 days and straight in 16 days. Many hatched in the 4 held at constant temperature and the 4

subsets transferred to 12.5°C, but none fed. Yellow perch eggs were exposed to *E. caeruleum* sperm 4 times. Only one of the three tests at 16.5°C gastrulated and that single egg developed no farther. The one at 9.5°C had eye pigmentation and heart beat but did not have a straight tail and did not feed; the subset transferred to 12.5°C also did not feed. Yellow perch eggs were exposed to *E. tetrazonum* sperm once at 9.5°C and the sample subdivided with one transferred to 12.5°C. Both lots developed pigmented eyes and heart beat but had curled tails and did not feed.

Yellow perch eggs were exposed to sperm from *Lepomis macrochirus, L. cyanellus, L. punctatus,* and *Chanenobryttus coronarius.* Embryos formed in all but the *L. macrochirus* tests. Those tests incubated at 16.5°C failed to gastrulate, but those at 9.5 and 12.5°C developed embryos in 5 or 6 days (9.5°C) and 4 days (12.5°C), the tails were free in 10 (9.5°C) and 6 (12.5°C) days, and straight in 17 (9.5°C) and 8 (12.5°C) days, the eyes formed in 8 (9.5°C) and 5 (12.5°C) days but none pigmented in any of the 3 possible combinations.

Yellow perch eggs were exposed to *Cichlasoma cyanoguttatum* sperm 3 times and only one lot gastrulated, but it did not progress farther.

Darter eggs were exposed to copious supplies of yellow perch semen several times. All were incubated at 16.5°C, because most darter eggs do not survive at lower temperatures (Hubbs, 1967b). The test with *Percina sciera* eggs had 8 gastrulated eggs on the fourth day and 3 embryos with pigmented eyes on the sixth day but no farther development. Six of the 7 tests with *Etheostoma caeruleum* had some eggs gastrulate but only one embryo developed pigmented eyes and it died the next day. All 4 tests with *E. blennioides* eggs had some eggs gastrulate but only one test had any (10) embryos develop pigmented eyes. None hatched or developed a heart beat. All 3 tests with *E. tetrazonum* eggs had more than 30 eggs gastrulate, of these 41% developed pigmented eyes but none developed further. The single test with *E. zonale* eggs did not gastrulate. Seven of the 8 tests with *E. spectabile* eggs had some gastrulate and of these 68% developed pigmented eyes in 6 to 7 days. One egg hatched in 8 days but the young was dead, 10 others had a heart beat.

Discussion

Hybridization tests may be plagued with problems associated with exclusive maternal development. Usually I have been concerned with stimulation of the egg by heterospecific sperm that did not contribute to subsequent ontogeny. The yellow perch tests showed another method by which this problem could develop. Several sets of yellow perch eggs not exposed to any sperm gastrulated and many proceeded to late embryonic stages. The first example was suspected to involve contamination through sloppy techniques, but later tests were done to reduce this potential to virtual zero. The females had been maintained in the laboratory, isolated from males for 10 to 18 days. The first eggs removed from the female were used for the nonfertilization experiment so that contamination could not come from that series of tests (Lindroth, 1947, showed that *Perca fluviatilis* sperm viability is measured in minutes), and the previous tests preceeded these by as much as 4 days. Contamination during experimental procedures is, therefore, essentially impossible.

If the eggs develop from uniparental sources, two alternative explanations remain. The female may have had an ovotestis (reported in *Perca* by Brunelli and Rizzo, 1928, and widely occuring in the related serranids as discussed by Smith, 1965), or the eggs may have developed without any sperm. The latter alternative is supported by the developmental data. All stages took longer to develop than they did in the fertilized controls and survival was exceedingly low. Although these circumstances could occur in self-fertilized eggs, the degree of expression is more likely to be extreme in functionally haploid development.

The exact causes were not an objective of this study, but the phenomenon relates to interpretations of hybrid experiments. Temperature shock seems not to be involved because most of the tests were run at 9.5°C, the temperature at which the parents had been maintained for up to 18 days. All hybrid tests were no more successful than those of nonfertilized controls, may have been based on that type of development.

None of the eggs in the numerous similar nonfertilized tests with darters and walleyes gastrulated. More than 2000 darter eggs (Hettler, 1970) and 500 walleye eggs without exposure to sperm were isolated and incubated in a manner similar to the perch eggs.

The close affinity of darters and walleyes is supported by all of the hybridization data. Although walleye eggs fertilized by darter sperm usually had severe prehatching mortality, the reciprocals often had more than 90% hatching. The young (both reciprocals) were vigorous and fed on available food supplies and could be reared through critical early feeding stages as well as or better than the walleye controls.

The experiments with yellow perch and darters were less successful. Only one of the 21 darter complements (5 species) fertilized by yellow perch sperm had any hatching. The single hatched young died less than 24 hours after hatching. No swimming was observed. The reciprocal experiments were more successful. Many young hatched and appeared normal. They developed most attributes at about the time the characters appeared in the controls but typically stayed on the bottom after hatching whereas the maternal controls were free swimming. All hybrids (136 incubating lots) died prior to feeding, but many lived until the yolk sac was absorbed.

The yellow perch × walleye hybrids had many vertebral deformities such as the severe dorsal bending of the vertebral column figured by Balon (1956) and reported by Kammerer (1907). The agreement of our results is likely to extend to the reciprocal experiment as Balon and Kammerer reported only on tests with *Perca* eggs and may have attributed the absence of development of *Stizostedion* = *Lucioperca* eggs to technique difficulties.* Our results are strikingly discordant with the putative hybrid reported by Peippo (1962). Because the specimen has not been located recently (Ryder, Koli, and Toivonen, all personal communications, 1971), the specimen has not been re-examined. The specimen was contrasted with kuhan (=*Stizostedion lucioperca*) and ahvenen (=*Perca fluviatilis*) and seemed intermediate in morphology. The soft dorsal rays were emphasized (13–14 in *P. fluviatilis*; 21–22 in *S. lucioperca*, and 17 in the specimen). Apparently *S. volgensis* was also examined as an alternative explanation; however, both *S. canadense* and *S. marina* may have 17 soft dorsal rays and the putative hybrid could merely be a *S. marina* that migrated to the Baltic.** Because the hybrid nature of this fish is discordant with all experimental evidence and other alternate explanations are available, I consider that the putative hybrid is a misidentified *Stizostedion*.

Yellow perch × walleye hybrids have drastically different results dependent upon the reciprocal tested. Walleye eggs crossed with yellow perch sperm do not gastrulate; the reciprocal tests typically gastrulated and the development was approximately parallel to that of the controls until tail free stage, after which development slowed. Those young that hatched remained on the bottom and did not swim unless the incubation pan was severely agitated. No feeding occured similar to that common in walleye—darter hybrids.

Walleye eggs exposed to serranid sperm did develop. The development was distinctly less than that shown by walleye—darter hybrids, but far better than that of the walleye eggs

* Balon (personal communication, 1971) confirms this hypothesis.

** Peippo (personal communication, 1971) reports that *S. marina* was not compared with the hybrid.

tested with *Perca* sperm. These results parallel the success of darter—serranid hybrids (Hubbs, 1967a). Unfortunately, no serranid males were available to test with the *Perca* eggs, and no tests have been done using serranid eggs.

Yellow perch eggs exposed to centrarchid sperm develop farther than the nonfertilized controls and the fraction to gastrulate was typically above 50%, whereas less than 10% of the nonfertilized controls produced embryos. This hybrid survival shows that yellow perch and sunfishes have a genetic similarity. Darter eggs fertilized by sunfish sperm reach an equivalent developmental stage (Hubbs and Strawn, 1957). The failure of the walleye eggs exposed to *Lepomis* sperm may be an artifact but is parallel to the *Perca* sperm results.

All tests with very differently related taxa failed. The failures may be due to phylogeny or technique problems. For example the *Cichlasoma*—percid hybrids might be unable to survive at low temperatures (*Cichlasoma* lethality) and high temperatures (*Stizostedion* or *Perca* lethality). Similar problems could ensue with many of the other tests attempted.

The genera of percid fishes have been grouped into several subfamilial units. If we exclude the genera (*Zingel* and *Romanichthys*) not incorporated into hybrid studies, the three groups, darters, walleyes, and perches have been arranged in three ways; (1) *Perca* and *Stizostedion* (and the fishes now in *Zingel*) in one family Percidae and the darters in another the Etheostomatidae; (2) *Perca* and darters in the Percinae and *Stizostedion* (and *Zingle*) in the Luciopercinae; and (3) *Perca* in the Percinae, *Stizostedion* in the Luciopercinae, and darters in the Etheostomatinae. The first two arrangements listed are discordant with the hybridization data. Although the *Perca* × darter and *Perca* × *Stizostedion* hybrids hatched, neither was seen to ingest the food that the *Stizostedion* × darter hybrids ate and metabolized. Therefore, the three groups should be considered to be of equivalent taxonomic ranking, or preferably the darters and walleyes to be grouped together.

The grouping of *Perca* and *Stizostedion* seems to be based on Jordan's (1923) statement about the Etheostomatidae "They differ from the Percidae in having six branchiostegals instead of seven, the head unarmed and the airbladder obsolete or nearly so." The airbladder of darters varies in its development and that variation (as yet not reported in detail in the literature) seems to be as great as the variation between darters with large air-bladders and *Stizostedion* or *Perca*. Collette's report that *Perca* and *Stizostedion* usually have 7 but occasionally 6 and darters usually have 6 but rarely 7 branchiostegals is in contrast to Jordan's use of that attribute to separate the taxa. The armored head (and large mouth) are attributes common in large piscivorous fishes but unexpected in small arthropod eaters and could be merely convergence on a similar adaptive peak. Because the *Perca* × *Stizostedion* hybrids have more difficulties than either other intergroup combination, the validity of this grouping is exceedingly dubious. In contrast, the morphological attributes cited linking them, seem equivocable.

The grouping of *Perca* and darters was proposed by Collette (1963) who summarized the Percinae "Percidae with the anteriormost interhaemal bone greatly enlarged; anal spines usually large and well developed; lateral lines usually not extending onto caudal fin and supplementary lateral lines on the caudal fin absent except sometimes in *G. schraester*." The morphological basis of the grouping apparently is limited to three structures. Two are stated to be equivocable by Collette which leaves one apparently unequivocable. Because the anterior interhaemals would be expected to support the anal spines, the size of the two should be linked. If so, the development of the interhaemals in darters with small anal spines might also be minimal and that character also equivocable. Although *Perca* × darter hybrids do better than *Perca* × walleye

hybrids, the present tests do not show development to stages typically achieved by *Stizostedion* × darter hybrids; therefore, if two groups are to be allied, it is the last two.

An extensive analysis of the morphological features of the three groups of percid fishes found in North America is beyond the scope of this investigation, but some seldom used characters have been noted. Male *Perca flavescens* has copious supplies of milt, but when that is expended no more could be removed in the following two weeks; in contrast, males of *Stizostedion vitreum* and a large variety of darters could renew their semen supply even if held without food. Eggs of *P. flavescens* are expelled in large numbers and adhere to each other and not to the substrate, in contrast, eggs of *Stizostedion vitreum* and darters are expelled in small numbers and adhere to the substrate. Eggs of *Perca flavescens* are enclosed in a soft gelatinous mass, whereas *Stizostedion vitreum* and darter eggs are enclosed in a hard chorionic shell. Developing embryos of *Perca flavescens* are typically capable of free swimming prior to eye pigmentation, whereas embryonic *Stizostedion* and darters have pigmented eyes long before normal hatching, and if hatched prematurely before eye pigmentation, are incapable of locomotion. The last attribute of *Perca* is similar to conditions prevalent in *Lepomis* ontogeny and *Perca* × *Lepomis* hybrids develop to late ontogenetic stages.

Conclusions

Survival of intergroup percid hybrids suggests that *Stizostedion* and darters are at least as closely related to each other as either group is to *Perca*. Therefore, if two groups are to be allied, the walleyes should be placed in the Etheostomatinae, and *Perca* and its allies would be the only tribe in the Percinae. The Etheostomatinae would then be divided into three tribes, the Etheostomatini, Luciopercini, and Romanichthyini.

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Percidae における tribes 間雑種の生残能力について

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ペルカ科 (Percidae) の三つの tribes, すなわち, Percini, Luciopercini および Etheostomatini の間での交雑合計 264 が行なわれたが, この中で Luciopercini と Etheostomatini (逆交雑両方向について) の組み合わせのもののみが生育した. このことは, この両 tribes が系統的に近縁であることを示す. 一連の形態学的形質も Percini が北部アメリカにおける Percidae の他の二つの tribes とはっきり区別されることを支持している.

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