

Document-ID: 2194476

Patron:

Note:

NOTICE:

Pages: 31 Printed: 01-18-12 11:47:21

Sender: Ariel/Windows

Texas A&M University Campus Libraries
Courier



ILLiad TN: 2194476

Journal Title: Journal of Experimental Zoology

1/18/2012 7:45 AM
(Please update within 24 hours)

Volume: 134

Issue: 1

Month/Year: 1957

Pages:

Call #: QL1 .J85

Location: evans

Article Author: Hubbs and Strawn

Not Wanted Date: 07/14/2012

Article Title: Survival of F1 Hybrids between fishes of the subfamily ethostominae

Note:

Status: TAES San Antonio
Phone: 830-214-5878
E-mail: mrbandel@ag.tamu.edu

Name: Bandel, Micaela

TAES San Antonio

2632 Broadway, Suite 301 South
San Antonio, TX 78215

SURVIVAL OF F₁ HYBRIDS BETWEEN FISHES OF THE SUBFAMILY ETHEOSTOMINAE

CLARK HUBBS AND KIRK STRAWN

Department of Zoology, The University of Texas, Austin

Natural hybridization of fishes has been reviewed by Carl L. Hubbs ('55). He reported numerous natural hybrid combinations among North American fresh-water fishes. The frequency of these hybrids and the degree of taxonomic difference between their parents (intergeneric but not intertribal) is far greater than that of other animal groups studied. Artificial hybrids have been produced between many fish species (Moenkhaus, '10; and Newman, '14). We have made a number of laboratory crosses between species of darters comprising the subfamily Etheostominae, which are known to have produced a few natural intergeneric hybrids. Intergeneric hybrids readily can be produced in the laboratory but no worker has succeeded in raising intertribal hybrids, thus the level of the most remote relationship of parents of potential laboratory hybrids approximates that of the parents of natural hybrids.

As hybridization of fishes is frequent, they can be used for the study of the effects of the interaction of diverse genotypes. Darters are among the many groups of fishes that have external fertilization. Moreover, parthenogenesis has not been demonstrated in this group. Therefore, it is possible to obtain uncontaminated eggs which can be observed closely from before fertilization until death.

The superspecific classification of the darters has undergone considerable change in the past decade (see Bailey, Winn and Smith, '54: 139). In view of the subjective nature of superspecific categories, it is impractical to discuss whether our crosses are inter- or intrageneric, because a cross that would be intergeneric according to one classification might be intrasubgeneric according to another. For nomenclatorial purposes and to demonstrate relative morphologic relationships, we follow the nomenclature outlined by Carl L. Hubbs ('55). In addition to the hybrid combinations he reported, we have produced hybrid offspring artificially from 5 intergeneric or intersubgeneric combinations not listed as having produced natural hybrids: (1) *Etheostoma (Microperca)* × *Hadropterus (Hadropterus)*, (2) *Etheostoma (Microperca)* × *Percina*, (3) *Etheostoma (Oligocephalus)* × *Etheostoma (Poecilichthys)*, (4) *Etheostoma (Oligocephalus)* × *Etheostoma (Nanostoma)*, and (5) *Etheostoma (Poecilichthys)* × *Etheostoma (Nanostoma)*.

MATERIALS AND METHODS

Hybridization has been carried out by stripping and mixing eggs and sperm from the desired parents. As internal fertilization and parthenogenesis are unknown in darters, we feel that this technique insures against contamination. Details of the technique are given by Strawn and Clark Hubbs ('56). Wild-caught parents have been used for most of the crosses because it is difficult to maintain stocks in breeding condition of all of the species used. Moreover, unless a "balanced diet" is fed to the parental stocks kept in the laboratory, survival rates of the offspring are drastically reduced. Eggs from laboratory stocks are often of a lighter shade than those from wild stocks. Apparently one or more essential metabolites are deficient in the diet (see below).

Most of the darter eggs studied by us can be handled during development. We have restricted the work to those darters which naturally inhabit limestone waters. Preliminary investigations indicate that many darters have restricted limits of

chemical toleration not occur in soft water. Their mortality rate decreases of viability in hard waters. They are pronounced during years that radically affect reservoir water darters" with to lack of time and the "soft-water darter".

The development of Most of the sample The percentage of has been selected development of pigment occurs two or three times (21-24°C.). A easily recognized. eggs which die prior An occasional egg, the others of the same any sign or development. Survival percentages pigmented eyes vary efficiency and technique the gametes. The young fish are removed see Strawn and

¹ We consider the streams to be hard-water streams. See ('52) for these waters, including Colorado River at Austin (Lake Nasworthy on the South Worth near Fort Worth (19 Texas to be soft-water streams total hardness for the following: 49 ppm.), Lake Cherokee near Marshall (31 ppm.), P. Tyler (41 ppm.).

the darters has undergone (see Bailey, Winn and objective nature of super- to discuss whether our cause a cross that would sification might be intra- nomenclatorial purposes gic relationships, we fol- L. Hubbs ('55). In addi- ported, we have produced ntergeneric or intersub- aving produced natural) × *Hadropterus* (*Had-* ca) × *Percina*, (3) *Ethe-* na (*Poecilichthys*), (4) stoma (*Nanostoma*), and heostoma (*Nanostoma*).

METHODS

by stripping and mixing parents. As internal fer- unknown in darters, we t contamination. Details awn and Clark Hubbs n used for most of the tain stocks in breeding d. Moreover, unless a ntal stocks kept in the fspring are drastically s are often of a lighter apparently one or more the diet (see below). s can be handled during work to those darters rs. Preliminary investi- ave restricted limits of

chemical toleration. Many of our "hard-water darters"¹ do not occur in soft waters, and when we have placed them there their mortality rate increased. We have noted a similar decrease of viability of "soft-water darters" when placed in hard waters. This phenomenon appears to be most pronounced during young stages. As water chemistry might radically affect results, we did not attempt to cross "hard-water darters" with darters restricted to soft waters. (Due to lack of time and space we have not made crosses among the "soft-water darters.")

The development of all eggs has been followed carefully. Most of the samples have been checked daily or twice daily. The percentage of the individuals that attained two stages has been selected for presentation. The first stage is the development of pigmented eyes, which, for the darters studied, occurs two or three days after stripping at room temperature (21–24°C.). At or near this stage the embryo is first easily recognized. We consider that the majority of the eggs which die prior to this stage have not been fertilized. An occasional egg, which lives for three or four days after the others of the same sample develop pigmented eyes without any sign or development, is also considered to be unfertilized. Survival percentages between stripping and development of pigmented eyes vary widely, in part because of the relative efficiency and technique of the workers stripping and mixing the gametes. The other stage listed is the time when the young fish are removed from their hatching pans (for description see Strawn and Hubbs, '56) and placed in aquaria.

¹ We consider the streams of the Edwards Plateau and adjacent limestone areas to be hard-water streams. Some figures for total hardness are given in Lohr *et al.* ('52) for these waters, including: Lake Waco near Waco (152 ppm. and 220 ppm.), Colorado River at Austin (160 ppm.), San Felipe Spring at Del Rio (218 ppm.), Lake Nasworthy on the South Concho River near San Angelo (219 ppm.), and Lake Worth near Fort Worth (197 ppm.). We consider most of the streams of eastern Texas to be soft-water streams. Lohr *et al.* ('52) gave the following figures for total hardness for the following waters: Neches River at Evadale (31 ppm. and 49 ppm.), Lake Cherokee near Longview (18 ppm. and 30 ppm.), Caddo Lake near Marshall (31 ppm.), Pine Creek near Paris (43 ppm.), and Lake Tyler near Tyler (41 ppm.).

This is usually shortly after the completion of the larval free-swimming stage and about one month after the eggs have been stripped (hatching is about one week after stripping). Obviously the time after stripping at which the various developmental stages occurs varies with the water temperature and the parents used. Survival rates between the development of pigmented eyes and the end of free swimming vary, probably due in part to variations in available food suitable for larval fish.

Incomplete records were taken on some of the early crosses and some samples were combined during development. Therefore, the number of samples listed may be slightly above the number used for any of the percentages. Our samples are made up of the eggs from one or more females fertilized by the sperm of one or more males. Each sample develops in a single pan.

The percentage of eggs in all the samples of each combination which attain the two stages is given. As all eggs in one pan are affected by the same adverse environmental conditions (if any are present) large samples subjected to abnormal environmental conditions bias the results. The survival percentage of each individual sample has been calculated and these percentages averaged for all of the samples of each combination. One death in small samples can greatly distort the results. Therefore, we feel that the true survival rate is somewhere between the two figures. The combinations which have more than one sample and which have been attempted the fewest times usually have the greatest difference between the total survival rate and the survival rate by sample. This is probably the result of chance difference in the success of large and small samples.

We suspect that our figures for survival are biased in favor of *E. lepidum*. Our hatching and rearing techniques are based primarily on knowledge gained by raising numerous spawns of naturally laid *E. lepidum* eggs. Techniques which are satisfactory for *E. lepidum* are not necessarily as efficient for

raising other species to those of *E. lepidum*.

We consider the combinations are a much higher frequency of the same combination of stripping technique of eggs that are fertilized by *E. fonticola* gametes. But they are small, and the experimental techniques. A large number of *E. fonticola* eggs are surprising, as no visible development have been reared between natural and experimental account for the large number of *E. fonticola* males present. The amounts are small. Therefore, fertilization of sperm would be efficient. In our experiments we have found that the survival of eggs is lowest when fertilized by *E. fonticola* sperm. We do not feel that the survival of *E. fonticola* hybrids is surprising. It is surprising.

Some of the offspring are inviable larvae. In our experimental and control samples the survival of laboratory stocks is low. In viability of offspring are stunted and they are absorbed. In some cases they are absorbed except perhaps for a few. In the mountain region. In the mountain region they are shapen and frequently they are unable to move.

completion of the larval month after the eggs at one week after stripping at which the varieties with the water temperature survival rates between the end of free swimming conditions in available food

Some of the early crosses show development. There may be slightly above the stages. Our samples are where females fertilized by each sample develops in

Some samples of each combination is given. As all eggs in diverse environmental conditions subjected to abnormal results. The survival has been calculated and of the samples of each combination can greatly distort the true survival rate is. The combinations which have been attempted show the greatest difference between survival rate by sample. This difference in the success of

Survival are biased in favor of stripping techniques are based on using numerous spawns. Techniques which are necessarily as efficient for

raising other species. Likewise, fish with requirements similar to those of *E. lepidum* have high survival percentages.

We consider that the survival percentages given for each combination are minimal. As naturally laid darter eggs have a much higher fertilization rate than stripped eggs of the same combination (Strawn and Hubbs, '56), refinement of stripping technique is expected to increase the percentage of eggs that are fertilized. This is especially true of *E. fonticola* gametes. Both the adults and eggs of *E. fonticola* are small, and the eggs might easily be damaged by stripping techniques. A large fraction of the crosses attempted using *E. fonticola* eggs had no visible development. This is not surprising, as none of the 6 *E. fonticola* controls had any visible development. Eggs of *E. fonticola* laid in aquaria have been reared repeatedly in our laboratory. Differences between natural egg laying and artificial fertilization may account for the lack of success in stripping eggs. *Etheostoma fonticola* males produce small quantities of sperm. Usually the amounts are too small to be visible to the naked eye. Therefore, fertilization percentages of eggs by *E. fonticola* sperm would be expected to be lower than those of the other darters we have used. Our fertilization percentages of any eggs is lowest when *E. fonticola* sperm is used (table 2). We do not feel that our small amount of success with *E. fonticola* hybrids is surprising, but that having any success at all is surprising.

Some of the darter spawns contain defective eggs and inviable larvae. These "bad" spawns occur in both hybrid and control samples. Almost all of them are from eggs of laboratory stocks. (We have not noted any similar decrease in viability of offspring due to sperm.) The larvae generally are stunted and have large yolk sacs which do not become absorbed. In some spawns the larvae appear almost normal except perhaps for a slightly angular profile in the yolk sac region. In the most severe cases the larvae are badly misshapen and frequently die before hatching. Those that hatch are unable to move when disturbed and die in the same spot

TABLE 1

Localities from which experimental stocks were obtained

	ETHIOSTOMA CAERULEUM STOREE	ETHIOSTOMA GRAHAMI (GIBBARD)	ETHIOSTOMA LEPIDUM (GIBBARD)	ETHIOSTOMA SPECTABILIS (AGASSIZ)	ETHIOSTOMA RIZONUM (HUBBS AND BLACK)	ETHIOSTOMA FONTICOLA (JORDAN AND GILBERT)	ETHIOSTOMA JULIAE (NEEK)	ETHIOSTOMA ZONALE (COPE)	HADROPTERUS SCIERUS (SWAIN)	PERCINA CAPRODES (RAPINESQUE)
1 Brush Cr. 2 mi. E. Goshen, Washington Co., Arkansas	X			X					?	?
2 Buffalo R. 1 mi. E. Ponea, Newton Co., Arkansas	X			P	X		X	P	?	P
3 Little Buffalo R. at Jasper, Newton Co., Arkansas	X			P	P		P	X	?	?
4 Lampasas R. at U. S. 81 crossing, Bell Co., Texas				X					X	P
5 Salado Cr. at Salado, Bell Co., Texas				X						
6 San Gabriel R. at Georgetown, Williamson Co., Texas				X					?	X
7 Colorado R. at Austin, Travis Co., Texas			?	X					P	X
8 S. Concho R., 4 mi. S. Christoval, Tom Green Co., Texas			X	P						
9 San Saba R. near Ft. McKavett, Menard Co., Texas			X	P						P
10 San Marcos R. at San Marcos, Hays Co., Texas				P		X			X	X
11 Comal Springs, at New Braunfels, Comal Co., Texas			P			X				

12
Guadalupe R. 3 mi. E.
Comfort, Kendall Co., Texa

13
Guadalupe R. 5 mi. E.
Center Pt., Kerr Co.,
Texas

14
Guadalupe R. 3 mi. E.
Kerrville, Kerr Co., Texas

15
Guadalupe R. at Kerrville,
Kerr Co., Texas

16
Guadalupe R. 2 mi. W.
Ingram, Kerr Co., Texas

17
S. Guadalupe R. at Hunt,
Kerr Co., Texas

18
S. Guadalupe R. 8 mi. S.W.
Hunt, Kerr Co., Texas

19
Kent Cr. mouth, 8 mi. N.
Leakey, Real Co., Texas

20
W. Frio R. 8 mi. N.
Leakey, Real Co., Texas

21
Live Oak Cr.
Kinney Co., Texas

22
San Felipe Spr., Del Rio,
Val Verde Co., Texas

23
Devil's R. at mouth of
Dolan Cr., Val Verde Co.,
Texas

X = stock used for experim
P = species known to occur
? = species may occur at lo
If blank, species not expect

TABLE 2

Summary of the survival of darter hybrids

	NUMBER OF SAMPLES ¹	NUMBER OF EGGS	NUMBER TO DEVELOP PIGMENT IN EYES	NUMBER PLACED IN TANK	% FERTILIZED		% OF EYED EGGS PLACED IN TANK		PARENTAL ORIGIN ²
					Total	By sample	Total	By sample	
<i>Etheostoma caeruleum</i> ♀									
<i>Etheostoma caeruleum</i> ♂	8 (2)	492	349	263	70.9	67.8	75.4	71.7	1 × 1, 2 × 2, 3 × 3
<i>Etheostoma lepidum</i> ♂	1	28	22	8	78.6	78.6	36.4	36.4	1 × 9
<i>Etheostoma euzonum</i> ♂	1 (1)	70	51	38	72.9	72.9	74.5	74.5	2 × 2
<i>Etheostoma juliae</i> ♂	5	233	89	25	38.2	49.0	28.1	20.1	2 × 2, 3 × 2
<i>Hadropterus scierus</i> ♂	1 (1)	52	6	0	11.5	11.5	0	0	2 × 10
<i>Percina caprodes</i> ♂	1 (1)	36	24	23	66.7	66.7	95.8	95.8	2 × 7
<i>Etheostoma lepidum</i> ♀									
<i>Etheostoma lepidum</i> ♂	3	449	217	164	48.3	47.7	75.6	73.4	16 × 16, 18 × 18
<i>Etheostoma spectabile</i> ♂	5	547	333 ³	240	60.9	54.2	68.4	50.2	9 × 7, 16 × 14, 16 × 16
<i>Percina caprodes</i> ♂	8	802	423 ⁴	126	52.7	52.2	28.4	26.9	9 × 7, 9 × 14, 16 × 14, 18 × 15
<i>Etheostoma spectabile</i> ♀									
<i>Etheostoma spectabile</i> ♂	33 (1)	2682	1861 ⁵	1244	69.4	64.7	67.3	59.7	4 × 5, 4 × 6, 5 × 5, 6 × 6, 6 × 7, 7 × 1, 7 × 6, 7 × 7, 12 × 12, 14 × 14, 16 × 16
<i>Etheostoma caeruleum</i> ♂	3 (1)	353	139	93	39.4	35.4	66.9	48.9	1 × 1, 7 × 1, 7 × 2
<i>Etheostoma grahami</i> ♂	2	19	9	1	47.4	39.2	11.1	50.0	7 × 23
<i>Etheostoma lepidum</i> ♂	16 (1)	2566	1377 ⁶	673	53.7	53.4	48.3	55.1	1 × 21, 7 × 9, 16 × 16
<i>Etheostoma fonticola</i> ♂	3 (1)	98	20	12	20.4	22.1	60.0	61.2	5 × 10, 7 × 10
<i>Hadropterus scierus</i> ♂	5 (1)	159	37 ⁷	4	23.3	41.8	33.0	44.4	4 × 4, 4 × 10, 12 × 12
<i>Percina caprodes</i> ♂	40 (4)	4702	2718 ⁸	1591	57.8	55.6	54.7	50.3	1 × 7, 6 × 6, 6 × 7, 7 × 6, 7 × 7, 12 × 12, 14 × 14
<i>Etheostoma euzonum</i> ♀									
<i>Etheostoma euzonum</i> ♂	1	106	39	0	36.8	36.8	0	0	2 × 2
<i>Etheostoma fonticola</i> ♀									
<i>Etheostoma fonticola</i> ♂	0 (6)	0	0	0	0	0	0	0	10 × 10
<i>Etheostoma caeruleum</i> ♂	1 (2)	26	8	0	30.8	30.8	0	0	10 × 1, 10 × 2, 10 × 3
<i>Etheostoma lepidum</i> ♂	1	70	3	0	4.3	4.3	0	0	11 × 17
<i>Etheostoma euzonum</i> ♂	1	12	1	0	8.3	8.3	0	0	10 × 2
<i>Etheostoma juliae</i> ♂	1	35	2	0	5.7	5.7	0	0	10 × 2
<i>Hadropterus scierus</i> ♂	2 (3)	46	13	1	28.2	28.2	7.7	5.0	10 × 4, 10 × 10
<i>Percina caprodes</i> ♂	1 (2)	37	2	1	5.4	5.4	50.0	50.0	10 × 10, 10 × 15
<i>Etheostoma juliae</i> ♀									
<i>Etheostoma juliae</i> ♂	1	77	1	0	1.3	1.3	0	0	2 × 2
<i>Etheostoma zonale</i> ♀									

										6 × 6, 6 × 7, 7 × 1, 7 × 6, 7 × 7, 12 × 12, 14 × 14, 16 × 16	STRAWN
<i>Etheostoma caeruleum</i> ♂	3 (1)	353	139	93	39.4	35.4	66.9	48.9	1 × 1, 7 × 1, 7 × 2		
<i>Etheostoma grahami</i> ♂	2	19	9	1	47.4	39.2	11.1	50.0	7 × 23		
<i>Etheostoma lepidum</i> ♂	16 (1)	2566	1377 ^a	673	53.7	53.4	48.3	55.1	1 × 21, 7 × 9, 16 × 16		
<i>Etheostoma fonticola</i> ♂	3 (1)	98	20	12	20.4	22.1	60.0	61.2	5 × 10, 7 × 10		
<i>Hadropterus scierus</i> ♂	5 (1)	159	37 ^r	4	23.3	41.8	33.0	44.4	4 × 4, 4 × 10, 12 × 12		
<i>Percina caprodes</i> ♂	40 (4)	4702	2718 ^s	1591	57.8	55.6	54.7	50.3	1 × 7, 6 × 6, 6 × 7, 7 × 6, 7 × 7, 12 × 12, 14 × 14		
<i>Etheostoma euzonum</i> ♀											
<i>Etheostoma euzonum</i> ♂	1	106	39	0	36.8	36.8	0	0	2 × 2		
<i>Etheostoma caeruleum</i> ♂	1	60	37	13	61.7	61.7	35.1	35.1	2 × 2		

<i>Etheostoma fonticola</i> ♀											
<i>Etheostoma fonticola</i> ♂	0 (6)	0	0	0	0	0	0	0	10 × 10		
<i>Etheostoma caeruleum</i> ♂	1 (2)	26	8	0	30.8	30.8	0	0	10 × 1, 10 × 2, 10 × 3		
<i>Etheostoma lepidum</i> ♂	1	70	3	0	4.3	4.3	0	0	11 × 17		
<i>Etheostoma euzonum</i> ♂	1	12	1	0	8.3	8.3	0	0	10 × 2		
<i>Etheostoma juliae</i> ♂	1	35	2	0	5.7	5.7	0	0	10 × 2		
<i>Hadropterus scierus</i> ♂	2 (3)	46	13	1	28.2	28.2	7.7	5.0	10 × 4, 10 × 10		
<i>Percina caprodes</i> ♂	1 (2)	37	2	1	5.4	5.4	50.0	50.0	10 × 10, 10 × 15		
<i>Etheostoma juliae</i> ♀											
<i>Etheostoma juliae</i> ♂	1	77	1	0	1.3	1.3	0	0	2 × 2		
<i>Etheostoma zonale</i> ♀											
<i>Etheostoma caeruleum</i> ♂	2	109	68	31	62.4	60.7	45.6	38.1	3 × 3		
<i>Etheostoma euzonum</i> ♂	1	46	21	0	45.7	45.7	0	0	3 × 2		
<i>Hadropterus scierus</i> ♀											
<i>Hadropterus scierus</i> ♂	4	467	253	0	54.2	46.0	0	0	10 × 4, 10 × 10		
<i>Etheostoma caeruleum</i> ♂	2 (1)	111	35	28	31.5	30.8	80.0	41.2	10 × 1		
<i>Etheostoma spectabile</i> ♂	1	144	15 ^r	11	10.4	10.4	91.7	91.7	10 × 5		
<i>Etheostoma fonticola</i> ♂	1 (1)	111	9	0	8.2	8.2	0	0	10 × 10		
<i>Etheostoma juliae</i> ♂	3	464	270	0	58.2	59.3	0	0	10 × 2		
<i>Percina caprodes</i> ♀											
<i>Percina caprodes</i> ♂	7	1147	776	166	67.7	70.3	21.4	26.6	7 × 7, 12 × 12, 12 × 13, 13 × 13, 15 × 13		
<i>Etheostoma lepidum</i> ♂	4	1316	325	122	24.7	44.0	37.5	58.8	7 × 18, 12 × 16, 13 × 16		
<i>Etheostoma spectabile</i> ♂	3	474	371	241	78.3	77.4	65.0	66.5	12 × 12, 12 × 14		

¹ The number of additional samples with apparently mature eggs that showed no development is listed in parentheses. These samples are not included in the figure listed outside of the parentheses.

² See table 2 for the localities designated by the numbers. The locality of the maternal parent is listed first.

³ Use 351 for denominator for percentage placed in tank.

⁴ Use 444 for denominator for percentage placed in tank.

⁵ Use 1848 for denominator for percentage placed in tank.

⁶ Use 1393 for denominator for percentage placed in tank.

⁷ Use 12 for denominator for percentage placed in tank.

⁸ Use 2906 for denominator for percentage placed in tank.

HYBRID SURVIVAL IN FISHES

in which they hatched. All of these inviable larvae share an inability to feed. They may strike repeatedly at a protozoan or newly-hatched brine shrimp but are unable to ingest it. Within a period of three days to two weeks after hatching, depending on temperature and the individual larvae, all are dead. A brood may be nearly uniform in the degree of deformity or considerable variation may occur with some of the larvae viable.

Laboratory stocks containing the parents of defective larvae are fed a diet of Houston dwarf white worms with very occasional feedings of *Daphnia magna* and mosquito larvae. Darters regularly fed mosquito larvae and given irregular supplementary feedings of white worms and daphnia produce a minimum of inviable young (Strawn, '56). These defective larvae probably result from an inadequate parental diet, even though laboratory females spawn regularly and lay large spawns of eggs. While no specific experiments have been made, extensive breeding experiments yield some clues. Eggs from freshly caught wild *E. lepidum* have never produced this type of larvae. The newly laid eggs of wild fish are bright yellow while the eggs of white worm-fed *E. lepidum* are colorless. The eggs of old laboratory stocks regularly given a diet including either brine shrimp or mosquito larvae are intermediate in color. The viability of the larvae is closely correlated with the amount of color of the newly laid eggs. We suspect that an essential metabolite contributes to or is associated with the egg color. The color intensity, thus, may indicate the amount of the essential metabolite.

Another contributing factor may be the nipping of the females by the males. In a 5 gallon aquarium such as the pairs are often bred in, some males pick on the females between spawns (occasionally the reverse is true) and after several spawns her fins, especially the anal and posterior part of the second dorsal, are chewed off. Eventually the female dies. Eggs from these ragged females rarely hatch into viable larvae.

Eggs from par occasionally can parents has been is primarily mos Some of the egg affected by a diet

Shortly after swimming stage, to species. The d are elongate and as water when a stage, the larvae primary food s we have fed the swimming stage bottom when adv absorption, they organisms, such daily. Our techn food organisms stage. We belie free-swimming la the conditions w offspring may h samples reported proximately the

As the hybrid ♂) has been a survival of each results but also causes for varia parents. We can the samples of th first is chance v December 7, 195 the complement were stripped an

inviabile larvae share an repeatedly at a protozoan are unable to ingest it. o weeks after hatching, individual larvae, all are m in the degree of de- y occur with some of the

parents of defective larvae white worms with very na and mosquito larvae. ae and given irregular ms and daphnia produce n, '56). These defective quate parental diet, even regularly and lay large riments have been made, some clues. Eggs from ve never produced this of wild fish are bright rm-fed *E. lepidum* are y stocks regularly given or mosquito larvae are of the larvae is closely the newly laid eggs. We ce contributes to or is olor intensity, thus, may metabolite.

be the nipping of the n aquarium such as the k on the females between true) and after several d and posterior part of eventually the female dies. rarely hatch into viable

Eggs from parents that have produced these inviable larvae occasionally can be reared to adult size after the diet of the parents has been supplemented with brine shrimp. If the diet is primarily mosquito larvae, the offspring often can be reared. Some of the eggs from wild females may also be adversely affected by a dietary deficiency in the parental habitat.

Shortly after hatching many darter larvae have a free-swimming stage, the duration of which varies from species to species. The darters with long free-swimming larval stages are elongate and of approximately the same specific gravity as water when advanced larvae. During their free-swimming stage, the larvae either require microscopic plankton as their primary food source or need to be fed more often than we have fed them. Other darter species have a short free-swimming stage and have to swim vigorously when off the bottom when advanced larvae. At, or shortly after, yolk sac absorption, they are able to survive on macroscopic food organisms, such as newly-hatched brine shrimp fed twice daily. Our techniques for providing quantities of microscopic food organisms to young fish are still in the developmental stage. We believe that many young of species with long free-swimming larval stages probably starve to death under the conditions we have used. Likewise, many of their hybrid offspring may have starved to death. However, all of the samples reported in this paper have been subjected to approximately the same feeding conditions.

As the hybrid combinations (*E. spectabile* ♀ × *P. caprodes* ♂) has been attempted most frequently, a study of the survival of each of its samples not only gives the most valid results but also can give an indication of additional possible causes for variation in the survival of spawns from wild parents. We can think of 5 major causes for variation within the samples of this single combination prior to hatching. The first is chance variation of the viability of the gametes. On December 7, 1954, three spawns (a spawn is considered to be the complement of eggs of one female at one laying) of eggs were stripped and mixed with sperm under apparently the

same conditions. They were reared separately until they were placed in the aquarium. The fertilization percentages were 56, 57, and 6. The developmental percentages were 61, 18, and 0 respectively. We know of no difference in techniques to account for the variation. Other combinations carried out under apparently identical techniques also vary. The second possible cause is the temperature at which the freshly-caught wild parents were stored and the gametes mixed. The parents of our first crosses were held at room temperature and the mixing of gametes was carried out at room temperature. About midway in the experimental work we began to keep the parents at cooler temperatures which were near that of their natural environment. We also mixed the gametes at that temperature. After these changes the success of our crosses rose. This increase may in part be due to a decrease in the metabolic rate of the gametes prolonging the time in which fertilization can occur. The third possible cause of variation is the time between the removal and the mixing of the gametes. With experience our dexterity increased as did the success of fertilization. Other factors being equal, the most recent crosses are the most successful. The fourth is the condition of the parents, particularly of the female. As stated above, eggs developing in laboratory-fed females vary in viability according to the variety of foods available to the female and the condition of the female. These laboratory females, however, do not cease laying eggs. It is possible that wild females also are subjected to dietary deficiencies. On April 2 and 3, 1955, we collected *E. spectabile* parents from Georgetown and Austin. Those from Austin were large and healthy. The Georgetown locality was warmer and the population density was very high. The individuals were all small and appeared to be emaciated. The survival rate of the eggs from Austin females was higher than that of the eggs from Georgetown females for all attempted combinations. The fifth possibility for variation in techniques causing different survival rates is the time after maturation at which the eggs are used. The parental stocks for the above crosses were first used on April

3 (the day on which they were first tested again until the date were far more than the date. Apparently the viable eggs within the date.

The stocks used in the crosses are listed in table 1.

Eight of the populations are from the genera of *Etheosoma*, *cephalus* (*caeruleum*), *Microperca* (*fontinalis*), *(juliae)*, and *Nannoperca*. They were placed in the subgenus *caeruleum* not divided into species.

Only one natural population was found in all of the field collections. This combination of parents neither has been previously reported during our experience. Natural History Commission, C. Jurgens and W. C. Jurgens, one-half mile west of Georgetown on November 6, 1955. William F. Herzog, Georgetown River in Austin, Texas. The populations of *E. spectabile* are sufficiently different to represent distinct species.

Comparisons

The results of the crosses are as follows. Although only a few

parately until they were
ation percentages were
ntages were 61, 18, and
erence in techniques to
mbinations carried out
also vary. The second
hich the freshly-caught
tes mixed. The parents
oom temperature and
t at room temperature.
ork we began to keep
hich were near that of
ked the gametes at that
success of our crosses
e to a decrease in the
ing the time in which
ible cause of variation
mixing of the gametes.
d as did the success of
ual, the most recent
ourth is the condition
male. As stated above,
ales vary in viability
able to the female and
oratory females, how-
sible that wild females
es. On April 2 and 3,
from Georgetown and
ge and healthy. The
he population density
ll small and appeared
the eggs from Austin
eggs from Georgetown
. The fifth possibility
fferent survival rates
ne eggs are used. The
ere first used on April

3 (the day on which the parents were collected or the next day). Due to the pressure of other work, the fish were not tested again until April 6. The crosses made on the former date were far more successful than those made on the latter date. Apparently there is a time limit on the retention of viable eggs within a female.

The stocks used for the experiments came from the localities listed in table 1.

Eight of the parental species belong to the following subgenera of *Etheostoma* listed by Carl L. Hubbs ('55): *Oligocephalus* (*caeruleum*, *grahami*, *lepidum*, and *spectabile*), *Microperca* (*fonticola*), *Poecilichthys* (*euzonum*), *Claricola* (*juliae*), and *Nanostoma* (*zonale*). *Hadropterus scierus* is placed in the subgenus *Hadropterus*. The genus *Percina* is not divided into subgenera.

Natural hybrids

Only one natural darter hybrid combination has been found in all of the field investigations at The University of Texas. This combination, *Hadropterus scierus* × *Percina caprodes*, neither has been previously reported nor has been attempted during our experimental work. A single specimen (Texas Natural History Collection No. 123) was collected by Kenneth C. Jurgens and William H. Brown from the San Marcos River, one-half mile west of Martindale, Caldwell County, Texas, on November 6, 1949. Another specimen that was collected by William F. Herzog and Billy D. Cooper from the Colorado River in Austin, Texas, during April, 1953, has been mislaid. The populations of *Hadropterus scierus* at these two localities are sufficiently distinct morphologically to be considered to represent distinct subspecies by Clark Hubbs ('54).

Comparison of darter hybrids produced by artificially mixing gametes

The results of our darter crosses are outlined in table 2. Although only a few crosses of all but two combinations have

been made, we feel that the sum of the various combinations is significant.

We have attempted 28 hybrid darter combinations (counting reciprocals) by stripping and mixing the gametes; some individuals of 20 (71%) of these have lived long enough to be placed in the aquaria. We have also attempted to raise 8 species under the same conditions; at least some individuals of four (50%) of these have lived long enough to be placed in aquaria. We believe that additional attempts with better techniques will result in the successful rearing of all of the darter crosses we have tested. Increased knowledge of raising and mixing gametes has resulted in higher percentages of fertilization and development within a single combination (Strawn and Hubbs, '56). Almost all of the successful hybrid combinations and controls have been carried to apparent adult size.

Although we have not been able to rear 8 of the attempted hybrid combinations, each of them has one (or both) parental species which we have not raised under the same conditions. Four of the hybrid failures have *Etheostoma fonticola* as the maternal parent. Eggs of *E. fonticola* are extremely delicate. The mortality may be due to "rough" handling, despite all of our care.

Two of the other four failures have *Hadropterus scierus* as the maternal parent. Two factors apparently contribute to this failure. One is that many embryos, both control and hybrid, within *H. scierus* egg shells appear badly deformed. (All of these eggs are from wild-caught female *H. scierus*.) These eggs seldom hatch, and if so, die almost immediately. We suspect that these wild females had a dietary deficiency. The other factor is the lack of food. *Hadropterus scierus* has a long free-swimming larval stage, and survival of darters with such larval stages is very low. The controls of both paternal species (*E. juliae* and *E. fonticola*) have not yet been raised successfully under the same conditions.

The other two unsuccessful hybrid combinations (*Etheostoma caeruleum* ♀ × *Hadropterus scierus* ♂ and *E. zonale*

♀ × *E. euzonum* maternal (latter our laboratory) larval stages and starved to death.

Two hybrid combinations and *E. spectabile* rates than either a single cross or parents would be offspring. The latter samples. Many techniques were that had not been latter sample in between the development in aquaria.

Seven hybrid combinations intermediate between (*E. spectabile* × *E. spectabile* survival rates. One of these used tank. All embryos in the aquarium percentages approximately. Two, both reciprocal are between a free-swimming species with a (*caprodes*). One combinations are in All embryos in aquaria. Excluding approximate the three combinations *euzonum* ♀ ×

the various combinations

combinations (counting the gametes; some in- e lived long enough to also attempted to raise at least some individuals ng enough to be placed al attempts with better ul rearing of all of the sed knowledge of raising higher percentages of n a single combination of the successful hybrid en carried to apparent

rear 8 of the attempted s one (or both) parental der the same conditions. *costoma fonticola* as the are extremely delicate. " handling, despite all

Hadropterus scierus as pparently contribute to ryos, both control and appear badly deformed. ight female *H. scierus*.) lie almost immediately. ad a dietary deficiency. l. *Hadropterus scierus* and survival of darters The controls of both *icola*) have not yet been nditions.

d combinations (*Etheo- scierus* ♂ and *E. zonale*

♀ × *E. euzonum* ♂) have either the paternal (former) or maternal (latter) parent that has not yet been raised under our laboratory conditions. Both parental species have long larval stages and the controls and hybrids appear to have starved to death.

Two hybrid combinations (*E. caeruleum* ♀ × *E. lepidum* ♂ and *E. spectabile* ♀ × *E. lepidum* ♂) have lower survival rates than either control species. The former is composed of a single cross using parents from laboratory stocks. Such parents would be expected to have a reduced survival of their offspring. The latter combination is composed of a number of samples. Many of them were made early in our work when techniques were not as efficient. One was made using females that had not been tested for ripeness for two days. If this latter sample is deleted, the overall survival percentage between the development of pigmented eyes and the time for placement in aquaria rises from 48.3 to 61.6%.

Seven hybrid combinations have a survival rate about intermediate between that of the two parental species. Two of these (*E. spectabile* ♀ × *E. caeruleum* ♂ and *E. lepidum* ♀ × *E. spectabile* ♂) have parents with approximately equal survival rates. The latter combination is based on 5 samples. One of these uses eggs from an *E. lepidum* laboratory stock tank. All embryos in this sample died before being placed in the aquarium. Excluding this sample, the survival percentages approximate those of the most successful parent. Two, both reciprocals of the *E. lepidum* × *P. caprodes* cross, are between a successful species with a moderately short free-swimming stage (*E. lepidum*) and a less successful species with a moderately long free swimming stage (*P. caprodes*). One fourth of the samples of both of these combinations are based on eggs from laboratory stock females. All embryos in these samples died before being placed in aquaria. Excluding these samples, the survival percentages approximate those of the most successful parent. The other three combinations (*E. caeruleum* ♀ × *E. juliae* ♂, *E. euzonum* ♀ × *E. caeruleum* ♂, and *E. spectabile* ♀ × *H.*

scierus ♂) have one parent, with a long free swimming larval stage, that has not yet been reared in our laboratory and one successful parent. Two of the 7 combinations have *E. lepidum* as one parent. As our techniques of rearing darters is based on experiments with that species, it is possible that the survival figures for the *E. lepidum* genotype are too high when compared with other genotypes, including those of *E. lepidum* hybrids.

Eight hybrid combinations have a survival rate about equal to that of the most successful parental species. Five of these have one parent which has not yet been raised, and 4 have one parent (*P. caprodes*) with a low success. They include both reciprocals of the *Percina caprodes* × *Etheostoma spectabile* cross. This combination is the one tested most frequently. The combinations have been made using parents from a number of localities as a part of a project on hybrid variability (Clark Hubbs and Strawn, in press). These fish not only have been carried through the larval stages, but also many have been raised to adult size. Our laboratory feeding conditions for older darters are much more favorable for *P. caprodes* than for *E. spectabile*. Stocks of the latter species require special foods for high survival, whereas the former eat almost any food used. The advanced hybrids have the feeding habits and survival rate of their most successful parent, *P. caprodes*. Therefore, during larval stages, the hybrids and *E. spectabile* have a higher survival rate than *P. caprodes* under our laboratory conditions; after completion of the larval stages, the hybrids and *P. caprodes* are easier to maintain in the laboratory than *E. spectabile*. Under our laboratory conditions the overall survival of the hybrids is higher than that of either species but does not exceed either during any one stage.

One hybrid combination has been raised (*Etheostoma fonticola* ♀ × *Hadropterus scierus* ♂) of which neither parent has been raised under the same conditions. This may be due to one or more of three factors. The first is chance. The second is that the two parental species are extreme with regard to a number

of characters su stage. The inter laboratory condi viving hybrid m. pigmentation an of pigmented eye formation of eye after eye pigmer

All of the da morphologically there may be co phologic interme their parental sp morphologically terns are interm *idum*, and *E. spe* themselves and have varying du room temperatur day for *E. spec* about 10 days for Both reciprocals settled in about 10 × *P. caprodes* c hybrid larvae can the naked eye sh and actions. Hy behavior patterns parental species. ily and *E. spectab* hybrids of both r mediate amount o

Although the hy follow the develop before hatching. eggs develop pigm *caprodes* eggs ha

g free swimming larval
our laboratory and one
ations have *E. lepidum*
earing darters is based
t is possible that the
otype are too high when
ing those of *E. lepidum*

Survival rate about equal
species. Five of these
been raised, and 4 have
success. They include
E. lepidum × *Etheostoma spec-*
e one tested most fre-
n made using parents
of a project on hybrid
(in press). These fish not
larval stages, but also
Our laboratory feeding
more favorable for *P.*
ks of the latter species
l, whereas the former
ned hybrids have the
their most successful
ing larval stages, the
ner survival rate than
tions; after completion
P. caprodes are easier
E. spectabile. Under our
vival of the hybrids is
does not exceed either

ed (*Etheostoma fontic-*
ich neither parent has
This may be due to one
nce. The second is that
th regard to a number

of characters such as the length of the free-swimming larval stage. The intermediate hybrid may be better suited to our laboratory conditions. The third factor is that the one surviving hybrid may have had *H. scierus* survival prior to eye pigmentation and *E. fonticola* survival after the development of pigmented eyes. The *E. fonticola* controls died prior to the formation of eye pigments and the *H. scierus* controls died after eye pigmentation.

All of the darter hybrids which have been reared are morphologically intermediate between their controls. Although there may be considerable variation in the amount of morphologic intermediacy, none of the hybrids can be placed in their parental species. Not only are the hybrids intermediate morphologically but after hatching their developmental patterns are intermediate. Three species (*P. caprodes*, *E. lepidum*, and *E. spectabile*) have been crossed repeatedly among themselves and all reciprocals reared. The three species have varying durations of their free-swimming stage. At room temperature the duration varies from less than one day for *E. spectabile* (if they free-swim at all), through about 10 days for *E. lepidum*, to over 30 days for *P. caprodes*. Both reciprocals of the *E. spectabile* × *P. caprodes* cross settled in about 10 days and both reciprocals of the *E. lepidum* × *P. caprodes* cross settle in about 20 days. Most of the hybrid larvae can be distinguished from their controls with the naked eye shortly after hatching by their appearance and actions. Hybrids reared to apparent adult size have behavior patterns intermediate between those of their two parental species. Whereas *P. caprodes* adults free-swim readily and *E. spectabile* adults seldom rise off of the bottom, their hybrids of both reciprocals remain on the bottom an intermediate amount of time.

Although the hybrids are intermediate after hatching, they follow the developmental pattern of their maternal controls before hatching. Under comparable conditions *E. spectabile* eggs develop pigmented eyes before *P. caprodes* eggs, and *P. caprodes* eggs hatch before those of *E. spectabile*. Their

hybrids from *E. spectabile* eggs develop pigmented eyes at the same time as the *E. spectabile* controls and the reciprocals develop pigmented eyes at the same time as the *Percina* controls. Likewise, the hatching time follows that of the maternal parent. Newman ('08) and Moenkhaus ('10) reported similar maternal development rates in *Fundulus* hybrids during early stages and intermediate development rates for both reciprocals later.

F₁ and F₂ hybrids from naturally laid eggs

The only F_2 hybrids obtained so far are from F_1 *Etheostoma lepidum* \times *E. grahami* and the reciprocal. All F_1 's and F_2 's of these crosses were produced by putting the desired parents together in an aquarium and letting them spawn naturally. The first attempts to obtain F_1 hybrids from the above parental species resulted in defective eggs and inviable larvae probably due to an inadequate diet. Later crosses from stocks fed chiefly on mosquito larvae succeeded and both reciprocals were obtained.

As the two nominal species are allopatric we desired to determine if the F_1 hybrids are fertile. Both reciprocals became sexually mature in midwinter when mosquito larvae are scarce in Austin. Since pairs fed white worms do not inevitably produce inviable larvae, breeders were placed in 5 gallon aquaria and the eggs produced were saved. Two female F_1 hybrids (West Frio River *E. lepidum* ♀ \times Devil's River *E. grahami* ♂) produced 9 spawns of defective eggs or severely deformed inviable larvae whether the zygotes were F_2 's or back crossed with their maternal stock. Five spawns of eggs taken from the F_1 stock tank of the reciprocal cross produced no viable larvae. Three F_1 females from this tank were placed in individual 5 gallon tanks. One female produced 5 bad spawns when bred with sibling males or back crossed to the paternal stock. The second female produced three inviable spawns when bred to a sibling male. The

third female who spawns that were hatched and 7 were regular feeding to the other two was not involved in a brood of deformed raised female warmer weather series of mating. Over one-half of and these are F_2 's. Female F_1 *lepidum* (West Frio River *E. grahami* West Frio) *E. lepidum* combination was back. A female of the Concho *E. lepidum* infertility between same habitat and habits. The female eggs. Although female *E. grahami* the tank, while filamentous algae number of hybrids been made. In species, the large the females. Wild populations in a ing sites or in experiments are are distinct or will be considered to

develop pigmented eyes at the same time as the *Percina* and the reciprocals. The time as the *Percina* follows that of the and Moenkhaus ('10) relative rates in *Fundulus* hybrid development rates

Naturally laid eggs

are from F_1 *Etheostoma* reciprocal. All F_1 's and F_2 's mating the desired parents them spawn naturally. from the above parental inviable larvae probably crosses from stocks fed and both reciprocals

allopatric we desired to fertile. Both reciprocals when mosquito larvae and white worms do not breeders were placed in and were saved. Two *E. lepidum* ♀ × Devil's spawns of defective eggs whether the zygotes or maternal stock. Five tank tank of the reciprocal three F_1 females from this gallon tanks. One female with sibling males or back second female produced to a sibling male. The

third female when placed with a sibling male produced three spawns that were failures and a spawn of 23 eggs of which 16 hatched and 7 were raised. This latter pair had been receiving regular feedings of brine shrimp which were not given to the other two pairs. This female was killed by the male and was not involved in later crosses. One of the F_1 males fathered a brood of deformed larvae when back crossed with a laboratory raised female of its paternal stock. With the advent of warmer weather and the increase of mosquito larvae, a new series of matings was tried using the same F_1 individuals. Over one-half of the spawns resulted in some viable larvae and these are discussed below. Both reciprocals produced F_2 's. Female F_1 's of the *E. grahami* (Devil's River) ♀ × *E. lepidum* (West Frio River) ♂ were backcrossed to a Devil's River *E. grahami* male and a Kent Creek (tributary to the West Frio) *E. lepidum* male. An F_1 male of the same combination was backcrossed with a West Frio *E. lepidum* female. A female of the reciprocal was backcrossed with a South Concho *E. lepidum* male. We find no indication of genetic infertility between these two species. Both species occupy the same habitat and in the aquarium have similar breeding habits. The female picks the site for the deposition of the eggs. Although there is complete overlap in spawning sites, female *E. grahami* tend to lay under rocks and on the side of the tank, while female *E. lepidum* tend to lay on glass wool or filamentous algae. Observations on mate selection and the number of hybrids produced in mixed populations have not been made. In 10 or 15 gallon tanks containing only one species, the largest male monopolizes the breeding site and the females. Whether this dominance would occur in mixed populations in a large tank with a number of alternate breeding sites or in the wild habitat is not known. Additional experiments are necessary to determine whether these species are distinct or whether these nominal allopatric species should be considered to be geographic variants.

TABLE 3
Summary of the survival of darter hybrids with varying degrees of allopatry

	NUMBER OF SAMPLES ¹	NUMBER OF EGGS	NUMBER TO DEVELOP PIGMENT IN EYES	NUMBER PLACED IN TANK	% FERTILIZED		% OF EYED EGGS PLACED IN TANK		PARENTAL ORIGIN ²
					Total	By sample	Total	By sample	
<i>Etheostoma caeruleum</i> ♀									
<i>Etheostoma juliae</i> ♂	3	114	76	23	66.7	69.2	30.3	28.6	2 × 2
<i>Etheostoma juliae</i> ♂	2	119	13	2	10.9	18.8	15.4	9.1	3 × 2 ¹⁰
<i>Etheostoma lepidum</i> ♀									
<i>Etheostoma spectabile</i> ♂	3	204	140 ³	122	68.6	69.6	77.2	62.5	16 × 16
<i>Etheostoma spectabile</i> ♂	2	343	193	118	56.3	38.8	61.1	31.7	9 × 7, 16 × 14 ¹⁰
<i>Percina caprodes</i> ♂	7	767	410 ⁴	126	53.5	54.7	29.2	30.7	9 × 7, 16 × 12, 16 × 14, 18 × 15 ¹⁰
<i>Percina caprodes</i> ♂	1	35	13	0	37.1	37.1	0	0	9 × 14 ¹¹
<i>Etheostoma spectabile</i> ♀									
<i>Etheostoma spectabile</i> ♂	26	2331	1660 ⁵	1103	71.2	67.9	65.6	59.8	5 × 5, 6 × 6, 7 × 7, 12 × 12, 14 × 14, 16 × 16
<i>Etheostoma spectabile</i> ♂	2	107	36 ⁶	0	33.6	39.7	0	0	4 × 5, 4 × 6 ¹⁰
<i>Etheostoma spectabile</i> ♂	5 (1)	244	165	141	67.6	59.2	85.5	71.2	6 × 7, 7 × 1, 7 × 6 ¹¹
<i>Etheostoma caeruleum</i> ♂	1	84	9	2	10.7	10.7	22.2	22.2	1 × 1
<i>Etheostoma caeruleum</i> ♂	2 (1)	269	130	91	48.3	47.7	70.0	62.3	7 × 1, 7 × 2 ¹¹
<i>Etheostoma lepidum</i> ♂	7	1332	884 ⁷	363	66.6	61.0	40.3	62.3	16 × 16
<i>Etheostoma lepidum</i> ♂	8	1097	486	310	44.3	53.6	63.8	56.5	7 × 9 ¹⁰
<i>Etheostoma lepidum</i> ♂	1 (1)	137	7	0	5.1	5.1	0	0	1 × 21 ¹¹
<i>Percina caprodes</i> ♂	3 (3)	174	66	58	37.9	48.8	87.9	46.8	1 × 7, 6 × 7, 7 × 6 ¹¹
<i>Etheostoma fonticola</i> ♀									
<i>Hadropterus scierus</i> ♂	1 (3)	23	3	0	13.0	13.0	0	0	10 × 10
<i>Hadropterus scierus</i> ♂	1	23	10	1	43.5	43.5	10.0	10.0	10 × 4 ¹¹
<i>Percina caprodes</i> ♂	0 (1)	0	0	0	0	0	0	0	10 × 10
<i>Percina caprodes</i> ♂	1 (1)	37	2	1	5.4	5.4	50.0	50.0	10 × 15 ¹⁰
<i>Hadropterus scierus</i> ♀									
<i>Hadropterus scierus</i> ♂	3	384	248	0	64.6	59.4	0	0	10 × 10
<i>Hadropterus scierus</i> ♂	1	83	5	0	6.0	6.0	0	0	10 × 4 ¹¹
<i>Percina caprodes</i> ♀									
<i>Percina caprodes</i> ♂	2	724	408	151	68.6	70.0	33.3	33.3	10 × 10

<i>Percina caprodes</i> ♂	1	35	13	0	37.1	37.1	0	0	9×14^{11}
<i>Etheostoma spectabile</i> ♀									$10 \times 14^7,$ 18×15^{10}
<i>Etheostoma spectabile</i> ♂	26	2331	1660 ⁵	1103	71.2	67.9	65.6	59.8	$5 \times 5, 6 \times 6,$ $7 \times 7, 12 \times 12,$ $14 \times 14, 16 \times 16$
<i>Etheostoma spectabile</i> ♂	2	107	36 ⁶	0	33.6	39.7	0	0	$4 \times 5, 4 \times 6^{10}$
<i>Etheostoma spectabile</i> ♂	5 (1)	244	165	141	67.6	59.2	85.5	71.2	$6 \times 7, 7 \times 1,$ 7×6^{11}
<i>Etheostoma caeruleum</i> ♂	1	84	9	2	10.7	10.7	22.2	22.2	1×1
<i>Etheostoma caeruleum</i> ♂	2 (1)	269	130	91	48.3	47.7	70.0	62.3	$7 \times 1, 7 \times 2^{11}$
<i>Etheostoma lepidum</i> ♂	7	1332	884 ⁷	363	66.6	61.0	40.3	62.3	16×16
<i>Etheostoma lepidum</i> ♂	8	1097	486	310	44.3	53.6	63.8	56.5	7×9^{10}
<i>Etheostoma lepidum</i> ♂	1 (1)	137	7	0	5.1	5.1	0	0	1×21^{11}
<i>Hadropterus scierus</i> ♂	4	95	36 ⁸	3	37.9	55.2	27.3	16.7	$12 \times 12, 4 \times 4$
<i>Hadropterus scierus</i> ♂	1 (1)	64	1	1	1.6	1.6	100.0	100.0	4×10^{11}
<i>Percina caprodes</i> ♂	37 (1)	4528	2652 ⁹	1533	58.7	56.3	84.0	80.8	$6 \times 6, 7 \times 7,$ $12 \times 12, 14 \times 14$
<i>Percina caprodes</i> ♂	3 (3)	174	66	58	37.9	48.8	87.9	46.8	$1 \times 7, 6 \times 7,$ 7×6^{11}
<i>Etheostoma fonticola</i> ♀									10×10
<i>Hadropterus scierus</i> ♂	1 (3)	23	3	0	13.0	13.0	0	0	10×4^{11}
<i>Hadropterus scierus</i> ♂	1	23	10	1	43.5	43.5	10.0	10.0	10×10
<i>Percina caprodes</i> ♂	0 (1)	0	0	0	0	0	0	0	10×10
<i>Percina caprodes</i> ♂	1 (1)	37	2	1	5.4	5.4	50.0	50.0	10×15^{10}
<i>Hadropterus scierus</i> ♀									10×10
<i>Hadropterus scierus</i> ♂	3	384	248	0	64.6	59.4	0	0	10×4^{11}
<i>Hadropterus scierus</i> ♂	1	83	5	0	6.0	6.0	0	0	
<i>Percina caprodes</i> ♀									$7 \times 7, 12 \times 12,$ 13×13
<i>Percina caprodes</i> ♂	2	724	498	151	68.6	70.8	30.3	32.8	$12 \times 13, 15 \times 12^{10}$ $12 \times 16, 13 \times 16^{10}$
<i>Percina caprodes</i> ♂	5	423	278	15	65.7	68.9	5.4	5.4	7×18^{11}
<i>Etheostoma lepidum</i> ♂	3	266	148	122	55.6	53.0	82.4	78.4	12×12
<i>Etheostoma lepidum</i> ♂	1	1050	177	0	16.7	16.7	0	0	12×14^{10}
<i>Etheostoma spectabile</i> ♂	2	300	215	119	71.7	71.3	55.3	60.7	
<i>Etheostoma spectabile</i> ♂	1	174	156	122	89.7	89.7	78.2	78.2	

¹ The number of additional samples with apparently mature eggs that showed no development is listed in parentheses. These samples are not included in the figures listed outside of the parentheses.

² See table 2 for the localities designated by the numbers. The locality of the maternal parent is listed first.

³ Use 158 for denominator for percentage placed in tank.

⁴ Use 431 for denominator for percentage placed in tank.

⁵ Use 1682 for denominator for percentage placed in tank.

⁶ Use 1 for denominator for percentage placed in tank.

⁷ Use 900 for denominator for percentage placed in tank.

⁸ Use 11 for denominator for percentage placed in tank.

⁹ Use 2840 for denominator for percentage placed in tank.

¹⁰ Both localities in same stream system.

¹¹ Parental localities in different stream systems.

STRAWN

HYBRID SURVIVAL IN FISHES

Comparison of allopatric and sympatric crosses

Eleven of the hybrid combinations (or reciprocals) and three of the controls have been carried out using parents with different degrees of allopatry (table 3). We have selected three levels for presentation: both parents from the same locality, the parents from different localities within the same stream system, and the parents from different stream systems. Darters have developed isolative mechanisms to prevent interbreeding between sympatric species but we find no strong indication that any such isolative mechanism between sympatric species of darters includes hybrid inviability. Of the 11 hybrid combinations, one (*Etheostoma spectabile* ♀ × *Percina caprodes* ♂) shows little or no difference in survival whether the gametes come from the same locality or different river systems, 5 appear to have better survival with increasing sympatry, and the other 5 seem to have better survival with decreasing sympatry. Four of the 5 combinations that have greater survival rates with decreasing sympatry are considered to be intergeneric, and thus are from distantly related parents. These species are expected to have developed many other isolative mechanisms. The remaining one (*E. spectabile* ♀ × *E. caeruleum* ♂) is between two closely related species. As *E. caeruleum* does not occur at or near the locality (Austin) from which the eggs were obtained for the cross between the parents from different systems, it appears as if Arkansas populations of *E. spectabile* have evolved a decrease in gamete compatibility with *E. caeruleum*. We do not believe that this is likely. *Etheostoma spectabile* eggs from our Arkansas stocks are small and delicate. In many respects they resemble *E. fonticola* eggs in their susceptibility to laboratory conditions. All of our *E. spectabile* crosses based on Arkansas eggs have a far lower survival rate than those based on eggs taken from Texas females. This is most apparent when the figures for *E. spectabile* ♀ × *E. lepidum* ♂ hybrids are studied. *Etheostoma lepidum* is another species closely related to *E. spectabile*. Over much of its range (and all of the range of

the males used from the same stream system attempted crosses from different systems in Arkansas. These crosses from the same locality show inviability with 1 pronounced in general, we find based upon the de parents that live makeup).

We believe that inhabiting limestone reared under probably can be attributed species has not *Etheostoma lepidum* questionably valid either is sympatric range or a form sympatric with the between forms which distinct genera in relationship necessarily appears to be the of natural hybrids hybridization in tribal hybrids. We between darters (family Centrarchidae) samples of darter *lepidum*, were expected *Lepomis punctatus* ther) respectively

sympatric crosses

as (or reciprocals) and
ried out using parents
ble 3). We have selected
parents from the same
localities within the same
different stream systems.
mechanisms to prevent in-
es but we find no strong
mechanism between sym-
brid inviability. Of the
Etheostoma spectabile ♀ ×
no difference in survival
ame locality or different
r survival with increas-
to have better survival
the 5 combinations that
creasing sympatry are
hus are from distantly
ected to have developed
The remaining one (*E.*
ween two closely related
ur at or near the locality
obtained for the cross
ystems, it appears as if
have evolved a decrease
E. lepidum. We do not believe
E. spectabile eggs from our
e. In many respects they
ceptibility to laboratory
osses based on Arkansas
han those based on eggs
st apparent when the fig-
E. lepidum ♂ hybrids are studied.
ies closely related to *E.*
and all of the range of

the males used for the crosses from the same locality or the same stream system) it is sympatric with *E. spectabile*. The attempted crosses of this combination between individuals from different systems used female *E. spectabile* from Arkansas. These crosses were far less successful than those from the same locality or the same stream system. If hybrid inviability with *E. lepidum* had developed, it should be most pronounced in sympatric populations of *E. spectabile*. In general, we find no strong indication of reduction of viability based upon the degree of allopatry (We have used only darter parents that live in water of the same general chemical makeup).

DISCUSSION

We believe that almost any darter hybrid from parents inhabiting limestone waters can be raised to mature size if reared under proper conditions. All of our failures reasonably can be attributed to technique, as one (or both) parental species has not been reared. With the exception of the *Etheostoma lepidum* × *E. grahamsi* hybrids, all involve unquestionably valid species. The parents of each combination either is sympatric with the other parent over part of its range or a form morphologically similar to one parent is sympatric with the other parent. The crosses include those between forms which are currently considered to represent distinct genera in all recent taxonomic works. The degree of relationship necessary to produce hybrids in the laboratory appears to be the same as that which limits the production of natural hybrids. In Carl L. Hubbs' ('55) review of natural hybridization in fishes, he reported no interfamilial or intertribal hybrids. We have attempted 6 interfamilial crosses between darters (family Percidae) and sunfishes of the related family Centrarchidae. In two of the attempts apparently good samples of darter eggs, *Hadropterus scierus* and *Etheostoma lepidum*, were exposed to visible quantities of sunfish sperm, *Lepomis punctatus* (Cuvier) and *Lepomis microlophus* (Günther) respectively. Although three of the *E. lepidum* eggs

showed some signs of development they died without the eyes developing pigment. In the other 4 attempts part of the spawn of sunfish eggs were exposed to sunfish sperm and darter sperm. Development was noted in reciprocals of the two crosses using darter eggs. Death occurred before or just after hatching. Other attempts to rear hybrids from distantly related parents experimentally have been unsuccessful (Clark and Moulton, '49; Moenkhaus, '03; Pinney, '18, '22, and '28; Newman, '15 and '17). Moenkhaus ('10) reared a number of hybrids between species now considered to be congeneric or to belong to closely related genera. He was unable to rear any hybrids between distantly related species. He reported decreasing success with decreasing morphologic relationship. Newman ('14) reported considerable success in rearing interspecific hybrids within the genus *Fundulus* and death at or before hatching of intersubfamilial hybrids between members of the genera *Fundulus* and *Cyprinodon*. Pinney ('28) had the most development of interfamilial hybrids with normal mitoses between parents belonging to relatively closely related families and hybrids with abnormal mitoses between distantly related parents. Probably the latter are parthenogenetic, a phenomenon not proven in darters.

The level of potential interfertility of darters (and other fishes) falls at a higher taxonomic rank than that of other animal groups. Blair ('41) has reported viable intergeneric offspring in tree frogs. However, the two species represented in this cross (*Hyla crucifer* × *Pseudacris triseriata*) are considered by some taxonomists to be congeneric. Both A. P. Blair and Hague S. Lindsay and William F. Pyburn, unpublished, have successfully reared crosses between other members of these nominal genera. Moore ('55) reported no other successful intergeneric crosses in anuran (salientian) amphibians. Many of the attempted intrageneric crosses reviewed by Moore are unsuccessful. Our results further differ from those Moore reviewed in that viable hybrids are produced by parents between which gene flow is extremely unlikely. We find that intergeneric or intrageneric hybridization of

fresh-water fish between the toad can be successful to cross members of the genus *Pe* (personal communication between members). Hybrids of species produced and occur between other parental species. Hybrids have been of the published the same species ('55) has informed 1952 also are being and that many may produce viable taxonomic rank such as the darter groups that have been due either to in the groups we

The ease by which produced in fishes (1950). In both groups made in the laboratory group in which the higher plants is

Mr. Allan D. I attempted many crosses *E. whipplei* (Girard) reciprocal using *E. whipplei* (*E. spectabile*) to be *whipplei*) a "soft

they died without the
er 4 attempts part of
posed to sunfish sperm
s noted in reciprocals
Death occurred before
s to rear hybrids from
ally have been unsuc-
oenkhaus, '03; Pinney,
'17). Moenkhaus ('10)
pecies now considered to
related genera. He was
stantly related species.
decreasing morphologic
d considerable success
n the genus *Fundulus*
intersubfamilial hybrids
Fundulus and *Cyprinodon*.
ent of interfamilial hy-
rents belonging to rela-
ybrids with abnormal
nts. Probably the latter
not proven in darters.
of darters (and other
nk than that of other
ted viable intergeneric
wo species represented
Peris triseriata) are con-
ongeneric. Both A. P.
am F. Pyburn, unpub-
s between other mem-
'55) reported no other
n (salientian) amphib-
neric crosses reviewed
ts further differ from
hybrids are produced
is extremely unlikely.
neric hybridization of

fresh-water fishes resembles that of intrageneric hybrids between the toads of the genus *Bufo* in that most combinations can be successfully reared. Dice ('33) reported that attempts to cross members of different species groups of deer mice of the genus *Peromyscus* had been unsuccessful. W. F. Blair (personal communication, '55) knows of no successful hybrids between members of different species groups of deer mice. Hybrids of species within the same species groups have been produced and occasionally produce fertile offspring. Hybrids between other mammals have been produced; however, the parental species are closely related and often allopatric. Many hybrids have been produced in the laboratory between fruit flies of the genus *Drosophila* (Patterson and Stone, '52). All of the published records, however, are between members of the same species groups. Patterson (personal communication, '55) has informed us that the additional crosses made since 1952 also are between members of the same species groups, and that many members of the same species groups will not produce viable hybrids. There is little question that the taxonomic rank at which hybrids may be produced in fishes, such as the darters, is much higher than that of other animal groups that have been studied. Although this difference may be due either to different degrees of splitting and lumping in the groups we believe that it is due to biologic factors.

The ease by which radical hybrid combinations can be produced in fishes resembles that of higher plants (Stebbins, '50). In both groups many intergeneric crosses have been made in the laboratory. Likewise, fishes are the only animal group in which the extensive natural hybridization typical of higher plants is known.

Mr. Allan D. Linder of Oklahoma A. and M. College has attempted many crosses between *Etheostoma spectabile* and *E. whipplei* (Girard). He has repeatedly succeeded with the reciprocal using *E. spectabile* females and never with that using *E. whipplei* females. As we consider one species (*E. spectabile*) to be a "hard-water darter" and the other (*E. whipplei*) a "softer-water darter," water chemistry may have

However, Newman ('14) which he raised only one mated a toad hybrid of Moreover, Linder has between *E. spectabile* and *E. radiosum* (Hubbs and tion of darters Carl L. is moderately common e less reduced and more ese species, in compari- small darters, have less e greater ease by which f the primitive species mber of eggs produced a indicates less control The small darters have the areas and habitats ference in the viability erence in the frequency om other causes. Tem- deemed significant, as arental species collected tion. Spatial isolating gnificance, as we have d females of the two ul. As those with less highest natural hybrid be a major isolating ear to have less control produce hybrids than rate. Therefore, Bailey, wn frequency of natural p may be invalid. One o darter genera is the s indicate that the high nly the result of their

close genetic relationship, but also may result from their having less control over the fate of their gametes.

We have found no significant variation in the viability of the hybrids which can be attributed to the degree of allopatry of the parents. Dobzhansky ('37: 258) suggested that sympatric forms are more likely to have intrinsic isolating mechanisms than allopatric forms. Any such intrinsic isolating mechanism within darters apparently does not include hybrid inviability.

The one demonstrated fertile F_1 is between allopatric forms currently recognized as distinct species (*E. lepidum* and *E. grahami*). Hybrids of this combination are as fertile as their controls. However, mating preference tests have not been made. Moreover, two sympatric species of minnows have hybrid offspring which are as fertile as their controls (Clark Hubbs and Strawn, '56). Further work is necessary before deciding whether the currently recognized specific ranking of these two forms is justified.

SUMMARY

1. Development has occurred in 113 hybridization experiments and 57 control experiments.
2. The taxonomic level at which laboratory hybrids can be produced in fishes approximates that of natural hybrids. This level is of a higher category than that of other animal groups.
3. Natural hybrids are less frequent between species that have more elaborate courtships and appear to have more control over the fate of any individual egg. Laboratory hybrids between these species are at least as easy to raise as those between species with less complicated courtships and less control over the deposition of individual eggs. Apparently mate selection is a major isolating factor in darters.
4. The degree of allopatry of parental stocks does not appear to affect hybrid viability.
5. The survival rate of many hybrids approximates that of their most successful parental species at any given stage

under laboratory conditions. When one parent is more successful at an early stage and the other more successful at a later stage, the hybrid can have the survival rate of the former during the early stage and the latter during its more advanced stage. Therefore, the overall survival of the hybrids are no more successful than the most successful parent at any one stage.

6. The hybrids are not only intermediate morphologically but also have intermediate behavior and developmental patterns. There is no doubt that the gene plasms have been mixed.

7. Interfertility has been demonstrated between two allopatric nominal species (*E. lepidum* and *E. grahami*).

8. Darter females fed only one type of food (Houston dwarf white worms) produce eggs as often as those fed white worms and mosquito larvae. However, the eggs from females fed a diet of white worms are less viable than those from females fed mainly on mosquito larvae.

ACKNOWLEDGMENTS

This research project has been supported by a grant from the National Science Foundation. Jack C. Kidd and Walter E. Fosberg have assisted in the care and maintenance of stocks.

Many individuals have assisted in the collection of specimens: Dr. A. P. Blair, Mr. Allan D. Linder, Dr. George A. Moore, and Mr. John Tilton assisted in the Arkansas collections. Mr. Jay Davis, Mr. Walter E. Fosberg, Mr. William F. Herzog, Mr. Clifford Johnson, Mr. David Pettus, Mr. William F. Pyburn, Mrs. Dorothy B. Treviño Robinson, Mr. Victor G. Springer, Mrs. Charlene Strawn, and Mr. Aaron Wasserman assisted in collecting the Texas parents. Many land owners have permitted us to collect specimens from their property.

We wish to thank Doctors Blair and Moore for assistance in identifying Arkansas specimens. Mr. Linder has kindly

made available the results and *E. whipplei* (Girard) and encouraged us during the work in which subgenera have experimented.

Doctors W. F. Blair, read and criticized this manuscript to them for their many

- BAILEY, R. M., H. E. WINN AND
River, Alabama and
Proc. Acad. Nat. Sci.
BLAIR, A. P. 1941 Isolating
U. S., 27: 14-17.
CLARK, E., AND J. M. MOULTON
1949: 152-154.
DICE, L. R. 1933 Fertility r
species of mice in
298-305.
DOBZHANSKY, TH. 1937 Gen
Press: xvi + 364 pp
HUBBS, CARL L. 1955 Hybrid
Zoology, 4: 1-20.
HUBBS, CLARK 1954 A new T
scierus, with a discu
Nat., 52: 211-220.
HUBBS, CLARK, AND K. STRAW
fishes, *Notropis lu*
341-344.
——— Relative variabili
spectabile and *Percin*
LOHR, E. W., J. R. AVRETT, B
1952 The industrial
central states, 1952.
MOENKHAUS, J. 1903 The dev
clitus and *Menidia n*
maternal and patern
——— 1910 Cross fertil
1910: 353-393.
NEWMAN, H. H. 1908 The pr
of *Fundulus* hybrids.
——— 1914 Modes of ir

ne parent is more suc-
 r more successful at a
 vival rate of the former
 rring its more advanced
 of the hybrids are no
 sful parent at any one

mediate morphologically
 nd developmental pat-
 ene plasms have been

ated between two allo-
 nd *E. grahami*).

pe of food (Houston
 ften as those fed white
 the eggs from females
 able than those from
 e.

s
 orted by a grant from
 k C. Kidd and Walter
 e and maintenance of

he collection of speci-
 Linder, Dr. George A.
 n the Arkansas collec-
 Fosberg, Mr. William
 r. David Pettus, Mr.
 reviño Robinson, Mr.
 rawn, and Mr. Aaron
 Texas parents. Many
 llect specimens from

Moore for assistance
 r. Linder has kindly

made available the results of his crosses between *E. spectabile* and *E. whipplei* (Girard). Dr. Carl L. Hubbs has advised and encouraged us during the investigation as well as telling us in which subgenera he placed the species with which we have experimented.

Doctors W. F. Blair, J. T. Patterson, and W. S. Stone have read and criticized this manuscript. We are deeply indebted to them for their many constructive criticisms.

LITERATURE CITED

- BAILEY, R. M., H. E. WINN AND C. L. SMITH 1954 Fishes from the Escambia River, Alabama and Florida, with ecological and taxonomic notes. Proc. Acad. Nat. Sci., Philadelphia, 106: 109-164.
- BLAIR, A. P. 1941 Isolating mechanisms in tree frogs. Proc. Natl. Acad. Sci., U. S., 27: 14-17.
- CLARK, E., AND J. M. MOULTON 1949 Embryological notes on *Menidia*. Copeia, 1949: 152-154.
- DICE, L. R. 1933 Fertility relationships between some of the species and subspecies of mice in the genus *Peromyscus*. J. of Mammalogy, 14: 298-305.
- DOBZHANSKY, TH. 1937 Genetics and the Origin of Species. Columbia Univ. Press: xvi + 364 pp.
- HUBBS, CARL L. 1955 Hybridization between fish species in nature. Systematic Zoology, 4: 1-20.
- HUBBS, CLARK 1954 A new Texas subspecies, *apristis*, of the darter *Hadropterus scierus*, with a discussion of variation within the species. Am. Midland Nat., 52: 211-220.
- HUBBS, CLARK, AND K. STRAWN 1956 Interfertility between two sympatric fishes, *Notropis lutrensis* and *Notropis venustus*. Evolution, 10: 341-344.
- Relative variability of hybrids between the darters, *Etheostoma spectabile* and *Percina caprodes*. Evolution, in press.
- LOHR, E. W., J. R. AVRETT, B. IRELAND, G. A. BILLINGSLEY AND T. B. DOVER 1952 The industrial utility of public water supplies in the west south-central states, 1952. Geol. Survey Circ., 221: vi + 123 pp.
- MOENKHAUS, J. 1903 The development of the hybrids between *Fundulus heteroclitus* and *Menidia notata* with especial reference to the behavior of the maternal and paternal chromatin. Am. J. Anat., 3: 29-66.
- 1910 Cross fertilization among fishes. Proc. Indiana Acad. Sci., 1910: 353-393.
- NEWMAN, H. H. 1908 The process of heredity as exhibited by the development of *Fundulus* hybrids. J.E.Z., 5: 503-561, pls. i-v.
- 1914 Modes of inheritance in teleost hybrids. J.E.Z., 16: 447-499.

- NEWMAN, H. H. 1915 Development and heredity in heterogenic teleost hybrids. *J.E.Z.*, 18: 511-576.
- 1917 On the production of monsters by hybridization. *Biol. Bull.*, 32: 306-321.
- PATTERSON, J. T., AND W. S. STONE 1952 Evolution in the genus *Drosophila*. Macmillan Co., New York: 610 pp.
- PINNEY, E. 1918 A study of the relation of the behavior of chromatin to development and heredity in teleost hybrids. *J. Morph.*, 31: 225-291.
- 1922 The initial block to normal development in cross fertilized eggs. *J. Morph.*, 36: 401-419.
- 1928 Developmental factors in teleost hybrids. *J. Morph.*, 36: 579-598.
- STEBBINS, G. L., JR. 1950 Variation and Evolution in Plants. Columbia Univ. Press: xx + 643 pp.
- STRAWN, K. 1956 A method of breeding and raising three Texas darters. Part II. *Aquarium Journal*, 27: 11, 13, 14, 17, 31, + 32.
- STRAWN, K., AND CLARK HUBBS 1956 Observations on stripping small fishes for experimental purposes. *Copeia*, 1956: 114-116.
- THORNTON, W. A. 1955 Interspecific hybridization in *Bufo woodhousei* and *Bufo valiceps*. *Evolution*, 9: 455-468.
- WINN, H. E. 1954 Comparative reproductive behavior and ecology of fourteen species of darters (Pisces-Percidae). Unpublished Ph.D. Dissertation, University of Michigan, 1954: vi + 234 pp., figs. 1-7.

DI
OF GROWTH
AND TE
H

Departm
Ma

The experimen
tion of the gro
flexuosa. The g
which it manife
zones of growth
temperature or
change condition
which might be p
uniformly throu
remain the same
affected than o
colony will be a

Each species o
pattern. The de
growth has long
by Kühn ('13).
sulted for brief

¹Contribution No. 6
This investigation was
Heart Institute, of th
by a grant-in-aid from
Committee on Growth
are in the text.