ILLINOIS NATURAL HISTORY SURVEY
Bulletin

Volume 29, Article 3
September, 1967

Printed by Authority of
the State of Illinois

Hybridization of Four Species of Sunfishes (Centrarchidae)

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DEPARTMENT OF REGISTRATION AND EDUCATION

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CONTENTS

This report is printed by authority of the State of Illinois, IRS Ch. 127, Par. 58.12. It is a contribution from the Section of Aquatic Biology of the Illinois Natural History Survey.

(39846-5000-9-67)

Frontispiece.—A green sunfish x bluegill F_1 hybrid produced by stocking male green sunfish and female bluegills in a pond containing no other fishes. Young-of-the-year F_1 hybrids were removed from the brood pond and stocked in a pond which contained only largemouth bass. When captured, this individual was 6 years old, had a total length of 12.2 inches, and weighed 2 pounds, 2 ounces.

Hybridization of Four Species of Sunfishes (Centrarchidae)

WILLIAM F. CHILDERS

HYBRIDIZATION is very common within many genera of plants and has been postulated as an important evolutionary mechanism of speciation in the plant kingdom (Anderson 1953:282-283; Heiser 1949:654; Stebbins 1959: 248). In the animal kingdom, the evolutionary importance of hybridization is less clear. Hybrids have been reported for most metazoan groups although in only a few cases are they known to be self-perpetuating (Ross 1958:337).

In fishes the incidence of natural hybridization appears to be low. Bailey (1960a:243) estimates that there are between 15,000 and 17,000 Recent fish species, and Slastenenko (1957:76-91) lists only 167 known natural interspecific fish hybrids of the world. Grossman & Buss (1965:1261) suggest the possibility of three additional hybrids in the Esocidae. Approximately 90 percent of these 170 hybrids were found in freshwaters; the rest occurred in marine or brackish water environments. About two-thirds of the freshwater hybrids were found in North America. Hubbs (1955:16,18-19) pointed out that hybridization has probably been most frequent in North American freshwaters where the existing fish fauna became established only as recently as the Miocene, Pliocene, and Pleistocene epochs. Hubbs also remarked that a considerable body of circumstantial evidence indicated that introgressive hybridization has been a significant factor in speciation in the tribe Lepomini of the Centrarchidae.

The term hybrid has been variously defined (Darwin 1897:1-2; Darlington 1958:40; Stebbins 1959:231). Unless otherwise specified, hybrid, as used here, refers to the offspring of interspecific matings.

ACKNOWLEDGMENTS

This paper is based upon research conducted at the Illinois Natural History Survey. Dr. George W. Bennett, Head of the Aquatic Biology Section, supervised this project and aided in all stages of the study. His advice, help, and encouragement were invaluable.

Most of the results presented in this paper were included in a thesis submitted to the University of Illinois in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Professor Charles S. Kendeigh directed my graduate studies, served as chairman of my doctoral committee, and assisted in the revision of my thesis manuscript.

Drs. Weldon R. Larimore and Donald F. Hansen of the Aquatic Biology Section of the Illinois Natural History Survey aided in part of the field work, criticized the manuscript, and made many valuable suggestions during all stages of this study. Mr. Robert M. Zewadski, Associate Technical Editor of the Survey, edited this paper, and his patience and skill are greatly appreciated. Professor Horace W. Norton of the University of Illinois advised and assisted in the statistical analysis of the data.

I also express my appreciation to my wife, Norma H. Childers, who assisted with laboratory experiments, and to staff members, too numerous to mention individually, of the Illinois Natural History Survey and the University of Illinois who contributed to this study.

Mr. William Utterback of Gibson

City, Mr. and Mrs. G. Maxfield of Fairmount, and Professor Fay H. Root, Assistant Director of Robert Allerton Park, donated the use of numerous ponds. The willing cooperation of these people is greatly appreciated.

THE CENTRARCHIDAE

The Centrarchidae (sunfishes) are a group of freshwater fishes of temperate North America whose present center of distribution is the Mississippi River basin. A complete classification of the extant Centrarchidae is given in the accompanying list. With one exception all 27 species were originally limited to freshwaters east of the Rocky Mountains. This exception, the Sacramento perch, *Archoplites interruptus* (Girard), is a relict form native to Califor-

Classification of the Centrarchidae. The scientific and common names are those suggested by Bailey (1960b: 27) and the subfamily and tribal classification follows that of Branson & Moore (1962).

Subfamily Centrarchinae	
Tribe Ambloplitini	
Archoplites interruptus (Girard)	Sacramento perch
Ambloplites cavifrons Cope	Roanoke bass
Ambloplites rupestris (Rafinesque)	Rock bass
Acantharchus pomotis (Baird)	Mud sunfish
Tribe Centrarchini	
Pomoxis nigromaculatus (Lesueur)	Black crappie
Pomoxis annularis Rafinesque	White crappie
Centrarchus macropterus (Lacépède)	Flier
Subfamily Lepominae	
Tribe Enneacanthini	
Enneacanthus obesus (Girard)	Banded sunfish
Enneacanthus gloriosus (Holbrook)	Bluespotted sunfish
Enneacanthus chaetodon (Baird)	Blackbanded sunfish
Tribe Lepomini	
Chaenobryttus gulosus (Cuvier)[1]	Warmouth
Lepomis symmetricus Forbes	Bantam sunfish
Lepomis cyanellus Rafinesque[1]	Green sunfish
Lepomis macrochirus Rafinesque[1]	Bluegill
Lepomis humilis (Girard)	Orangespotted sunfish
Lepomis gibbosus (Linnaeus)	Pumpkinseed
Lepomis microlophus (Günther)[1]	Redear sunfish
Lepomis punctatus (Valenciennes)	Spotted sunfish
Lepomis marginatus (Holbrook)	Dollar sunfish
Lepomis auritus (Linnaeus)	Redbreast sunfish
Lepomis megalotis (Rafinesque)	Longear sunfish
Tribe Micropterini	
Micropterus salmoides (Lacépède)	Largemouth bass
Micropterus dolomieui Lacépède	Smallmouth bass
Micropterus coosae Hubbs & Bailey	Redeye bass
Micropterus notius Bailey & Hubbs	Suwannee bass
Micropterus punctulatus (Rafinesque)	Spotted bass
Micropterus treculi (Vaillant & Bocourt)	Guadalupe bass

[1]Species used in hybridization experiments.

nia. In the last 100 years many species have been widely introduced into freshwaters throughout North America and in other parts of the world.

Lepomini Evolution

On the basis of the fossil record, current natural geographic distribution, and comparative morphology, the evolution of the Lepomini can be hypothesized as follows:

1) The Centrarchidae date from the early Cenozoic and are closely related to the sea basses (Serranidae) (Miller 1958:199).
2) The Mississippi River basin was probably their center of origin (Branson & Moore 1962:88).
3) A relative abundance of extinct centrarchids in Miocene and Pliocene rocks of Oregon, Nevada, and Utah indicates that the Centrarchids' range was much larger then than it is now (Miller 1958: 193,199).
4) The Rocky Mountain uplift, beginning in the Miocene or early Pliocene and increasing to the end of the era (Schuchert & Dunbar 1941:386) isolated west coast Centrarchids from those east of the Rocky Mountains.
5) Fossils of the extant species warmouth and black crappie have been found in middle Pliocene deposits in Logan County, Kan. (Branson & Moore 1962:96).
6) Late Pliocene to early Pleistocene deposits in southern Idaho and eastern Oregon contain a fossilized sunfish which is probably of the genus *Lepomis* (Miller 1958: 194).
7) During the Pleistocene the west

Lepomini hybrids known to occur in nature.

Kind of Hybrid	*Reference*
Warmouth x Pumpkinseed	Radcliffe (1914:27)[2]
Warmouth x Redbreast sunfish	McAtee & Weed (1915:13)[1]
Warmouth x Green sunfish	McAtee & Weed (1915:13)[1]
Warmouth x Bluegill	Hubbs (1920:102)[2]
Green sunfish x Bluegill	Bailey & Lagler (1938:588-604)[2]
Green sunfish x Pumpkinseed	Bailey & Lagler (1938:588-604)[2]
Green sunfish x Longear sunfish	Cross & Moore (1952:410-411)[2]
Green sunfish x Redbreast sunfish	Raney (1940:364)[1]
Green sunfish x Red-ear sunfish	Trautman (1957:501)1
Green sunfish x Orangespotted sunfish	Hubbs & Ortenburger (1929:42)[1]
Bluegill x Red-ear sunfish	Cross & Moore (1952:411)[2]
Bluegill x Pumpkinseed	Bailey & Lagler (1938:588-604)[2]
Bluegill x Orangespotted sunfish	Cross & Moore (1952:411)[2]
Bluegill x Longear sunfish	Cross & Moore (1952:411)[2]
Bluegill x Redbreast sunfish	Bailey & Lagler (1938:577)[1]
Pumpkinseed x Orangespotted sunfish	O'Donnell (1953:487)[1]
Pumpkinseed x Redbreast sunfish	Greeley & Bishop (1933:101)
Pumpkinseed x Longear sunfish	Hubbs (1926:72)
Longear sunfish x Orangespotted sunfish	O'Donnell (1935.487)[1]
Warmouth x Red-ear sunfish	Childers (unpublished)
Bluegill x Spotted sunfish	Stinauer & Childers (unpublished)

'Contains no description.
'Contains description.

coast species were probably restricted to a southern coastal distribution and are represented today by one relic species, the Sacramento perch.

8) During the Pleistocene northern species east of the Rocky Mountains withdrew in a southeasterly direction or became extinct.
9) Speciation in the genus Lepomis probably has proceeded at a rapid rate during the Recent epoch.

Reported Natural Lepomini Hybrids

It is theoretically possible for the 11 species of Lepomini to hybridize in 110 different F_1 combinations; however, since it appears impossible morphologically to differentiate between hybrids of reciprocal crosses (Hubbs & Hubbs 1932:433), only 55 morphologically different Lepomini F_1 hybrids could be identified. Of this number at least 21 have been found in nature. In the list of naturally occurring Lepomini hybrids (page 161), an attempt has been made to give credit to the author of the first published description of each kind.

SPECIES SELECTED FOR STUDY

Four species of sunfishes in the tribe Lepomini (red-ear sunfish, bluegill, green sunfish, and warmouth) were selected as experimental species because of local availability; importance to sport fishing; taxonomic relationships; and similarities and differences in their morphology, habitat selection, and reproductive behavior.

Geographic Distribution

The natural geographic ranges of the four species greatly overlap one another (Tautman 1957: 497, 500, 504, 517). All four species are sympatric in east-central Illinois, and they are quite abundant in a number of lakes and ponds in this area. Bluegills, green sunfish, and warmouths are indigenous to east-central Illinois, and the red-ear sunfish, a more southern species, was successfully introduced into this area in 1946 (Bennett 1958:177).

Diagnostic Morphological Characters

Forbes & Richardson (1920 : 245-251, 257-259) and Trautman (1957:496—504, 516-518) give good morphological descriptions of the four experimental species. Certain key morphological characteristics of the four species are presented in Table 1.

Habitat Selection

Larimore (1957:2), in discussing the distribution of the warmouth in Illinois, stated that although the warmouth is principally a pond and lake fish, it occurs in the Rock, Mississippi, and Illinois rivers and is reported as common in small, sluggish streams in the southern part of the state. In east-central Illinois warmouth are only occasionally found in streams. Many of the creeks and some larger streams in this area have been dredged and are unsuitable for most species of fishes. The undredged portions of these streams are probably unsuitable for warmouths because their current velocities are greater than warmouths can tolerate. Trautman (1957:498) reports that in Ohio

> The Warmouth Sunfish was most numerous in lakes, ponds, oxbows, marshes, and stre:-ms of base or very low gradients which had silt-free water, an abundance of aquatic vegetation, and a mucky bottom which was often covered with organic debris. The species was present only in small numbers in weedless oxbows and ponds which had a yellow-silt bottom, and although its colloquial name was "Mud Bass" it seemed to be less tolerant to turbidity and siltation than was the Green Sunfish.

The green sunfish is abundant in creeks and small rivers in east-central Illinois (Forbes & Richardson 1920:250; Larimore & Smith 1963:325). This species is adept at ascending small temporary streams formed by overflow waters from lakes and ponds. Green sunfish are prolific and frequently gain access to a new lake or pond before other species of sunfishes. When this occurs, they commonly produce such large populations that the individuals become stunted. Green sunfish are usually unable to compete successfully with other species of sunfishes which typically inhabit clear-water lakes and ponds. Trautman (1957:501) stated that the green sunfish is more tolerant of turbidity and siltation than other sunfishes except the orangespotted; however, the largest populations were found in clear-water habitats under conditions of low competition with other sunfish species.

In east-central Illinois the largest populations of bluegills and red-ear sunfish occur in lakes and ponds which have relatively clear waters. Forbes & Richardson (1920:258) found that the bluegill occurred throughout Illinois, but it was generally limited to the larger streams and their principal tributaries and was common in northeastern glacial lakes. During the past 30 years bluegills have been stocked in thousands of Illinois lakes and farm ponds by federal and state agencies (Bennett 1962:104).

In 1951 the Illinois Department of Conservation obtained red-ear sunfish breeding stock from Dr. G. W. Bennett of the Illinois Natural History Survey. These adult fish were offspring of the red-ear sunfish which were introduced into east-central Illinois from Indiana in 1946 (Lopinot 1961:3). From 1951 to 1964 the Illinois Department of Conservation stocked 1,383 lakes and ponds with red-ear sunfish, and this species has been widely distributed throughout the state (W. J. Harth, personal communication).

Trautman (1957:518) remarked that wherever the red-ear sunfish has been introduced into waters which are north of its natural range, it has essentially inhabited nonflowing waters which were relatively clear and contained at least some aquatic vegetation. Trautman also stated that at Buckeye Lake, Ohio, the red-ear sunfish seemed to require as much as, or more aquatic vegetation than, did the bluegill, and that although both species frequented open water, the red-ear congregated about brush, stumps, and logs more than the bluegill.

Reproduction

The reproductive activities of the four kinds of sunfishes were observed over a 7-year period, from 1958 through 1964, in a number of lakes and ponds within 50 miles of Urbana, Ill. The most frequent observations were made in Big Pond (owned by William Utterback and located 5 miles southeast of Gibson City, Ill.) and Lake Italy (owned by the Material Service Corporation and located 3 miles south of Fairmount, Ill.) Big Pond contained bluegill, red-ear, and green sunfishes, and Lake Italy contained all four species.

TIME OF SPAWNING.—For all four species, males in spawning condition were first collected each year during late April or early May. The first ripe females were collected during the 2nd or 3rd week of May. The first fish to become ripe were invariably large individuals. Ripe individuals from stunted populations of bluegills and green sunfish were first collected 2-4 weeks later than from nonstunted populations. Ripe males and females of all four species were collected each month, May through August; however, ripe individuals were much less abundant during July and August than during May and June. The latest observed fall spawning occurred in Big Pond during the 1st week of September, 1960.

Big Pond is naturally divided into three areas which are connected by two short, narrow, shallow channels. On August 24, 1960, the three areas were separated by placing heavy canvas barriers across both channels. Two areas were treated with rotenone. On September 5 both treated areas were inspected to determine if any fish had

Table 1.—Certain key morphological characters of typical adult red-ear sunfish, bluegills, green sunfish, and warmouths (modified slightly from Forbes & Richardson 1920; Trautman 1957).

Morphological Character	Red-ear Sunfish	Bluegill	Green Sunfish	Warmouth
Teeth on tongue	None	None	Rarely a few	Well developed
Supramaxilla	Rudimentary or lacking	Rudimentary or lacking	Small or rudimentary	Well developed
Orange-red spot at bases of last 3 dorsal rays	Absent	Absent	Absent	Well developed in males, less so in females
Mouth size	Moderately small	Very small	Large	Large
Posterior edge of upper jaw extends	Almost to or to anterior edge of eye	Rarely to anterior edge of eye	To center of eye or beyond	To center of eye or beyond
Length of pectoral fin	Very long	Long	Short	Moderately short
Tip of pectoral fin, when laid forward across the cheek, reaches	Almost to or to tip of snout	Beyond anterior edge of eye	Posterior edge of eye	Posterior edge of eye
Tip of pectoral fin	Very pointed	Pointed	Round	Moderately round
Length of longest dorsal spine	Long	Long	Short	Moderately short
Length of longest dorsal spine/head length°	About 1/2	About 1/2	Usually 1/4	About 1/4

Gill rakers on first gill arch	Short, blunt, and often crooked	Moderately long, straight, and thin	Long, straight, and thin	Long and straight
Longest gill raker	About [illegible] diameter of eye	Almost 1/3 diameter of eye	Fully 1/2 diameter of eye	Fully 1/2 diameter of eye
Teeth on lower pharyngeal arches	Molar-like, bluntly pointed or rounded	Conical, thin, and sharply pointed	Conical, blunt with heavy basal portion	Conical, blunt with heavy basal portion
Lower pharyngeal arches	Broad and strong	Narrow and strong	Narrow and strong	Narrow and strong
Bony portion of opercle flap	Moderately flexible	Very flexible	Inflexible	Inflexible
Color of opercle membrane	White or slate gray with red spot on posterior edge	Black	Coppery to purplish	Coppery above to lavender below
Markings on side of head	Reddish-brown, irregular spots	2 broad, bluish bands, ventrally located	3-5 narrow, wavy, broken emerald lines	3-5 dark bands
Dusky spot in soft dorsal fin	None	Above bases of last 3 rays, sometimes absent	At bases of last 3 rays, sometimes absent	None
Dusky spot in soft anal fin	None	If present, usually poorly developed	At bases of last 3 rays, sometimes absent	None
Color of ventral fins	Dusky yellow	Dusky	Yellow	Transparent to light olive
Soft-rayed dorsal fin edged with	Nothing	Faint iridescent blue	Whitish to yellowish orange	Red in breeding males, white to absent in others

*Head length: the distance from the tip of the snout to the posterior margin of the bony portion of the opercle flap.

survived the rotenone treatment. The fish kill appeared to be complete in one area, but in the other approximately 30 male bluegills were occupying nests. All nests contained either eggs or larval young. A careful inspection of the untreated area failed to reveal a single nesting sunfish.

Swingle (1956:865) suggested that certain species of fishes secrete or excrete a hormone-like substance which acts as a repressive factor and inhibits reproduction in ponds containing dense fish populations. Apparently the rotenone treatment with its resulting drastic reduction of the fish population stimulated the few surviving bluegills to reproduce within about 9-11 days during a period which was somewhat later than their normal spawning season in east-central Illinois.

In Alabama, red-ear sunfish spawned in the spring when surface water reached a temperature of about 24° C.(75° F.), reproduced sparingly or not at all during the summer, and again spawned heavily in the early fall (Swingle 1949:299). I have observed no extensive fall spawning of red-ear sunfish in any east-central Illinois lakes and ponds.

In Fork Lake, Ill., during 1939, the bluegills of both sexes had gonads in spawning condition during June, July, and August. Males matured earlier than females and large males became sexually mature earlier than smaller males. Nests were first observed on May 28 when the water temperature at 3 feet was 25° C.(77° F.). Occupied nests were last observed on September 18 (Bennett, Thompson, & Parr 1940:17—18).

In the Gardner Ponds at the University of Wisconsin Arboretum the spawning season of green sunfish commenced in late May or early June when the water temperature reached about 21° C.(70° F.), continued through June and July, and terminated in early August. Apparently larger males spawned earlier and more frequently than smaller males (Hunter 1963: 16-18).

In Park Pond, Vermilion County, Ill., warmouth spawning was initiated during the 2nd week in May, 1949, when the water temperature at 12 inches was approximately 21° C.(70° F.). Gonadal weight-body weight ratios indicated that most spawning was completed by early July. Warmouths of less than 89 mm (3.5 inches) total length failed to spawn. Males ripened earlier in the season than females and large fish spawned earlier than smaller ones (Larimore 1957:31-35).

LOCATION OF NESTS.—The first evidence of reproductive activity in the spring was the movement of males into shallow water. As the length of the photoperiod and the temperature of the water increased, males constructed nests (saucer-shaped depressions in the substrate) which they defended with great vigor.

All four species usually nested in areas where the water was less than 3 feet deep. Red-ear sunfish, bluegills, and green sunfish normally nested in colonies, on firm substrates, and often in locations exposed to the sun. Warmouths were more solitary in their nest site selections. They frequently nested on soft substrates even when firm substrates were available. Larimore (1957:40) reported that warmouths were not as consistent in selecting a particular type of substrate as they were in selecting a spot near a stump, rock, root, clump of vegetation, or some similar object, and that nests were never found on an area of bottom completely exposed, such as was usually chosen by the bluegill.

In Utterback's Big Pond and Lake Italy, red-ear sunfish males and bluegill males were frequently found nesting together in the same colony. Green sunfish males were less commonly found nesting with males of the other species; however, this difference may have been related to the smaller numbers of green sunfish in both bodies of water. Warmouth males were never observed nesting in colonies. In colonies of nests occupied by more than one species, males of the minority species formed a subcolony within the larger group.

SPAWNING AND CARE OF YOUNG.—In general the four species are remarkably similar in their spawning and parental behavior. During spawning a pair slowly swims side by side in tight circles over the male's nest. Fertilization is external and the demersal eggs adhere to the material forming the bottom of the nest. After spawning, the female leaves or is driven from the nest by the male, and the male fans the eggs and larval young until they become free-swimming fry. During fanning, the male hovers over the nest while undulating his body in such a way that currents of water are directed downward into the nest. Fanning can best be described as stationary swimming. The water currents thus produced are probably important in cleansing and oxygenating the developing embryos. During this period, the male also protects the eggs and young and will viciously attack predators much larger than himself.

DURATION OF FERTILITY OF GAMETES.—Since three of the four species selected for study sometimes nest in mixed colonies, the functional life spans of gametes could be very important in controlling hybridization between these species. If gametes are capable of fertilizing and being fertilized over long periods of time, sperm driftage could result in the production of hybrid individuals. Experiments were conducted to determine the functional life spans of bluegill, green sunfish, and warmouth gametes. In one set of experiments, both sperm and eggs were aged for various periods of time prior to fertilization; in another group of experiments only eggs were aged.

Ripe male and female bluegills, green sunfish, and warmouths were captured by seining and trapping in local ponds.[1] These fish were moved into the laboratory and separated in aquaria according to species and sex. Fish were held in these aquaria for one-half hour to 2 hours before gametes were stripped from them. Care was taken to avoid any temperature shock to the fish prior to their use in the experiments.

In the experiments in which both sperm and eggs were aged prior to fertilization, the method was: Five clean glass petri dishes were individually numbered from 1 to 5. Each dish was then partially filled by adding 20 ml of water. All of the water used in these experiments was obtained from the well on Parkhill's Lake Park Subdivision Number Two. The water was moved into the Illinois Natural History Survey laboratory and stored in a 210-gallon aquarium. It was aerated and filtered through activitated charcoal for at least 1 week prior to its use. A partial chemical analysis of this water is presented in Table Al in the appendix.

Starting with dish 1 and ending with dish 5, eggs from a ripe female warmouth were stripped into each of the five dishes. Immediately after eggs were stripped into a dish, the dish was gently shaken to scatter the eggs over the bottom. Eggs were stripped into consecutive dishes at approximately 7—second intervals, and so the entire egg-stripping process was completed in about 30 seconds. During the next 15 seconds, one-half ml of seminal fluid was stripped from a ripe male warmouth and diluted with 10 ml of water. One ml of this solution was then added to the water and eggs in dish 1, and to the

[1]All sunfishes used in these experiments were obtained from these Illinois ponds: bluegills and green sunfish from Pifers Pond, about 3 miles southeast of Sullivan, and Utterback's Big Pond, 5 miles southeast of Gibson City; bluegills from Redhead's Pond, 4 miles east of Homer; warmouths and green sunfish from Lake of the Woods, 2 miles northeast of Mahomet; warmouths from Taylor's Pond, 3 miles southwest of Fairmount.

other dishes in sequence after intervals of 2.5, 5.0, 7.5, and 10.0 minutes. From 5 to 10 minutes after the gametes were mixed in each dish, the zygotes were washed three times (by decanting and refilling each dish with clean water) and then enough water was added to each dish to cover the eggs.

Water in each dish was changed several times during incubation, and dead eggs and embryos were removed. Newly hatched larvae were transferred to clean, numbered dishes. The number of eggs in each dish at time of fertilization, the number of eggs which hatched, and the number of larvae which developed into normal-appearing swim-up fry were recorded. The incubation temperature was recorded with an air thermograph located directly alongside the petri dishes.

The same procedure was followed in measuring the functional life spans of bluegill and green sunfish sperm and eggs, except that six egg samples were stripped from each female and the time interval separating the mixing of gametes in the sequence of dishes was 1.0 minute instead of 2.5 minutes.

In the second group of experiments only the eggs of the three species were aged prior to fertilization. The procedure of the first group of experiments was used, except that two drops of undiluted seminal fluid were stripped directly on the eggs after they had been aged for 0.5, 30.0, 60.0, 120.0, and 180.0 minutes.

The results of individual experiments concerning the functional life spans of activated gametes of bluegills, green sunfish, and warmouths are presented in Tables A2—A13 of the appendix. Data from experiments for each species were pooled and are presented in Tables 2-4.

Under the conditions of these experiments "average functional lives" (length of time gametes were aged that resulted in a 50—percent reduction in fry viability) of warmouth, bluegill, and green sunfish eggs were interpolated to be 94, 60, and 47 minutes, respectively. Specific differences may have been the result of variation in the physiological state of the mature eggs and the exposure of the various samples to uncontrollable environmental differences.

All of the eggs in an individual exper-

Table 2.—Duration of fertility of activated warmouth gametes. Data from experiments W1–W4 are combined.

Age of Eggs in Minutes	*Age of Sperm in Minutes*	*Number of Eggs*	*Percent of Eggs that Hatched*	*Percent of Eggs that Developed into Fry*°
		Aging of Sperm and Eggs		
0.75	0.25	118	46	44
3.15	2.75	231	10	9
5.55	5.25	150	0	
8.45	7.75	178	0	
10.35	10.25	123	0	
		Aging of Eggs		
0.50		128	92	80
30.00		161	53	52
60.00		172	59	56
120.00		150	33	29
180.00		178	3	1

°Fry able to swim for short periods but not completely free swimming. Only normal fry are included in this figure.

Table 3.—Duration of fertility of activated bluegill gametes. Data from experiments B1–B4 are combined.

Age of Eggs in Minutes	*Age of Sperm in Minutes*	*Number of Eggs*	*Percent of Eggs that Hatched*	*Percent of Eggs that Developed into Fry*[a]
		Aging of Sperm and Eggs		
1.00	0.25	92	65	48
1.90	1.25	74	49	24
2.80	2.25	76	12	12
3.70	3.25	85	1	1
4.60	4.25	65		
5.50	5.25	64		
		Aging of Eggs		
0.50		210	81	80
30.00		143	69	67
60.00		123	42	40
120.00		196	19	16
180.00		124	9	7

[a]Fry able to swim for short periods but not completely free swimming. Only normal fry are included in this figure.

Table 4.—Duration of fertility of activated green sunfish gametes. Data from experiments G1–G4 are combined.

Age of Eggs in Minutes	*Age of Sperm in Minutes*	*Number of Eggs*	*Percent of Eggs that Hatched*	*Percent of Eggs that Developed into Fry*[a]
		Aging of Sperm and Eggs		
1.00-2.25	0.25	163	48	44
1.90-3.15	1.25	141	18	18
2.80-4.05	2.25	105	3	3
3.70-4.95	3.25	223	3	2
4.60-5.85	4.25	139	1	1
5.50-6.75	5.25	158	2	2
		Aging of Eggs		
0.50		141	58	57
30.00		222	39	37
60.00		216	23	21
120.00		152	20	18
180.00		171	13	12

[a]Fry able to swim for short periods but not completely free swimming. Only normal fry are included in this figure.

iment were from a single female; however, eggs from one female may vary in their physiological response to fertilization. The first eggs to flow often appeared to be more nearly ripe than those which followed. Consequently if eggs from a female were slightly green, serial samples of her eggs would exhibit a decrease in the percentage hatching, but if her eggs were slightly past optimum ripeness, serial samples might increase in hatchability. Even though dead zygotes were removed and the water in the dishes was changed at frequent intervals during each experiment, carbon dioxide and dissolved oxygen tensions were probably variable and more critical in dishes where mortality was high. Chance contamination of some samples by bacteria and protozoans also may have resulted in environmental differences in various samples.

The average functional life spans of sperm (based on the age of the sperm in the experiments in which both eggs and sperm were aged) from warmouths, green sunfish, and bluegills were interpolated to be 1.1, 1.0, and 1.0 minutes, respectively. Specific differences in the results of these experiments were probably not valid because of the factors previously described.

The average functional life span of eggs from the three species was 67 minutes, and for sperm it was 1 minute. Functional life spans of gametes from red-ear sunfish were not investigated; however, they are probably similar to those of warmouths, bluegills, and green sunfish.

The brief functional life spans of the spermatozoans of these species are undoubtedly very important in reducing hybridization caused by sperm drifting from nest to nest.

HYBRIDIZATION EXPERIMENTS

Two types of experiments were used to produce hybrid sunfishes. In the first, referred to as "stripping experiments," gametes were stripped from ripe adults and manually mixed. With this method it was possible to determine species isolation due to incompatibilities between sperm and eggs (primary genetic isolation). In the second type, designated "isolation experiments," one or more pairs of fish composed of a male of one species and a female of another were isolated in small ponds to determine if they would hybridize when mates of their own species were absent.

In this paper R refers to red-ear sunfish, B to bluegill, G to green sunfish, and W to warmouth. Matings between individuals of different species are designated as P_1 crosses, and the resultant hybrids are designated as F_1 hybrids. F_2 hybrids are those produced by mating an F_1 male with an F_1 female. The male parent species is always given first; thus, the P_1 cross of a male bluegill and a female green sunfish is designated B x G and the resultant hybrids are designated BG F_1 hybrids; GB F_1 designates the reciprocal hybrids.

Stripping Experiments

Sperm and eggs stripped from the four parent species were paired in 16 different combinations to produce zygotes representing the four parent species and 12 hybrids. These experiments were designed to allow comparisons of rates of embryological development and the extent of viability of F_1 hybrids and their maternal parent species.

METHODS AND MATERIALS.—Ripe males and females of the four species were brought into the laboratory from nine local ponds. Laboratory treatment of these fish was the same as for those used in experiments concerned with functional life spans of gametes.

A ripe female of one of the four species and one ripe male of each of the four species were used in each experiment. No individual fish was used more than once. Fish selected for an experiment were individually isolated for at least 30 minutes before gametes were stripped, and the person doing the stripping rinsed and dried his hands after handling each fish.

Twelve clean petri dishes were individually marked and 20 ml of aged, filtered well water were added to each dish. A sample of eggs from one ripe female was stripped into each of the 12 petri dishes, and the eggs were scattered by gently shaking the dishes. Two drops of milt were then stripped into each dish. Milt from one male of each of the four species was used to fertilize the eggs in three dishes. An entire stripping program for the five fish was completed in less than 5 minutes. Approximately 10 minutes after the stripping was completed, the zygotes were washed three times by decanting and refilling each dish with clean water. During incubation the amount of water in each dish was regulated so that the developing embryos were always covered with a thin layer (2-8 mm) of water.

Dead embryos were removed, and the water covering the living embryos was changed several times during each experiment. The frequency with which dead embryos were removed and water was changed was varied according to the incubation temperature. In the experiments with the highest (28.6° C.) and the lowest (22.3° C.) mean incubation temperatures the intervals were approximately 5 and 24 hours, respectively. Larvae were transferred to clean, numbered dishes within 1 hour after hatching.

An air thermograph was used to record temperatures adjacent to the dishes containing the embryos. The maximum range of fluctuation of air temperature during any one experiment was 3° C. Hourly air temperature fluctuations never exceeded 0.7° C. Since the petri dishes contained relatively small amounts of water and since air temperature fluctuations were slight, water temperatures were considered to be the same as air temperatures in these experiments. Records were made of the number of eggs in each dish at the time sperm and eggs were mixed, the number of eggs that hatched each hour, and the number of larvae that developed into morphologically normal-appearing swim-up fry. Upon termination of each experiment, all living fry were killed with a 4-percent aqueous solution of formaldehyde and stored in a 1-percent solution. Total body lengths of 25 morphologically normal-appearing fry of each kind of viable fry from each experiment were measured to the nearest 0.03 mm with an ocular micrometer.

A total of 11 stripping experiments was conducted: Eggs from three red-ear sunfish, three bluegills, three green sunfish, and two warmouths were fertilized with sperm from males of all four species. The temperatures at which these experiments were conducted were well within the range of temperatures that embryos of the four species are subjected to under natural conditions. Nine of the experiments were terminated when the zygotes developed into swim-up fry. The other two experiments, both of which were conducted with red-ear sunfish eggs, were terminated shortly after the fry became free swimming.

RESULTS AND DISCUSSION.—The percentages of eggs that hatched and the percentages of eggs that developed into morphologically normal-appearing fry were calculated for each of the 132 samples of the 11 experiments (Tables A14—A24 of the appendix). These percentages were transformed into degrees of a right angle to minimize bias inherent in using weighted percentages in an analysis of variance (Fisher & Yates 1963:74–75). A 7094 digital computer was used in analyzing these data. Data from the 11 stripping experiments were condensed and are presented in Tables 5-8.

Preliminary tests revealed that high percentages of eggs hatched in some petri dishes containing as many as 500 eggs; however, mortality was higher in dishes containing 400-500 eggs than in dishes containing 200-300 eggs. Since the number of eggs per sample was a variable in these experiments, the number was purposely kept low (mean number of eggs per sample was 65) to minimize the effect of crowding. An analysis of variance revealed that there was no significant correlation between the number of eggs per sample and the percentage that hatched. Consequently, the number of eggs per sample was used as a statistical weight in the analysis of the viabilities of the 16 different kinds of zygotes.

Data from the 11 stripping experiments pertaining to the percentages of eggs that hatched and the percentages

Table 5.—Some aspects of the embryonic development of red-ear sunfish and BR,* GR, and WB F_1 hybrid sunfishes.

Parents	Number of Eggs	Percent† Hatched	Hatching Time in Hours		Incubation Temperature in Degrees C.‡		Percent† Normal Fry	Length of Fry in mm		
♂			Mean	Standard Deviation	Mean	Standard Deviation		Number Measured	Mean	Standard Deviation
Experiment S1, 161 hours, average temperature 23.6° C., standard deviation 0.77° C.										
R x R	135	45	50.3	5.36	23.8	0.41	38	25	4.78	0.300
B x R	147	58	52.1	4.45	23.8	0.40	50	25	4.86	0.139
G x R	117	57	49.6	5.33	23.8	0.41	50	25	4.98	0.165
W x R	123	36§	49.1	4.38	23.8	0.41	0	0	. . .	. . .
Experiment S2, 120 hours, average temperature 28.7° C., standard deviation 0.44° C.										
R x R	234	33	26.6	1.94	28.5	0.29	23	25	4.99	0.155
B x R	214	30	28.4	1.80	28.6	0.31	25	25	5.13	0.192
G x R	231	38	27.7	2.31	28.6	0.31	27	25	5.10	0.189
W x R	184	35§	27.9	2.65	28.6	0.31	5**	0	. . .	
Experiment S3, 120 hours, average temperature 28.7° C., standard deviation 0.44° C.										
R x R	143	44	28.1	2.18	28.6	0.31	24	25	5.28	0.166
B x R	151	41	28.2	1.79	28.6	0.31	29	25	5.58	0.137
G x R	204	49	28.2	1.94	28.6	0.31	41	25	5.54	0.185
W x R	241	27§	26.5	2.67	28.5	0.27	20*	0	. . .	. . .

*R = red-ear sunfish, B = bluegill, G = green sunfish, and W = warmouth.
†Percentage based on eggs in each sample at the time sperm and eggs were mixed.
‡Incubation temperature from time of fertilization to mean time of hatching.
§More than 90 percent of these larvae were morphologically abnormal and all were behaviorally abnormal.
**Appeared morphologically normal, but all were behaviorally abnormal.

Table 6.–Some aspects of the embryonic development of bluegill and RB,* GB, and WB F_1 hybrid sunfishes.

Parents	Number of Eggs	Percent† Hatched	Hatching Time in Hours		Incubation Temperature in Degrees C.‡		Percent† Normal Fry	Length of Fry in mm		
♂ x ♀			Mean	Standard Deviation	Mean	Standard Deviation		Number Measured	Mean	Standard Deviation
Experiment S4, 184 hours, average temperature 22.6° C., standard deviation 0.68° C.										
B x B	261	87	71.1	3.32	22.3	0.24	56	25	4.28	0.172
R x B	363	81	67.9	2.80	22.3	0.24	76	25	4.42	0.108
G x B	271	82	73.4	4.78	22.3	0.23	41	25	4.48	0.144
W x B	302	22§	59.8	4.04	22.3	0.23	0	0		
Experiment S5, 113 hours, average temperature 26.9° C., standard deviation 0.48° C.										
B x B	257	84	33.9	2.06	26.8	0.37	83	25	4.98	0.110
R x B	195	94	34.6	1.66	26.8	0.37	93	25	4.98	0.151
G x B	219	92	34.3	2.14	26.8	0.37	88	25	5.00	0.164
W x B	214	90§	29.4	1.49	26.8	0.40	0	0		. . .
Experiment S6, 105 hours, average temperature 27.3° C., standard deviation 0.65° C.										
B x B	163	90	32.5	0.83	27.0	0.42	90	25	4.69	0.076
R x B	184	88	31.8	0.83	27.0	0.40	85	25	4.63	0.069
G x B	149	91	32.3	1.01	27.0	0.40	91	25	4.61	0.095
W x B	183	93§	27.9	1.77	26.8	0.28	3**	0		

*R = red-ear sunfish, B = bluegill, G = green sunfish, and W = warmouth.
†Percentage based on eggs in each sample at the time sperm and eggs were mixed.
‡Incubation temperature from time of fertilization to mean time of hatching.
§More than 90 percent of these larvae were morphologically abnormal and all were behaviorally abnormal.
**Appeared morphologically normal, but all were behaviorally abnormal.

Table 7.—Some aspects of the embryonic development of green sunfish and RG,* BG, and WG F_1 hybrid sunfishes.

Parents ♂ x ♀	Number of Eggs	Percent† Hatched	Hatching Time in Hours: Mean	Hatching Time in Hours: Standard Deviation	Incubation Temperature in Degrees C.‡: Mean	Incubation Temperature in Degrees C.‡: Standard Deviation	Percent† Normal Fry	Length of Fry in mm: Number Measured	Length of Fry in mm: Mean	Length of Fry in mm: Standard Deviation
Experiment S7, 151 hours, average temperature 24.4° C., standard deviation 0.46° C.										
G x G	156	76	50.0	2.37	23.8	0.27	74	25	4.79	0.095
R x G	134	86	48.0	1.64	23.8	0.26	86	25	4.62	0.097
B x G	182	74	49.5	2.52	23.8	0.27	71	25	4.74	0.079
W x G	152	84	48.7	2.64	23.8	0.27	61	25	4.60	0.132
Experiment S8, 104 hours, average temperature 27.3° C., standard deviation 0.66° C.										
G x G	306	76	31.5	2.08	27.1	0.44	74	25	4.72	0.237
R x G	206	81	31.7	0.95	27.1	0.44	79	25	4.84	0.091
B x G	192	74	31.4	1.20	27.0	0.42	74	25	4.85	0.118
W x G	214	81	29.8	1.96	27.0	0.41	50	25	4.68	0.098
Experiment S9, 78 hours, average temperature 28.1° C., standard deviation 0.51° C.										
G x G	177	82	29.1	1.75	27.6	0.32	78	25	4.64	0.187
R x G	257	77	27.4	1.45	27.6	0.31	77	25	4.53	0.201
B x G	215	72	28.0	1.57	27.6	0.31	66	25	4.69	0.204
W x G	312	68	29.0	1.46	27.6	0.32	57	25	4.53	0.182

*R = red-ear sunfish, B = bluegill, G = green sunfish, and W = warmouth.
†Percentage based on eggs in each sample at the time sperm and eggs were mixed.
‡Incubation temperature from time of fertilization to mean time of hatching.

Table 8.-Some aspects of the embryonic development of warmouth and RW,* BW, and GW F_1 hybrid sunfishes.

Parents	Number of Eggs	Percent† Hatched	Hatching Time in Hours		Incubation Temperature in Degrees C‡		Percent† Normal Fry	Length of Fry in mm		
			Mean	Standard Deviation	Mean	Standard Deviation		Number Measured	Mean	Standard Deviation
Experiment S10, 115 hours, average temperature 27.2° C., standard deviation 0.45° C.										
W x W	179	51	29.4	1.55	27.3	0.33	45	25	4.75	0.176
R x W	175	47	29.4	1.31	27.3	0.33	43	25	4.63	0.139
B x W	197	53	30.8	1.29	27.3	0.33	45	25	4.74	0.180
G x W	146	55	30.9	1.37	27.3	0.33	35	25	4.76	0.212
Experiment S11, 78 hours, average temperature 28.1° C., standard deviation 0.51° C.										
W x W	116	69	28.9	1.56	27.6	0.31	56	25	4.72	0.204
R x W	142	80	29.2	1.03	27.6	0.31	68	25	4.60	0.199
B x W	114	68	28.9	1.24	27.6	0.31	54	25	4.77	0.141
G x W	130	71	29.7	1.00	27.6	0.32	62	25	4.90	0.203

*R = red-ear sunfish, B = bluegill, G = green sunfish, and W = warmouth.
†Percentage based on eggs in each sample at the time sperm and eggs were mixed.
‡Incubation temperature from time of fertilization to mean time of hatching.

of eggs that developed into morphologically normal-appearing fry were combined and are presented in Table 9. The percentages of hatched zygotes of the four pure species were compared with one another. Statistically, the hatching success of red-ear sunfish zygotes (39 percent) was significantly less (0.01 level) than that of warmouth (58 percent), green sunfish (78 percent), and bluegill zygotes (87 percent). The warmouth hatching percentage was significantly less than those of the green sunfish (0.05 level) and the bluegill (0.01 level). The hatching success of green sunfish zygotes was not significantly different from that of bluegill zygotes. These differences are not believed to represent valid differences between the hatchabilities of eggs of the four species, but are probably the result of differences in the maturity of eggs from the females used in these experiments. Consequently, to minimize such differences, the percentage of eggs that hatched and the percentage of normal-appearing fry of each hybrid type were compared with those of its maternal parent species.

No hybrid type was significantly different from its maternal parent species in the percentage of zygotes that hatched; however, more than 90 percent of the WR and WB F_1 hybrids were morphologically abnormal (Fig. 1 and 2).

Both WR and WB F_1 hybrids exhibited high mortality between the hatching and swim-up fry stages. At the time the experiments were terminated, only 2 percent of the WR hybrids and 1 percent of the WB hybrids appeared to be morphologically normal. All of these morphologically normal-appearing WR and WB F_1 hybrid fry were very sluggish. When petri dishes containing these hybrid fry were tapped with a pencil, the fry responded with weak swimming movements or not at all, and it is very doubtful that

Table 9.—The degree of viability of 16 different kinds of fishes produced by pairing gametes from red-ear sunfish, bluegills, green sunfish, and warmouths. Data from experiments S1-S11 are combined.

Parent Species x	Number of Eggs	Percentt Hatched	Percentt Normal Fry
R x R	512	39	27
B x R	512	41	33
G x R	552	46	37
W x R	548	321	2**
B x B	681	87	75
R x B	742	86	83
G x B	639	87	69
W x B	699	611	
G x G	639	78	75
R x G	597	80	79
B x G	589	73	70
W x G	678	76	55
W x W	295	58	49
R x W	317	62	58
B x W	311	58	44
G x W	276	62	47

*R = red-ear sunfish, B = bluegill, G = green sunfish, W = warmouth.

tPercentage based on number of eggs at the time sperm and eggs were mixed together and the number that hatched.

$Percentage based on number of eggs at the time sperm and eggs were mixed together and the number of morphologically normal-appearing fry.

§More than 90 percent of these larvae were morphologically deformed.

**These fry appeared morphologically normal, but all were behaviorally abnormal.

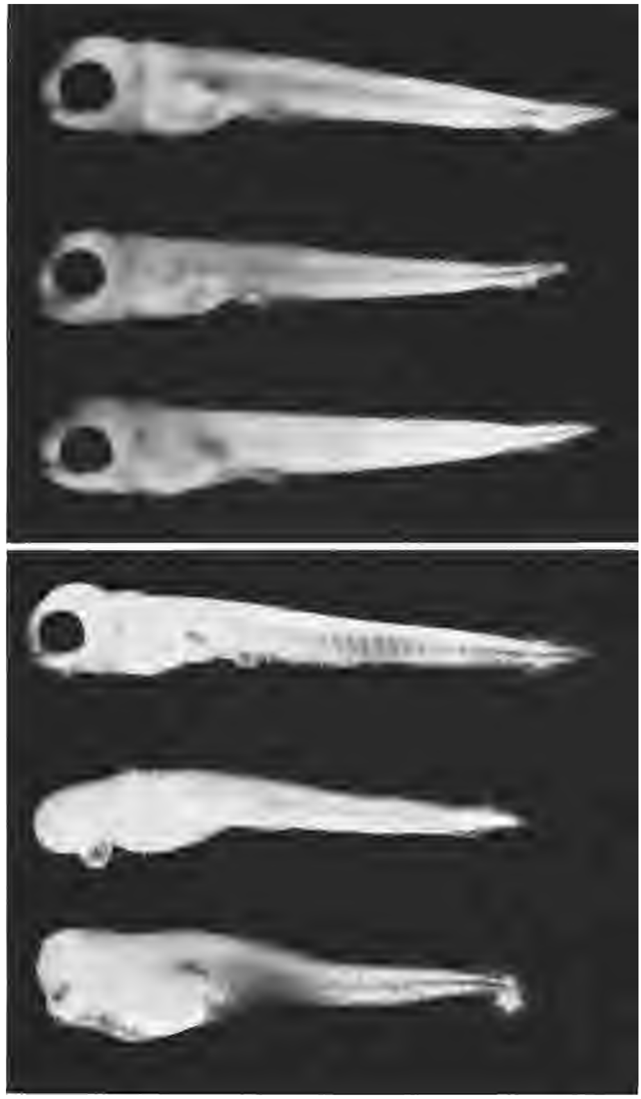

Fig. 1 —Red-ear sunfish fry (above) and WR F_1 hybrid fry (below) selected to show the range of morphological deformities of the hybrid fry. All fry were produced from eggs from one female red-ear sunfish. Both kinds of zygotes were allowed to develop simultaneously under similar conditions for 120 hours before being sacrificed. The mean hourly temperature was 28.7° C. (83.6° F.), standard deviation, 0.44° C. (0.79° F.).

any of these fry would have become free swimming. Fifty-five percent of the WG hybrid and 75 percent of the pure green sunfish zygotes developed into normal-appearing swim-up fry (difference significant to 0.05 level). The WG hybrid swim-up fry appeared to be behaviorally normal. The remaining nine kinds of hybrids were not significantly different from their maternal parent species in the percentages that developed into normal swim-up fry.

The mean hatching time and standard deviation were calculated for each of the 132 samples of eggs in the 11 experiments (Tables A14—A24 of the appendix). A statistical weight was calculated for the mean hatching time of each sample by dividing the number of eggs that hatched by the variance of the mean hatching time. A 7094 digital computer was used in the analysis of variance of these data.

The statistical analysis, in which the weighted mean hatching time of each kind of hybrid was compared with that

Fig. 2. — Bluegill fry (above) and WB F_1 hybrid fry (below) selected to show the range of morphological deformities of the hybrid fry. All fry were produced from eggs from one female bluegill. Both kinds of zygotes were allowed to develop simultaneously under similar conditions for 113 hours before being sacrificed. The mean hourly temperature was 26.9° C. (80.4° F.), standard deviation, 0.48° C. (0.86° F.).

of its maternal parent species, revealed that WB F_1 hybrid zygotes hatched significantly sooner than pure bluegill zygotes when both kinds of zygotes were incubated at the same temperatures. Although WB zygotes hatched in less time, the newly emerged WB larvae were not as advanced in their development as the unhatched pure bluegill embryos. WR F_1 hybrids were not significantly different from pure red-ears in hatching time; however, the newly emerged WR larvae were not as advanced in their development as the pure red-ear larvae. There were no statistically significant differences in the time of hatching between the other 10 kinds of hybrids and their respective maternal parent species, and differences in the degree of development between the hybrids and their respective maternal parent species were not pronounced.

The mean body length and standard deviation were calculated for each kind of viable fry from each experiment (Tables 5-8). An analysis of variance in which the mean body length of each kind of viable hybrid fry was compared to that of its maternal parent species revealed that BR and GR were significantly (0.05 level of probability) longer bodied than the pure red-ear fry. The other eight kinds of hybrids were not significantly different from their respective maternal parent species fry in total body length.

The alpha temperature threshold of development (Shelford 1927:357) and the mean number of developmental units (degree-hours of effective temperature) necessary for 50-percent hatching were calculated for each of the 16 kinds of zygotes. The statistical method was designed by Dr. H. W. Norton[1] to determine the linear correlation of two variables (T and R) when both variables are subject to error.

Given: Equation I

$$(T - A)t = U$$

where

- T = mean hourly incubation temperature
- A = alpha threshold of development
- t = mean hours of incubation necessary for 50-percent hatching
- U = number of developmental units necessary for 50-percent hatching

Then: Equation II

$$A = \frac{(W + V/U^2)\,(T - UR)}{(W\ V/U^2)}$$

[1]Professor of Statistical Design and Analysis, Animal Science Department, University of Illinois, Urbana.

where

$$W = 4$$

(estimated on the basis of the accuracy with which the thermograph could be adjusted and read)

$$V = \frac{3n_t t^4}{n v_b + 3v_w}$$

(three times the number of zygotes which hatched, times the fourth power of the mean hours of incubation, divided by the number which hatched, times the pooled between variance of the hatching times, plus three times the within variance of the hatching times)

$$R = 1/t$$

(reciprocal of the mean hours of incubation)

Then: Equation III

$$2[(W+V/U^2)\ R\ (A+UR-T) - V/U^3(A+UR-T)^2] = 0$$

T, V, R, and W values were calculated for each kind of larvae for each of the 11 experiments. A 7094 digital computer was programmed to select all T, V, R, and W values for one kind of larva and to determine by a trial-and-error method the U value that best satisfied equation III.

An estimate of the goodness of fit of T and R values to the linear regression line U was determined by this equation:

$$S = (W+V/U^2)\ (A+UR-T)^2$$

Only two experiments were conducted using warmouth eggs, and the mean incubation temperatures of these two experiments differed by only 0.3° C. Consequently, the alpha thresholds and the numbers of developmental units necessary for 50-percent hatching of warmouth and the three kinds of hybrid zygotes produced from warmouth eggs are not reliable. The alpha thresholds, numbers of developmental units necessary for 50-percent hatching, and S values for the other 12 kinds of zygotes are presented in Table 10.

The t test comparisons revealed that

Table 10.—Alpha temperature thresholds of development and numbers of developmental units (degree-hours of effective temperature) necessary for 50-percent hatching of red-ear sunfish, bluegill, green sunfish, and nine different kinds of hybrid sunfish zygotes. (See Tables 5-8 for mean incubation temperatures.)

Parent Species[a] ♂ x ♀	Number of Eggs that Hatched	Alpha Threshold Degrees Centigrade	Alpha Threshold Degrees Fahrenheit	U Values (Degree-hours of Effective Temperature) Centigrade Scale	U Values (Degree-hours of Effective Temperature) Fahrenheit Scale	S Values[b]
R x R	202	18.3	64.9	280	504	7.76
B x R	211	18.3	64.9	290	522	0.16
G x R	255	17.8	64.0	302	543	1.05
W x R	175	18.1	64.5	284	511	6.32
B x B	592	18.3	64.9	287	516	1.52
R x B	638	17.9	64.2	299	538	20.73
G x B	559	18.5	65.3	279	503	6.58
W x B	429	18.1	64.6	249	448	7.33
G x G	498	18.5	65.3	266	478	0.54
R x G	481	18.3	64.9	267	481	24.21
B x G	432	18.6	65.5	258	464	7.80
W x G	513	18.6	65.4	258	464	5.08

[a] R = red-ear sunfish, B = bluegill, G = green sunfish, W = warmouth.
[b] S values can be considered equivalent to Chi square values, and S values larger than 3.83 indicate that the deviations of T and R values from their linear regression line U are significantly greater (0.05 probability level) than deviations due to chance.

the alpha thresholds of development of the 12 kinds of fishes were not significantly different from one another. The alpha thresholds ranged from 17.8° to 18.6°C. (64.0°-65.5° F.) and the mean alpha threshold for all 12 kinds of fishes was 18.3° C. (64.9° F.). Approximately 280 developmental units centigrade scale or 500 units Fahrenheit scale were necessary for 50-percent hatching.

The S values for bluegills, green sunfish, BR F_1 hybrids, and GR F_1 hybrids indicate that the magnitude of the deviations of the weighted T and R values from their respective linear regression lines, U, are within the range that can be attributed to chance. S values for the other eight kinds of fishes indicate that the deviations of their T and R values from U are greater than can be attributed to chance.

Why these greater-than-expected deviations occurred is not known; however, if the relation of incubation temperature to the reciprocal of hatching time is linear for median effective temperatures (Allee et al. 1949:109) and if the values of the statistical weights V and W were correctly estimated, differences in the sizes of eggs from different females or environmental differences such as carbon dioxide and oxygen tensions could be responsible for such deviations.

Differences in the sizes of eggs undoubtedly result in differences in time of hatching. Newly hatched larvae from large eggs are larger than those from small eggs, and Larimore (1957: 45-46) noted that warmouth larvae emerging from eggs early during the hatching period were smaller (2.30-2.60 mm in total length) than those emerging later (2.65-2.85 mm in total length). Consequently, if eggs were incubated under similar conditions, small eggs would hatch in less time than large eggs.

Alderdice, Wickett, & Brett (1958: 229) found that Pacific salmon eggs exposed to low dissolved oxygen levels just prior to hatching hatched prematurely. Low dissolved oxygen levels may affect sunfish eggs in the same manner.

In the 11 experiments reported here, a high positive correlation between high mortality prior to hatching and premature hatching should be apparent if environmental dissolved oxygen levels became critical enough to affect the time of hatching. An inspection of the data revealed no such correlation; consequently, in these experiments differences in the sizes of eggs from different females appear to be a more likely source of experimental error than low levels of environmental dissolved oxygen.

Isolation Experiments

Thirty-two isolation experiments were conducted from 1957 through 1963. Sixteen of these experiments, all of which involved intrageneric *Lepomis* matings, were reported by Childers & Bennett (1961:6).

METHODS AND MATERIALS.—Males of one species and females of a different species were isolated in small earthen ponds (0.02-0.90 acres). Each of the 12 possible hybrid-producing combinations was tested in one or more ponds. Shortly before the ponds were stocked with the parent species, each pond was drained or treated with rotenone to eliminate any fish which might be present. Parent species were stocked during late May or early June and the ponds were checked at approximately monthly intervals throughout the summer.

The following August or September each pond was drained, seined, or treated with rotenone, and small fish (if present) were identified. Three of the 32 ponds had become contaminated with other fish, and two ponds dried up during the late summer. No hybrids were found in these five ponds. Results of the other 27 experiments are reported in Table 11.

RESULTS AND DISCUSSION.—R x G, G x B, and W x G pairings successfully hybridized each time they were tested. R x G and G x B were each tested in two ponds and W x G was tested in one.

The B x G cross, not included in Table 11, was attempted in two ponds. Both ponds became contaminated with male green sunfish, and large numbers of green sunfish young were produced in both ponds. Consequently, both experiments gave no test of hybridization between bluegill males and green sunfish females.

The R x B cross was attempted in four ponds. No hybrids were produced in three ponds although the ponds remained full and were uncontaminated by other fishes. Eleven small fish were found when the fourth pond was drained, and these fish were believed to have been RB F_1 hybrids although they were not positively identified as such (Childers & Bennett 1961:6). The water in this pond contained a high and constant clay turbidity that reduced the transparency of the water and caused the parent fish to be extremely pale in body color. The normally scarlet portions of the opercle tabs of the red-ear males appeared as a faint rose color. The R x B cross has been attempted three times in Indiana without obtaining offspring (Krumholz 1950:113).

The B x R cross was set up in four ponds. Three of these (not included in Table 11) were improper because of contamination by bluegill females in one and complete loss of water in the other two during the late summer. The fourth test appeared to have been valid but no hybrids were produced.

Smitherman & Hester (1962: 335, 337) attempted R x B and B x R crosses by stocking single pairs in plastic pools 9 feet in diameter and 2.5 feet deep. Each cross was attempted in two pools, and hybridization failed to occur in all four trials. In contrast to these results, the B x R cross has been productive of hybrids six times in Indiana (Krumholz 1950:113).

Adults for the R x W cross were stocked in two ponds. One pond became contaminated with male and female green sunfish, and when the

Table 11.—Results of isolation experiments. Adult males of one species and females of another were isolated in each pond in May or June, and ponds were censused in August or September, 1957-1963.

P_1 Cross*	Pond Surface Area in Acres	Number of Adults Stocked		Results
♂ x ♀		♂	♀	
R x B	0.02	4	6	11 young found†
R x B	0.10‡	15	10	No hybrids
R x B		13	15	No hybrids
R x B	0.84	16	11	No hybrids
B x R	0.25‡	8	22	No hybrids
R x G	0.02	4	7	Hybrids abundant
R x G	0.04	8	3	Hybrids abundant
G x R	0.10	2	8	No hybrids
G x R	0.02	4	6	No hybrids
G x R	0.10‡	8	11	No hybrids
G x B	0.02	4	6	Hybrids abundant
G x B	0.90	30	35	Hybrids abundant
W x R	0.20	12	4	No hybrids
W x R	0.20	6	19	No hybrids
R x W	0.75‡	13	6	No hybrids
R x W	0.25‡	12	28	Contaminated§
W x B	0.02	4	5	No hybrids
W x B	0.02	4	5	No hybrids
W x B	0.02	4	5	No hybrids
W x B	0.02	4	5	No hybrids
B x W	0.02	6	5	No hybrids
B x W	0.02	5	3	No hybrids
B x W	0.02	5	3	No hybrids
B x W	0.02	3	2	No hybrids
W x G	0.02	4	6	Hybrids abundant
G x W	0.10‡	8	8	No hybrids
G x W	0.02	4	6	No hybrids

*R = red-ear sunfish, B = bluegill, G = green sunfish, W = warmouth.
†The 11 young sunfish were believed to be hybrids but were not positively identified as such.
‡Size of pond estimated.
§This pond was contaminated with green sunfish. No RW hybrids were found, but young RG hybrids were found.

pond was seined during September, it contained large numbers of young-of-the-year green sunfish and RG or GR F_1 hybrids. No RW hybrids were found in either pond.

Although the R x W cross was unsuccessful in these experiments, I found a large natural population of RW hybrids in a 2-acre farm pond near Fairmount, Ill. This pond was stocked in 1952 with warmouths, red-ear sunfish, and smallmouth bass. No hybrids were found during 1953 or 1954. During March, 1955, all three species in this pond suffered extensive mortality because of critical dissolved oxygen conditions. A large population of RW hybrids was produced following this spring mortality. Since stripping experiments indicated the W x R cross resulted in nonviable hybrids, the natural hybrid population was apparently produced by male red-ears and female warmouths.

The G x R (three tests), W x R (two tests), W x B (four tests), B x W (four tests), and G x W (two tests) all failed to produce hybrid populations although the parent species had good opportunities to hybridize in each test.

In each of the 32 isolation experiments males constructed nests and remained in spawning condition for extended periods throughout the summer. The failure of certain P_1 crosses to hybridize was believed to result from incompatibilities in the reproductive behavior patterns of the various species and not because males and females failed to be in spawning condition at the same time.

Fish hybridization might result from sperm driftage or interspecific matings. Sperm driftage is an important cause of hybridization among certain species of fishes, particularly minnows and darters, which live in flowing water habitats and simultaneously spawn in close proximity to one another (Hubbs 1955: 10, 16). Sperm driftage may also account for some hybridization between pond- or lake-dwelling centrarchids; however, since average functional life spans of sunfish spermatozoans are so brief (Tables 2-4) and since there is such good synchronization in the release of sperm and eggs by a spawning pair, most hybrid sunfish are probably the result of interspecific pair formation.

The four experimental species are sexually dimorphic, closely allied, sympatric species. Signals that are in some way involved in reproductive isolation of such species are likely to be highly divergent (Marler 1957:35) and may involve specific differences in shape, color, special movements, sounds, scents, etc. (Tinbergen 1951:56). The precise signals which are operative in conspecific pair formation of the four experimental species are not known; however, specific differences in color of opercle tabs, eyes, cheeks, and pelvic fins of nest-guarding males (Table 1) may be important in controlling the behavior of ripe females. When a female ready to spawn approaches a nest-guarding male, she usually stops some distance from the nest and the male exhibits a courtship display (Miller 1963:118). Species recognition apparently occurs during this short time, and the female flees or remains in the vicinity of the nest and accepts the advances of the male.

Since in one isolation experiment there was an indication that the scarlet portions of the opercular tabs of male red-ear sunfish might possibly prevent hybridization between male red-ears and female bluegills, an experiment was conducted during 1964 to test this hypothesis. Two small earthen ponds (25 feet wide, 45 feet long, with a maximum depth of 4 feet) were each stocked during July with three ripe adult male red-ear sunfish and three adult female bluegills. The opercular tabs were clipped from all males stocked in one pond, and the tabs were left intact on the males stocked in the other pond.

The ponds were drained during early October, and several thousand small (0.5-1.0 inch in total length) hybrid fry were collected from the pond containing red-ear males whose opercular tabs had been removed. No small fish were found in the control pond. An examination of the clipped males revealed that the blue portion of the opercular tabs had regenerated to almost normal size but the scarlet portions had not regenerated. One tab on each of these males had a small, narrow, yellowish-orange margin.

One such test cannot, of course, be considered conclusive proof that specific differences in the color of the opercular tabs of male red-ears are highly functional in preventing their hybridization with female bluegills; however, additional investigation of the importance of color as a reproductive isolating mechanism in the sunfishes might prove rewarding.

According to Hubbs (1957:17), fish hybridization is controlled to a large extent by environmental factors. Sunfish hybrids appear to be more common in ponds which are choked with aquatic vegetation or have high turbidities than in clear-water ponds which have extensive spawning areas free from vegetation. In weed-choked ponds or ponds with high turbidities the range of visibility must be short, and under these conditions ripe females might occasionally spawn with males without observing preliminary courtship displays believed to be important in conspecific pair formation.

HYBRIDS REARED IN PONDS

Large numbers of each of the 10 viable F_1 hybrid types were stocked in one or more ponds. Most of these hybrids were produced in the laboratory by combining sex products stripped from ripe adults of the four parent species. However, a few were produced by isolating males of one species and females of another species in ponds which contained no other fish. The laboratory hybrids were stocked on the day they became free-swimming; the pond-produced hybrids were seined and stocked in other ponds when they had attained a length of about 1 inch.

The F_1 hybrids were reared to maturity in their respective ponds and the sex ratio, fecundity, and degree of heterosis of each F_1 hybrid population were studied.

Sex Ratios

After the F_1 hybrids had grown to sexual maturity, fish were collected from each population and sexed, mostly by dissection. However, some were sexed by stripping eggs or milt from ripe individuals, and fewer still were sexed by reference to color, size of abdomen, and comparative size and shape of anus and urogenital openings.

The sex of a mature sunfish is not difficult to determine by observation during the spawning period. Male sunfish are usually more vividly colored than females. The abdominal region of females becomes greatly distended with eggs shortly before spawning occurs. The diameter of the urogenital opening in male sunfish is usually less than one-half the diameter of the anus, but in females it is equal to or larger than the diameter of the anus. In male sunfish the urogenital opening forms a cup-shaped depression and in females it forms a small papilla.

If there was any doubt as to the sex of a particular individual, that individual was dissected. The accuracy of determining sex by observation was checked several times by dissecting all fish in a particular sample in which the fish had been previously sexed by observation. No errors were revealed.

The sex ratios, expressed as the percentage of males, for each of the 10 different kinds of viable F_1 hybrids produced from the four experimental species are reported in Table 12. Sex ratios determined for population of the six intrageneric *Lepomis* hybrids reported by Childers & Bennett (1961:7) are included in Table 12.

Table 12.—Sex ratios of F_1 hybrid sunfishes expressed as the percentage of males in individual populations.

F_1 Hybrid Populations*	Where Produced†	Number Sexed	Percent Male
RB	L	178	100
RB	L	197	100‡
RB	L	95	87
		470	97
BR	L	110	97
RG	L	457	69
GR	L	252	48
BG	L	142	97
GB	P	44	70
GB	P	22	64
		66	68
RW	L	174	55
BW	L	101	66
BW	L	302	70
		403	69
GW	L	147	16
WG	P	104	84

*R=red-ear sunfish, B=bluegill, G=green sunfish, W=Warmouth. In names of hybrids the male parent species is given first.

†L=laboratory-produced hybrids and P=pond-produced hybrids.

‡One F_2 hybrid was collected from this pond; so at least one female was in this population.

Of the 10 kinds of viable F_1 hybrids, seven were predominately males (**RB, BR,** and BG were 97 percent males; WG were 84 percent males; and RG, GB, and BW were approximately 70 percent males), two were approximately 50 percent males (GR and RW), and one was predominately female (GW was 16 percent males). Ricker (1948:93-94) determined the sex of 428 BR F_1 hybrids in Indiana and found them to be 97.7 percent males.

Sex determination in sunfishes is very poorly understood. Bluegills, green sunfish, and their F_1 hybrids apparently have 24 pairs of chromosomes, and the sex chromosomes are indistinguishable from the autosomes (Bright 1937:36). Bright (1937:26) also reported that the chromosomes are so similar in shape and size that he was unable to detect specific differences. Roberts (1964:402) found that red-ear, bluegill, and warmouth sunfishes each have 24 pairs of chromosomes; green sunfish from Wake County, N.C., had 24 pairs; but green sunfish from Leetown, W.Va., had only 23 pairs.

The unbalanced phenotypic tertiary[*] sex ratios of the F_1 hybrid sunfish could result from unbalance primary genetic sex ratios, specific differences in the strength of sex-determining factors, an overriding of the genetic sex by environmental factors, or differential mortality of the sexes.

Since the WG F_1 hybrids were 84 percent males and the reciprocal cross hybrids were 16 percent males, it is possible that the strength of sex-determining factors of warmouths are 5.25 times more powerful than those of green sunfish. Specific differences in the strength of sex-determining factors cannot alone explain the sex ratios of the remaining eight kinds of viable hybrids, since none of these were predominantly females.

RB and BG F_1 hybrids were both 97 percent males. If differential mortality were the cause of these unbalanced sex ratios, much of the mortality would have had to occur after the swim-up fry stage, since in the stripping experiments total mortality between fertilization and the swim-up fry stages was only 14 percent for the RB and 27 percent for the BG F_1 hybrids.

It is not known which sex is the heterozygous condition for the sex chromosomes of the four experimental species; however, Haldane (1922:108) formulated a rule which furnishes a clue.

> When in the F_1 offspring of a cross between two animal species or races, one sex is absent, rare, or sterile, that sex is always the heterozygous sex.

Using Haldane's rule, Krumholz (1950:114), in a study concerning BR F_1 hybrids, pointed out that the males of both bluegills and red-ear sunfish are probably homozygous for sex and the females heterozygous. The application of Haldane's rule to all possible F_1 hybrids produced from red-ear sunfish, bluegills, and green sunfish indicates that the female is the heterozygous sex in these three species. Hybridization of male warmouths with females of the three *Lepomis* species resulted in partial or complete lethals, suggesting that in the warmouth the male is the heterogametic sex.

Fecundities

The reproductive success of each of the 10 kinds of viable F_1 hybrids was investigated in one or more ponds. The occurrence and abundance of F_2 hybrids were determined by seining, trapping, shocking, poisoning, or draining the ponds after the F_1 hybrids were 1 or more years of age. Eighteen separate populations were studied. The results of these studies are presented in Table 13.

Of the 10 kinds of viable F_1 hybrids,

[*] In this paper the terms primary, secondary, and tertiary sex ratios refer to sex ratios at the time of fertilization, time of hatching, and time of sexual maturity, respectively. Genetic sex refers to the type of sex chromosomes an individual receives from its parents, and phenotypic sex refers to whether its gonads are testes or ovaries.

Table 13.—The occurrence and estimated abundance of F_2 hybrids in ponds stocked with F_1 hybrids.

Hybrid*	Pond	Abundance of F_2 Hybrids	Other Species Present
RB	1	None found	None
RB	2	None found	None
RB	3	One individual found	Hybrid crappies
BR	4	None found	None
RG	5	Abundant	None
RG	6	Abundant	None
RG	7	Scarce	Largemouth bass
GR	8	Scarce to abundant†	None
BG	9	None found	None
GB	10	Abundant	None
GB	11	Scarce	Largemouth bass
RW	18	Abundant	None
BW	15	None found	Largemouth bass
BW	16	Abundant	Hybrid crappies
BW	17	Scarce‡	Largemouth bass, bluegills, lake chubsuckers, warmouths, channel catfish
GW	12	Abundant	None
GW	13	Fairly abundant	Largemouth bass and bluegills
WG	14	Abundant	None

*R = red-ear sunfish, B = bluegill, G = green sunfish, W = warmouth. In names of hybrids the male parent species is given first.

†When the F_1 hybrid population was dense, only a few F_2 hybrids were found. The number of F_1 hybrids was drastically reduced and the few remaining F_1 hybrids produced a large number of F_2 hybrids the following year.

‡These fish were believed to be F_2 hybrids; however, they could have been produced by the F_1 hybrids backcrossing to either of their parent species.

RB, BR, and BG failed to produce abundant F2 generations when in ponds which contained no other species of fishes. In contrast to these results, BR F_1 hybrids produced abundant F2 generations in two ponds in Indiana (Ricker 1948:94). Six of the other seven kinds of F_1 hybrids produced abundant F2 hybrids when in ponds which contained no other species of fishes, and the seventh, stocked in a pond with F_1 hybrid crappies, also produced a large F2 generation.

Three of the seven kinds of F_1 hybrids which produced large F2 populations when stocked in ponds containing either no other fishes or hybrid crappies were also stocked in ponds with largemouth bass. RG F1 hybrids and GB F_1 hybrids, when stocked with largemouth bass, produced only a few F_2 hybrids. No F2 hybrids were found in the pond stocked with BW F_1 hybrids and largemouth bass. Only a few BW F2 hybrids were found when an 18-acre lake containing BW F_1 hybrids; largemouth bass; warmouths; bluegills; channel catfish, *Ictalurus punctatus* (Rafinesque); and lake chubsuckers, *Erimyzon sucetta* (Lacépède), was drained.

The results of these experiments, although not conclusive because of the small number of trials, do indicate that RG, GB, and BW F_1 hybrids which are capable of producing large F2 populations in ponds containing either no other species or hybrid crappies are unable to do so in ponds containing largemouth bass. It is not known whether scarcities of F2 hybrids in ponds containing largemouth bass are the result of low fecundity of F_1 hybrids or a high vulnerability of F2 hybrids to bass predation. Both possible causes may be important. Hale (1956: 105) found that green sunfish with forebrain lesions exhibited a marked reduction in their ability to learn to negotiate a maze. It would be interesting to know if the forebrains of the F_2 hybrids are structurally or functionally different from those of F_1 hybrids or their parent species.

WG F_2 hybrids, stocked in a pond which contained no other fishes, produced a large F_3 generation. GW F_2 hybrids, which were stocked in a pond containing no other fishes, also produced a large F_3 population.

Backcrosses, outcrosses, a four-species cross, and a three-species cross involving F_1 hybrids are listed in Table 14. The BW x B backcross was made by stocking adult male BW F_1 hybrids and adult female bluegills in a pond which contained no other fishes. The other 12 crosses listed in Table 14 were made by stripping gametes from ripe adults and rearing the young to the free-swimming fry stage in the laboratory.

R x RW, W x RW, B x RW, G x RW, R x GB, and RB x W young were killed after they developed into free-swimming fry because of the lack of ponds in which they could be stocked. All six kinds of fry appeared to be normal and probably would have developed into adults. Free-swimming fry of the remaining six crosses in the laboratory were stocked in ponds and did develop into adult fishes. BW x B, G x GW, and B x RG populations produced large numbers of young.

Hubbs & Hubbs (1933:631-636) reported that in Michigan F_1 hybrids of bluegills, green sunfish, longear sunfish, pumpkinseeds, and orangespotted sunfish were unable to reproduce because males were sterile and ova stripped from the few adult females used in the experiments appeared distinctly abnormal. This study, often cited in the literature, has resulted in a rather widespread belief that all male hybrid sunfish are sterile. Results of my experiments conclusively establish that a number of different kinds of hybrid sunfishes produced in Illinois are not sterile, are fully capable of producing abundant F_2 and F_3 generations, and can be successfully backcrossed to parent species and even outcrossed to nonparental species.

Table 14.—Successful backcrosses, outcrosses, four-way cross, and another cross involving F_1 hybrid sunfishes.[a]

Backcrosses ♂ x ♀	Outcrosses ♂ x ♀	Four-Species Cross ♂ x ♀	Three-Species Cross ♂ x ♀
R x RW	R x GB	RB x GW	BW x GW
G x GW	R x BW		
W x RW	R x GW		
BW x B	B x RG		
	B x RW		
	G x RW		
	RB x W		

[a] R = red-ear sunfish, B = bluegill, G = green sunfish, W = warmouth.

Hybrid Vigor

Heterosis has been defined (Manwell, Baker, & Childers 1963:103) as

> that condition where, with respect to one or more particular characteristics, the values for most, if not all, of the individual hybrids fall significantly outside the range formed from the means for both parent populations. In cases of positive heterosis—hybrid vigor—the hybrid shows a faster growth rate than either of the parents, or it possesses some other characteristic, often an economically significant one, at a "better" level than the parents do.

RATE OF GROWTH.—The growth of the different kinds of hybrids stocked in ponds was recorded for each of the populations mentioned in Tables 12 and 13. Since various numbers (200–10,000 per surface acre) of hybrids were stocked and since the ponds differed in size, depth, shape of basin, and fertility, no valid comparisons can be made between the growth rates of the hybrids or between those of the hybrids and their parent species. In general the rates of growth of these hybrids were inversely proportional to their population densities. The most rapid growth during the 1st year of life occurred in a 1-acre pond stocked on May 24, 1957, with approximately 1,000 free.swimming, laboratory-produced, 1-day-old RW F_1 hybrid fry. Approximately 4½ months later, October 8, 66 hybrids from this population averaged 145 mm (5.7 inches) in total length and 69 grams (0.15 pound).

The slowest growth occurred in a 0.1-acre pond stocked on May 27, 1957, with approximately 1,000 (10,000 per surface acre) 1-day-old, free-swimming, laboratory-produced GR F_1 hybrid fry. On August 30, 106 hybrids from this population averaged only 94 mm (3.7 inches) total length. The fish in this sample were not weighed; however, based on the weights of fish of similar lengths from later samples, the calculated average weight of fish from the August 30th collection was about 15 grams (0.03 pound).

The largest hybrid captured to date (May, 1965) was a 6-year-old GB F_1 hybrid whose total length was 310 mm and weight was 965 grams (Frontispiece).

Hubbs & Hubbs (1931:291, 296-297) during 1929 and 1930 studied the growth of pumpkinseed, green sunfish, and the naturally produced hybrid of these two species in Wiard's pond near Ypsilanti, Mich. Average lengths of both 1- and 2-year-old hybrids in this pond were greater than those of either parent species. The effect on growth of the relative abundance of the three kinds of fishes in this pond (pumpkinseeds, green sunfish, and their hybrids) was not considered. Based on the total number of individuals reported, pumpkinseeds were approximately 10 times more abundant than green sunfish and green sunfish twice as abundant as hybrids. Intraspecific competition is usually keener than interspecific competition because individuals of the same species are more nearly equal in their structural, functional, and behavioral adaptations (Kendeigh 1961: 183). Consequently, the greater growth of the hybrid sunfish in Wiard's pond may have been the result of less intraspecific competition among the hybrids than among the parent species and not because the hybrids exhibited hybrid vigor.

In an attempt to determine whether certain F_1 hybrid sunfishes actually grow faster than their parent species, two experiments were conducted in which equal numbers of uniformly sized F_1 hybrids and parent species were stocked in ponds which contained no other fishes (Childers & Bennett 1961:11-13). In the first experiment, 171 BG F_1 hybrids and 171 green sunfish averaging about 25 mm (1.0 inch) and 19 mm (0.75 inch), respectively, were stocked on July 10, 1958, in an 0.8-acre gravel pit pond. Ten months later (during 4 months of which the waters were warm enough for fish growth) fish were removed from the pond by trapping and rotenone poisoning.

In the second experiment 200 GR F_1 hybrids, 200 green sunfish, and 200 redear sunfish averaging 117 mm (4.6 inches), 107 mm (4.2 inches), and 89 mm (3.5 inches), respectively, were stocked in a 1.1-acre farm pond during early August, 1958. Fish were removed from this pond April 20 through May 25, 1959, by hook-and-line fishing, trapping, and rotenone poisoning.

In both experiments the average increase in total length of the hybrids was not significantly different from the increases of the parental species. The population densities of the fishes in both ponds were much lower than would be found in most normal natural populations. In both experiments intraspecific and interspecific competition was undoubtedly quite light; consequently, the question of whether certain F_1 hybrid sunfishes are superior to their parent species in rate of growth cannot be answered until high density populations containing equal numbers of equal-sized hybrids and parent species are studied.

ELECTROPHORETIC PATTERNS OF HEMOGLOBINS.—Manwell, Baker, & Childers (1963:118-119) determined that redear sunfish, bluegills, green sunfish, and warmouths each have a hemoglobin pattern in vertical starch gel electrophoresis that is unique. Almost every one of the F_1 hybrids of these species yields a hemoglobin pattern that is identical with that obtained by simply mixing hemoglobins of the two parental species; however, from 25 to 40 percent of the hemoglobin from BW, GW, and WG F_1 hybrids has electrophoretic properties different from the hemoglobins of the parental species. Oxygen equilibria for the hemoglobins from these three hybrids show greater heme-heme interactions than those for hemoglobin from any of their parental species. As a result of this greater heme-heme interaction, hemoglobins from these three hybrids have better blood gas transport properties than those of their parental species, and in this respect each of these three hybrids is believed to exhibit hybrid vigor.

VULNERABILITY TO HOOK-AND-LINE CAPTURE.—Although no controlled experiment has tested whether F_1 hybrid sunfishes are more vulnerable to angling than their parental species, certain F_1 hybrids are so easily caught that at several locations sport fishermen have almost completely eliminated substantial hybrid sunfish populations in a few days of angling.

For example, on May 30, 1958, the Illinois Department of Conservation opened the lake at Lincoln Trail State Park near Marshall to public fishing. From May 30 through September 29, fishermen caught and removed approximately 10,800 naturally produced BG or GB F_1 hybrids of which 50 percent (5,400) were caught during the 1st day of fishing, and 82 percent (8,600) were removed during the first 3 days. This hybrid sunfish population was almost completely eliminated during the 1st week of fishing.

Ridge Lake, an 18-acre lake in Fox Ridge State Park near Charleston, Ill., was drained during the fall of 1959 and fish were moved to other waters. In the spring of 1960, the lake was restocked with 4,503 BW F_1 hybrids, 299 largemouth bass, 41 channel catfish, and 585 lake chubsuckers. An additional 448 RW F_1 hybrids were stocked in May, 1961.

A limited number of fishermen was permitted to fish during June, July, and August of 1960, 1961, and 1962. During 1960, fishermen totaled 1,583 man-hours of fishing and caught 3,772 of these hybrids, of which 64 were removed and 3,708 returned to the lake. In 1961 fishermen totaled 2,830 man-hours of fishing and caught 4,890 hybrids, of which 194 BW and 6 RW hybrids were removed. The remaining 4,690 hybrids were returned to the lake. The next season (1962) fishermen were permitted to remove all the hybrids they caught, and during 2,817 man-hours of fishing they removed 1,075 BW and 134 RW F_1 hybrids. Of these 1,209 hybrids, 65 percent were caught during the first 5 days of fishing, 81 percent during the first 10 days, and 88 percent during the first 15 days. The lake was drained during March, 1963, and was found to contain 8 BW and 64 RW F_1 hybrids.

HYBRID SUNFISHES FOR SPORT FISHING

Overpopulation of sunfish is the single greatest problem encountered in the management of Illinois lakes and ponds containing largemouth bass and one or more of the *Lepomis* species. The *Lepomis* species have such high reproductive capacities and survival capabilities that they commonly become so abundant that they are unable to grow to sizes large enough to be of value to fishermen. Because certain kinds of F_1 hybrid sunfishes appear to be unable to produce sizable F2 populations in ponds containing largemouth bass, a number of experiments are now in progress to test the usefulness of hybrids in combination with largemouths. Preliminary results indicate that several types of hybrids in combination with bass furnish fishing superior to that furnished by bass in combination with the hybrids' parent species.

SUMMARY

1.—Red-ear sunfish, Lepomis *microlophus* (Gunther); bluegill, L. macrochirus Rafinesque; green sunfish, L. *cyanellus* Rafinesque; and warmouth, Chaenobryttus gulosus (Cuvier) are present in a number of east-central Illinois lakes and ponds and are known to hybridize occasionally. During 1957 through 1964, the spawning seasons of these species were observed to extend from mid-May to August or September.

2.—Red-ear sunfish, bluegills, and green sunfish usually nested in colonies, and mixed colonies containing two and, less frequently, all three of these species were not uncommon. Warmouths tended to be more solitary in their nest site selections.

3.—Results of laboratory experiments indicate that average functional life spans of bluegill, green sunfish, and warmouth gametes are approximately 1 hour for ova and 1 minute for spermatozoa. The brief functional life spans of spermatozoa are undoubtedly important in reducing hybridization caused by sperm driftage.

4.—Gametes stripped from the four species were paired in 16 different combinations to produce zygotes representing 12 kinds of F_1 hybrids and the four parental species. W ♂ x B ♀ and W ♂ x R crosses were 100 percent lethal and the W ♂ x G ♀ cross was partially lethal. Based on the percentages of zygotes that hatched and developed into normal-appearing fry, the viability of each of the remaining nine kinds of hybrids was not significantly different from that of its maternal parent. B ♂ R 9 and G ♂ R hybrid fry were significantly longer bodied than pure red-ear fry of comparable ages. The other eight kinds of hybrid fry were not significantly different in length from their respective maternal parent fry of comparable ages.

5.—Alpha temperature thresholds of development for red-ear sunfish, bluegills, green sunfish, and nine kinds of hybrids were not significantly different from one another. The mean alpha threshold for all 12 kinds of fishes was 18.3° C. (64.9° F.). Approximately 280 developmental units (degree-hours of effective temperature) centigrade scale or 500 units Fahrenheit scale were necessary for 50-percent hatching.

6.—Adult males of one species and adult females of another species were isolated in ponds to determine which of the 12 possible crosses may occur in nature. Thirty-four such experiments were conducted. Only R ♂ x G ♀, G ♂ x B ♀, and W ♂ x G ♀ crosses (two, two, and one experiment, respectively) produced large F_1 hybrid populations. Female bluegills successfully spawned with red-ear males whose opercular tabs had been removed (one experiment). Spawning did not occur in ponds containing normal red-ear males and female bluegills (five experiments). Results of the remaining 23 experiments were either negative or inconclusive.

7.—Large numbers of each of the 10 kinds of viable F_1 hybrids were stocked in ponds, and after they grew to maturity, the sex ratio and fecundity exhibited by each population were investigated. R ♂ B ♀, B R ♀, and B ♂ G ♀ were 97 percent males; W ♂ G 9 was 84 percent males; R G ♀, G ♂ B 9, and B ♂ W ♀ were approximately 70 percent males; G R ♀ and R ♂ W ♀

= red-ear sunfish, B = bluegill, G = green sunfish, W = warmouth.

were 50 percent males; and G W 9 were only 16 percent males. When in ponds which contained either no other fishes or only hybrid crappies, only three (R♂ B♀, B♂ R♀, and B G 9) of the 10 kinds of viable F_1 hybrids failed to produce abundant F2 generations. R♂ G♀, GaB2, and B♂ W♀ F_1 hybrids were also stocked in ponds containing largemouth bass. These three kinds of F_1 hybrids were unable to produce abundant F2 generations when in combination with largemouths although they did so in ponds where largemouth bass were absent.

G 9 F2 hybrids isolated in one pond and G ♂ W ♀ F2 hybrids isolated in another pond produced abundant F_3 generations. Thirteen other crosses involving F_1 hybrids were successful. Free-swimming fry from six of these were sacrificed because ponds were not available in which to stock them. Young from the other seven crosses were reared to 1 or more years of age in ponds, and successful reproduction occurred in three ponds.

9.—Equal numbers of relatively equal-sized G♂ R♀ F_1 hybrids, green sunfish, and red-ear sunfish were stocked in one pond, and B G♀ F_1 hybrids and green sunfish were stocked in another pond. When fishes in both ponds were poisoned at later dates, the average increases in total lengths of the hybrids were not significantly different from those of their parental species.

10.—The hemoglobin patterns in vertical starch gel electrophoresis of B♂ W 9, G W♀ and W♂ G♀ F_1 hybrids show marked differences from those of their parental species. The hybrid hemoglobins have better gas transport properties than those of the parental species, and in this respect these three hybrids are believed to exhibit hybrid vigor. The electrophoretic pattern of the hemoglobins of each of the other seven hybrids is identical to that obtained by mixing equal amounts of hemoglobins of the two parental species.

11.—F_1 hybrid sunfishes appear to be highly vulnerable to capture by hook-and-line fishing. Creel censuses indicate that large populations of hybrids can be almost completely eliminated in only a few days when subjected to moderate fishing pressure.

LITERATURE CITED

Alderdice, D. F., W. P. Wickett, and J. R. Brett. 1958. Some effects of temporary exposure to low dissolved oxygen levels on Pacific salmon eggs. Journal of the Fisheries Research Board of Canada 15(2): 229-250.

Allee, W. C., Alfred E. Emerson, Orlando Park, Thomas Park, and Karl P. Schmidt. 1949. Principles of animal ecology. W. B. Saunders Company, Philadelphia and London. 837 p.

Anderson, Edgar. 1953. Introgressive hybridization. Biological Reviews of the Cambridge Philosophical Society 28(3) :280-307.

Bailey, Reeve M. 1960a. Pisces (zoology), p. 242-243. *In* Encylopedia of science and technology. Volume 10. McGraw-Hill Book Company, Inc., New York.

______, *Chairman.* 1960b. A list of common and scientific names of fishes from the United States and Canada. Second ed. American Fisheries Society Special Publication 2. 102 p.

______, and Karl F. Lagler. 1938. An analysis of hybridization in a population of stunted sunfishes in New York. Michigan Academy of Science, Arts, and Letters Papers for 1937, 23:577-606.

Bennett, George W. 1958. Aquatic biology, p. 163-178. *In* A century of biological research. Illinois Natural History Survey Bulletin 27(2):85-234.

______ 1962. Management of artificial lakes and ponds. Reinhold Publishing Coporation, New York. 283 p.

______, David H. Thompson, and Sam A. Parr. 1940. Lake management reports, 4. A second year of fisheries investigations at Fork Lake, 1939. Illinois Natural History Survey Biological Notes 14. 24 p.

Branson, Branley A., and George A. Moore. 1962. The lateralis components of the acoustico-lateralis system in the sunfish family Centrarchidae. Copeia (1) :1-108.

Bright, William Milton. 1937. Spermatogenesis in sunfish. Ph.D. Thesis. University of Illinois, Urbana. 49 p.

Childers, William F., and George W. Bennett. 1961. Hybridization between three species of sunfish (Lepomis). Illinois Natural History Survey Biological Notes 46. 15 p.

Cross, Frank Bernard, and George A. Moore. 1952. The fishes of the Poteau River, Oklahoma and Arkansas. American Midland Naturalist 47(2):396.-412.

Crossman, E. J., and Keen Buss. 1965. Hybridization in the family Esocidae. Journal of the Fisheries Research Board of Canada 22(5):1261-1292.

Darlington, C. D. 1958. Evolution of genetic systems. Second ed. Oliver & Boyd, London. 265 p.

Darwin, Charles. 1897. The origin of species. Reprint of sixth ed. Volume 2. D. Appleton and Company, New York. 339 p.

Fisher, Ronald A., and Frank Yates. 1963. Statistical tables for biological, agricultural and medical research. Sixth ed. Hafner Publishing Company, Inc., New York. 146 p.

Forbes, Stephen Alfred, and Robert Earl Richardson. 1920. The fishes of Illinois. Second ed. Illinois Natural History Survey, Urbana. 357 p.

Greeley, J. R., and S. C. Bishop. 1933. Fishes of the upper Hudson watershed, p. 64-101. *In* A biological survey of the upper Hudson watershed. New York Conservation Department Biological Survey, Supplemental to Twenty-Second Annual Report, 1932, Volume 7.

Haldane, J. B. S. 1922. Sex ratio and unisexual sterility in hybrid animals. Journal of Genetics 12(2):101-109.

Hale, E. B. 1956. Social facilitation and forebrain function in maze performance of green sunfish, Lepomis cyanellus. Physiological Zoology 29(2) :93-107.

Heiser, Charles B., Jr. 1949. Natural hybridization with particular reference to introgression. Botanical Review 15(10):645-687.

Hubbs, Carl L. 1920. Notes on hybrid sunfishes. Aquatic Life 5(9) :101-103.

______. 1926. A check-list of the fishes of the Great Lakes and tributary waters, with nomenclatorial notes and analytical keys. University of Michigan Museum of Zoology Miscellaneous Publication 15. 85 p.

______. 1955. Hybridization between fish species in nature. Systematic Zoology 4(1) :1-20.

______, and Laura C. Hubbs. 1931. Increased growth in hybrid sunfishes. Michigan Academy of Science, Arts and Letters Papers for 1930, 13:291-301.

______, and ______ 1932. Experimental verification of natural hybridization between distinct genera of sunfishes. Michigan Academy of Science, Arts and Letters Papers for 1931, 15:427-437.

______, and ______ 1933. The increased growth, predominant maleness, and apparent infertility of hybrid sunfishes. Michigan Academy of Science, Arts and Letters Papers for 1932, 17:613-641.

______, and A. I. Ortenburger. 1929. Further notes on the fishes of Oklahoma with description of new species of Cyprinidae, p. 17-43. *In* University of Oklahoma Bulletin, new series 434. (Also listed as University of Oklahoma Biological Survey Publications 1(2) :17-43.)

Hunter, John R. 1963. The reproductive behavior of the green sunfish, Lepomis cyanellus. Zoologica 48(1) :13-24.

Kendeigh, S. Charles. 1961. Animal ecology. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. 468 p.

Krumholz, Louis A. 1950. Further observations on the use of hybrid sunfish in stocking small ponds. American Fisheries Society Transactions for 1949, 79:112-124.

Larimore, R. Weldon. 1957. Ecological life history of the warmouth (Centrarchidae). Illinois Natural History Survey Bulletin 27(1):1-83.

———, and Philip W. Smith. 1963. The fishes of Champaign County, Illinois, as affected by 60 years of stream changes. Illinois Natural History Survey Bulletin 28: (2) :299-382.

Lopinot, Al. 1961. The red-ear sunfish Illinois Wildlife 17(1) :3-4.

Manwell, Clyde, C. M. Ann Baker, and William Childers. 1963. The genetics of hemoglobin in hybrids-I. A molecular basis for hybrid vigor. Comparative Biochemistry and Physiology 10:103-120.

Marler, P. 1957. Specific distinctiveness in the communication signals of birds. Behaviour 11(1) :13-39.

McAtee, W. L., and A. C. Weed. 1915. First list of the fishes of the vicinity of Plummers Island, Maryland. Biological Society of Washington Proceedings 28:1-14.

Miller, Helen Carter. 1963. The behavior of the pumpkinseed sunfish, Lepomis gibbosus (Linneaus), with notes on the behavior of other species of Lepomis and the pigmy sunfish, Elassoma evergladei. Behaviour 22 (1-2) :88-151.

Miller, Robert Rush. 1958. Origin and affinities of the freshwater fish fauna of western North America, p. 187-222. In Carl L. Hubbs, editor, Zoogeography. American Association for the Advancement of Science Publication 51, Washington, D. C. 509 p.

O'Donnell, D. John. 1935. Annotated list of the fishes of Illinois. Illinois Natural History Survey Bulletin 20(5):473-491.

Radcliffe, Lewis. 1914. A hybrid centrarchid. Copeia (7) :26-28.

Raney, Edward C. 1940. Reproductive activities of a hybrid minnow, Notropis cornutus x Notropis rubellus. Zoologica 25 (24) :361-367.

Ricker, William E. 1948. Hybrid sunfish for stocking small ponds. American Fisheries Society Transactions for 1945, 75:84-96.

Roberts, Franklin L. 1964. A chromosome study of twenty species of Centrarchidae. Journal of Morphology 115(3) :401-417.

Ross, Herbert H. 1958. Evidence suggesting a hybrid origin for certain leafhopper species. Evolution 12(3) :337-346.

Schuchert, Charles, and Carl O. Dunbar. 1941. A textbook of geology. Part II-historical geology. Fourth ed. John Wiley & Sons, Inc., New York. 544 p.

Shelford, Victor E. 1927. An experimental investigation of the relations of the codling moth to weather and climate. Illinois Natural History Survey Bulletin 16(5) :311-440.

Smitherman, R. Oneal, and F. Eugene Hester. 1962. Artificial propagation of sunfishes, with meristic comparisons of three species of Lepomis and five of their hybrids. American Fisheries Society Transactions 91(4): 333-341.

Slastenenko, E. P. 1957. A list of natural fish hybrids of the world. Publication of the Hydrobiological Research Institute, Faculty of Science, University of Istanbul. Series B. 4(2-3): 76-97.

Stebbins, G. Ledyard. 1959. The role of hybridization in evolution. American Philosophical Society Proceedings 103:231-251.

Swingle, H. S. 1949. Some recent developments in pond management. North American Wildlife Conference Transactions 14: 295-312.

———. 1956. A repressive factor controlling reproduction in fishes. Eighth Pacific Science Congress Proceedings 3A:865-871.

Tinbergen, N. 1951. The study of instinct. Oxford University Press, London. 228 p.

Trautman, Milton B. 1957. The fishes of Ohio. The Ohio State University Press, Columbus. 683 p.

Listed below are some recent papers on hybrid sunfishes. These papers have been published since the completion of the manuscript of this bulletin.

Birdsong, Ray S., and Ralph W. Yerger. 1967. A natural population of hybrid sunfishes: Lepomis macrochirus x Chaenobryttus gulosus. Copeia (1) :62-71.

Childers, William F., and George W. Bennett. 1967. Hook-and-line yield of largemouth bass and redear x green sunfish hybrids in a one-acre pond. Progressive Fish-Culturist 29(1):27-35.

Clark, Francis W., and Miles H. A. Keenleyside. 1967. Reproductive isolation between the sunfish Lepomis gibbosus and L. macrochirus. Journal of the Fisheries Research Board of Canada 24(3) :495-514.

West, Jerry L., and F. Eugene Hester. 1966. Intergeneric hybridization of centrarchids. Transactions of the American Fisheries Society 95(3) :280-288.

APPENDIX

Table A1.—Partial chemical analysis of water taken August 2, 1963, from the well on Parkhill's Lake Park Subdivision Number Two, approximately 3 miles south of Champaign, Ill. The water was aerated and filtered through activated charcoal for 1 week prior to the analysis. The water had no measurable color or odor. The pH was 8.8. The analysis was made by the Illinois Water Survey.

Chemical Composition	*Parts Per Million*
Iron (total)	Trace
Calcium	24
Magnesium	21
Chloride	3
Phenothaline alkalinity (as $CaCO_3$)	12
Methyl orange alkalinity (as $CaCO_3$)	204
Hardness (as $CaCO_3$)	148
Total dissolved minerals	239

Table A2.—Experiment W1: duration of fertility of activated gametes from one pair of warmouths.*

Age of Eggs in Minutes	*Age of Sperm in Minutes*	*Number of Eggs*	*Percent of Eggs that Hatched*	*Percent of Eggs that Developed into Fry†*
		Aging of Sperm and Eggs		
0.75	0.25	93	47	46
3.15	2.75	211	11	10
5.55	5.25	102	0	0
8.45	7.75	149	0	0
10.35	10.25	86	0	0

*The temperature at time of fertilization was 27.8° C. (82.0° F.). During the entire experiment, 132 hours, the temperature ranged from 26.1° to 27.8° C. (79.0° to 82.0° F.) and averaged 27.2° C. (80.9° F.).
†Fry were free swimming. Only normal fry are included in this figure.

Table A3.—Experiment **W2:** duration of fertility of activated gametes from one pair of warmouths.*

Age of Eggs in Minutes	*Age of Sperm in Minutes*	*Number of Eggs*	*Percent of Eggs that Hatched*	*Percent of Eggs that Developed into Fry†*
		Aging of Sperm and Eggs		
0.75	0.25	25	42	35
3.15	2.75	20	0	0
5.55	5.25	48	0	0
8.45	7.75	29	0	0
10.55	10.25	37	0	0

*The temperature at time of fertilization was 27.8° C. (82.0° F.). During the entire experiment, 128 hours, the temperature ranged from 26.1° to 27.8° C. (79.0° to 82.0° F.) and averaged 27.2° C. (80.9° F.).
†Fry were free swimming. Only normal fry are included in this figure.

Table A4.—Experiment W3: duration of fertility of activated eggs from a single female warmouth. Sperm used in this experiment were from a single male.*

Age of Eggs in Minutes	*Age of Sperm in Minutes*	*Number of Eggs*	*Percent of Eggs that Hatched*	*Percent of Eggs that Developed into Fry*†
		Aging of Eggs		
0.50	0	39	100	95
30.00	0	79	92	90
60.00	0	91	72	70
120.00	0	81	49	48
180.00	0	89	7	2

*The temperature at time of fertilization was 27.2° C.(81.0° F.). During the entire experiment, 100 hours, the temperature ranged from 26.1° to 28.3° C.(79.0° to 83.0° F.) and averaged 27.3° C.(81.1° F.).

†Fry able to swim for short periods but not completely free swimming. Only normal fry are included in this figure.

Table A5.—Experiment W4: duration of fertility of activated eggs from a single female warmouth. Sperm used in this experiment were from a single male.*

Age of Eggs in Minutes	*Age of Sperm in Minutes*	*Number of Eggs*	*Percent of Eggs that Hatched*	*Percent of Eggs that Developed into Fry*†
		Aging of Eggs		
0.50	0	89	89	73
30.00	0	82	16	16
60.00	0	81	44	41
120.00	0	69	14	6
180.00	0	89	0	0

*The temperature at time of fertilization was 27.5°C.(81.5° F.). During the entire experiment, 125 hours, the temperature ranged from 26.4° to 27.8° C.(79.5° to 82.0° F.) and averaged 27.0° C.(80.6° F.).

† Fry were free swimming. Only normal fry are included in this figure.

Table A6.—Experiment B1: duration of fertility of activated gametes from one pair of bluegills.*

Age of Eggs in Minutes	Age of Sperm in Minutes	Number of Eggs	Percent of Eggs that Hatched	Percent of Eggs that Developed into Fry†
		Aging of Sperm and Eggs		
1.00	0.25	48	50	33
1.90	1.25	40	45	8
2.80	2.25	38	18	18
3.70	3.25	63	2	2
4.60	4.25	42	0	0
5.50	5.25	27	0	0
		Aging of Eggs		
0.50	0	26	85	85
30.00	0	44	75	68
60.00	0	26	38	27
120.00	0	59	22	10
180.00	0	32	0	0

*The temperature at time of fertilization was 23.3° C.(74.0° F.). During the entire experiment, 102 hours, the temperature ranged from 23.0° to 27.2° C.(73.5° to 81.0° F.) and averaged 25.3° C.(77.6° F.).
†Fry able to swim for short periods but not completely free swimming. Only normal fry are included in this figure.

Table A7.-Experiment B2: duration of fertility of activated gametes from one pair of

Age of Eggs in Minutes	Age of Sperm in Minutes	Number of Eggs	Percent of Eggs that Hatched	Percent of Eggs that Developed into Fait
		Aging of Sperm and Eggs		
1.00	0.25	44	82	64
1.90	1.25	34	53	44
2.80	2.25	38	5	5
3.70	3.25	22	0	0
4.60	4.25	23	0	0
5.50	5.25	37	0	0

°The temperature at time of fertilization was 23.6° C. (74.5° F.). During the entire experiment, 102 hours, the temperature ranged from 23.0° to 27.2° C.(73.5° to 81.0° F.) and averaged 25.3° C.(77.6° F.).
tFry able to swim for short periods but not completely free swimming. Only normal fry are included in this figure.

Table A8.—Experiment B3: duration of fertility of activated eggs from one female bluegill. Sperm used in this experiment were from a single male.*

Age of Eggs in Minutes	*Age of Sperm in Minutes*	*Number of Eggs*	*Percent of Eggs that Hatched*	*Percent of Eggs that Developed into Fry*†
		Aging of Eggs		
0.50	0	96	96	96
30.00	0	71	82	82
60.00	0	59	51	51
120.00	0	93	16	16
180.00	0	44	20	20

*The temperature at time of fertilization was 26.9° C.(80.5° F.). During the entire experiment, 98 hours, the temperature ranged from 26.1° to 28.3° C.(79.0° to 83.0° F.) and averaged 27.3° C.(81.1° F.).

†Fry able to swim for short periods but not completely free swimming. Only normal fry are included in this figure.

Table A9.—Experiment B4: duration of fertility of activated eggs from a single female bluegill. Sperm used in this experiment were from a single male.*

Age of Eggs in Minutes	*Age of Sperm in Minutes*	*Number of Eggs*	*Percent of Eggs that Hatched*	*Percent of Eggs that Developed into Fry*†
		Aging of Eggs		
0.50	0	88	65	61
30.00	0	28	28	28
60.00	0	38	32	32
120.00	0	44	23	23
180.00	0	48	4	0

*The temperature at time of fertilization was 23.9° C.(75.0° F.). During the entire experiment, 138 hours, the temperature ranged from 22.8° to 25.0° C.(73.0° to 77.0° F.) and averaged 24.2° C.(75.5° F.).

†Fry able to swim for short periods but not completely free swimming. Only normal fry are included in this figure.

Table A10.—Experiment G1: duration of fertility of activated gametes from one pair of green sunfish.*

Age of Eggs in Minutes	*Age of Sperm in Minutes*	*Number of Eggs*	*Percent of Eggs that Hatched*	*Percent of Eggs that Developed into Fry†*
		Aging of Sperm and Eggs		
1.00	0.25	40	52	50
1.90	1.25	32	6	6
2.80	2.25	34	1	1
3.70	3.25	31	0	0
4.60	4.25	43	0	0
5.50	5.25	38	5	5
		Aging of Eggs		
0.50	0	33	91	88
30.00	0	57	24	23
60.00	0	74	8	7
120.00	0	49	4	2
180.00	0	27	7	4

*The temperature at time of fertilization was 23.6° C. (74.5° F.). During the entire experiment, 185 hours, the temperature ranged from 22.8° to 25.0° C. (73.0° to 77.0° F.) and averaged 24.2C. (75.6° F.).
†Fry able to swim for short periods but not completely free swimming. Only normal fry are included in this figure.

Table A11.—Experiment G2: duration of fertility of activated gametes from one pair of green sunfish.*

Age of Eggs in Minutes	*Age of Sperm in Minutes*	*Number of Eggs*	*Percent of Eggs that Hatched*	*Percent of Eggs that Developed into Fry†*
		Aging of Sperm and Eggs		
2.25	0.25	79	52	47
3.15	1.25	57	19	18
4.05	2.25	55	2	2
4.95	3.25	81	0	0
5.85	4.25	71	1	1
6.75	5.25	74	0	0
		Aging of Eggs		
0.50	0	31	32	32
30.00	0	39	41	36
60.00	0	78	26	23
120.00	0	46	13	13
180.00	0	70	7	6

*The temperature at time of fertilization was 23.6° C. (74.5° F.). During the entire experiment, 185 hours, the temperature ranged from 22.8° to 25.0° C. (73.0° to 77.0° F.) and averaged 24.2° C. (75.6° F.).
†Fry able to swim for short periods but not completely free swimming. Only normal fry are included in this figure.

Table A12.—Experiment G3: duration of fertility of activated gametes from one pair of green sunfish.*

Age of Eggs in Minutes	Age of Sperm in Minutes	Number of Eggs	Percent of Eggs that Hatched	Percent of Eggs that Developed into Fry†
		Aging of Sperm and Eggs		
1.00	0.25	44	36	34
1.90	1.25	52	25	25
2.80	2.25	16	0	0
3.70	3.25	111	5	4
4.60	4.25	25	4	4
5.50	5.25	46	2	2
		Aging of Eggs		
0.50	0	41	36	34
30.00	0	75	39	39
60.00	0	31	[illegible]2	22
120.00	0	26	54	50
180.00	0	26	42	42

*The temperature at time of fertilization was 24.4° C.(76.0° F.). During the entire experiment, 118 hours, the temperature ranged from 23.0° to 27.2° C.(73.5° to 81.0° F.) and averaged 25.1° C.(77.2° F.).
†Fry able to swim for short periods but not completely free swimming. Only normal fry are included in this figure.

Table A13.—Experiment G4: duration of fertility of activated eggs from a single female green sunfish. Sperm used in this experiment were from a single male.*

Age of Eggs in Minutes	Age of Sperm in Minutes	Number of Eggs	Percent of Eggs that Hatched	Percent of Eggs that Developed into Fry†
		Aging of Eggs		
0.50	0	36	75	75
30.00	0	51	53	53
60.00	0	33	48	48
120.00	0	31	26	26
180.00	0	48	10	8

*The temperature at time of fertilization was 26.9° C.(80.5° F.). During the entire experiment, 101 hours, the temperature ranged from 26.1° to 28.3° C.(79.0° to 83.0° F.) and averaged 27.3° C.(81.1° F.).
†Fry able to swim for short periods but not completely free swimming. Only normal fry are included in this figure.

Table A14.-Experiment 51: some aspects of the development of red-ear sunfish zygotes and hybrid zygotes during the first 161 hours of their development. Throughout the experiment hourly temperatures averaged 23.6° C. (84.5° F.), standard deviation 0.77° C. (1.38° F.).

Parents* ♀ x ♂	Sample Number	Number of Eggs	Percent† Hatched	Hatching Time in Hours: Mean	Hatching Time in Hours: Standard Deviation	Incubation Temperature in Degrees C.‡: Mean	Incubation Temperature in Degrees C.‡: Standard Deviation	Percent† Normal Fry	Length of Fry in mm: Number Measured	Length of Fry in mm: Mean	Length of Fry in mm: Standard Deviation
R x R	1	41	34	52.1	4.22			24			
	2	39	46	49.3	5.29			46			
	3	55	53	50.1	5.67			44			
	Combined samples	*135*	*45*	*50.3*	*5.36*	*23.8*	*0.41*	*38*	25	4.78	*0.300*
B x R	1	50	46	52.8	4.38			36			
	2	53	55	49.9	5.15			47			
	3	44	75	53.5	2.77			68			
	Combined samples	*147*	*58*	*52.1*	*4.45*	*23.8*	*0.40*	*50*	*25*	4.86	0.139
G x R	1	31	29	52.1	4.50			29			
	2	43	70	49.9	5.26			56			
	3	43	65	48.4	5.34			*60*			
	Combined samples	*117*	*57*	49.6	5.33	*23.8*	*0.41*	*50*	*25*	*4.98*	*0.165*
W x R	1	45	13	48.8	4.78			0			
	2	42	45	48.2	2.68			0			
	3	36	56	50.2	5.25			0			
	Combined samples	*123*	*36§*	*49.1*	*4.38*	*23.8*	*0.41*	*0*	*0*		

*R = red-ear sunfish, B bluegill, G = green sunfish, and W = warmouth.
†Percentage based on eggs in each sample at the time sperm and eggs were mixed.
‡Incubation temperature from time of fertilization to mean time of hatching.
§More than 90 percent of these larvae were morphologically abnormal and all were behaviorally abnormal.

Table A15.—Experiment S 2: some aspects of the development of red-ear sunfish zygotes and hybrid zygotes during the first 120 hours of their development. Throughout the experiment hourly temperatures averaged 28.7° C.(83.6° F.), standard deviation 0.44° C.(0.79° F.).

*Parents**				*Hatching Time in Hours*		*Incubation Temperature in Degrees C.‡*			*Length of Fry in mm*		
5 9	*Sample Number*	*Number of Eggs*	*Percent† Hatched*	Mean	Standard Deviation	Mean	Standard Deviation	*Percent† Normal Fry*	Number Measured	Mean	Standard Deviation
R x R	1	73	29	26.6	1.58			20			
	2	59	34	27.4	2.53			20			
	3	102	36	26.2	1.58			25			
Combined samples		*234*	*33*	*26.6*	*1.94*	*28.5*	*0.29*	*23*	*25*	*4.99*	*0.155*
B x R	1	81	23	29.1	2.08			20			
	2	93	25	27.8	1.22			19			
	3	40	55	28.4	1.82			48			
Combined samples		*214*	*30*	*28.4*	*1.80*	*28.6*	*0.31*	*25*	*25*	*5.13*	*0.192*
G x R	1	89	42	27.2	2.25			32			
	2	91	35	28.3	2.35			23			
	3	51	37	27.6	2.14			24			
Combined samples		*231*	*38*	*27.7*	*2.31*	*28.6*	*0.31*	*27*	*25*	*5.10*	*0.189*
W x R	1	73	38	28.4	3.03			5			
	2	42	19	28.0	2.29			2			
	3	69	40	27.2	2.15			6			
Combined samples		*184*	*35§*	*27.9*	*2.65*	*28.6*	*0.31*	*5***	0		

*R = red-ear sunfish, B = bluegill, G = green sunfish, and W = warmouth.
†Percentage based on eggs in each sample at the time sperm and eggs were mixed.
‡Incubation temperature from time of fertilization to mean time of hatching.
§More than 90 percent of these larvae were morphologically abnormal and all were behaviorally abnormal.
**Appeared morphologically normal, but all were behaviorally abnormal.

Table A16.—Experiment S 3: some aspects of the development of red-ear sunfish zygotes and hybrid zygotes during the first 120 hours of their development. Throughout the experiment hourly temperatures averaged 28.7° C.(83.6° F.), standard deviation 0.44° C.(0.79° F.).

Parents° ♂ x ♀	Sample Number	Number of Eggs	Percent† Hatched	Hatching Time in Hours: Mean	Hatching Time in Hours: Standard Deviation	Incubation Temperature in Degrees C‡: Mean	Incubation Temperature in Degrees C‡: Standard Deviation	Percent† Normal Fry	Length of Fry in mm: Number Measured	Length of Fry in mm: Mean	Length of Fry in mm: Standard Deviation
R x R	1	32	66	28.4	2.14			41			
	2	59	39	27.7	2.14			22			
	3	52	36	28.3	2.18			15			
Combined samples		*143*	*44*	*28.1*	*2.18*	*28.6*	*0.31*	*24*	*25*	*5.28*	*0.166*
B x R	1	57	30	27.6	1.21			23			
	2	44	41	27.8	2.02			32			
	3	50	54	28.8	1.73			34			
Combined samples		*151*	*41*	*28.2*	*1.79*	*28.6*	*0.31*	*29*	*25*	*5.58*	*0.137*
G x R	1	76	43	28.6	2.04			37			
	2	67	42	27.9	2.00			33			
	3	61	64	28.2	1.74			56			
Combined samples		*204*	*49*	*28.2*	*1.94*	*28.6*	*0.31*	*41*	*25*	*5.54*	*0.185*
W x R	1	145	19	25.7	2.38			3			
	2	61	26	26.2	2.57			0			
	3	35	66	27.6	2.70			0			
Combined samples		*241*	*27§*	*26.5*	*2.67*	*28.5*	0.27	V°	*0*		

°R = red-ear sunfish, B = bluegill, G = green sunfish, and W = warmouth.
†Percentage based on eggs in each sample at the time sperm and eggs were mixed.
‡Incubation temperature from time of fertilization to mean time of hatching.
§More than 90 percent of these larvae were morphologically abnormal and all were behaviorally abnormal.
°°Appeared morphologically normal, but all were behaviorally abnormal.

Table A17.-Experiment S 4: some aspects of the development of bluegill zygotes and hybrid zygotes during the first 184 hours of their development. Throughout the experiment hourly temperatures averaged 22.6° C.(72.7° F.), standard deviation 0.68° C.(1.22° F.).

*Parents** ♂ x ♀	*Sample Number*	*Number of Eggs*	*Percent† Hatched*	*Hatching Time in Hours* Mean	Standard Deviation	*Incubation Temperature in Degrees C.‡* Mean	Standard Deviation	*Percent† Normal Fry*	*Length of Fry in mm* Number Measured	Mean	Standard Deviation
B x B	1	75	77	69.3	4.44			8			
	2	94	90	71.8	2.87			63			
	3	92	92	71.7	2.20			89			
Combined samples		*261*	*87*	*71.1*	*3.32*	*22.3*	*0.24*	*56*	*25*	*4.28*	*0.172*
R x B	1	78	81	66.9	3.47			77			
	2	174	76	68.3	2.44			68			
	3	111	88	67.9	2.62			88			
Combined samples		*363*	*81*	*67.9*	*2.80*	*22.3*	*0.24*	*76*	*25*	*4.42*	*0.108*
G x B	1	48	77	74.8	4.37			71			
	2	144	80	72.7	4.51			6			
	3	79	90	73.9	5.18			87			
Combined samples		*271*	*82*	*73.4*	*4.78*	*22.3*	*0.23*	*41*	*25*	*4.48*	*0.144*
W x B	1	68	24	59.6	5.23			0			
	2	108	16	59.4	3.92			0			
	3	126	25	60.1	3.33			0			
Combined samples		*302*	22§	59.8	*4.04*	*22.3*	*0.23*	*0*	*0*		

*R = red-ear sunfish, B = bluegill, G = green sunfish, and W = warmouth.
Percentage based on eggs in each sample at the time sperm and eggs were mixed.
‡Incubation temperature from time of fertilization to mean time of hatching.
§More than 90 percent of these larvae were morphologically abnormal and all were behaviorally abnormal.

Table A18.—Experiment S 5: some aspects of the development of bluegill zygotes and hybrid zygotes during the first 113 hours of their development. Throughout the experiment hourly temperatures averaged 26.9° C. (80.4° F.), standard deviation 0.48° C. (0.86° F.).

Parents* ♂ x ♀	Sample Number	Number of Eggs	Percent† Hatched	Hatching Time in Hours: Mean	Hatching Time in Hours: Standard Deviation	Incubation Temperature in Degrees C.‡: Mean	Incubation Temperature in Degrees C.‡: Standard Deviation	Percent† Normal Fry	Length of Fry in mm: Number Measured	Length of Fry in mm: Mean	Length of Fry in mm: Standard Deviation
B x B	1	53	79	33.7	1.29			79			
	2	131	86	34.2	2.20			85			
	3	73	85	33.4	2.11			82			
Combined samples		*257*	*84*	33.9	2.06	*26.8*	*0.37*	*83*	*25*	*4.98*	*0.110*
R x B	1	77	91	34.3	1.75			90			
	2	66	94	34.8	1.69			92			
	3	52	98	34.9	1.37			98			
Combined samples		*195*	*94*	*34.6*	*1.66*	*26.8*	0.37	93	*25*	*4.98*	*0.151*
G x B	1	73	88	32.8	1.59			78			
	2	88	93	34.4	2.09			93			
	3	58	95	35.7	1.64			93			
Combined samples		*219*	*92*	*34.3*	*2.14*	*26.8*	*0.37*	*88*	*25*	*5.00*	*0.164*
W x B	1	86	86	29.3	1.81			0			
	2	76	92	29.6	1.20			0			
	3	52	94	29.0	1.23			0			
Combined samples		*214*	*90§*	*29.4*	*1.49*	*26.8*	*0.40*	*0*	*0*		

*R = red-ear sunfish, B = bluegill, G = green sunfish, and W = warmouth.
†Percentage based on eggs in each sample at the time sperm and eggs were mixed.
‡Incubation temperature from time of fertilization to mean time of hatching.
§More than 90 percent of these larvae were morphologically abnormal and all were behaviorally abnormal.

Table A19.-Experiment S 6: some aspects of the development of bluegill zygotes and hybrid zygotes during the first 105 hours of their development. Throughout the experiment hourly temperatures averaged 27.3° C. (81.1° F.), standard deviation 0.65° C. (1.17° F.).

*Parents** ♂ x ♀	*Sample Number*	*Number of Eggs*	*Percent†* *Hatched*	*Hatching Time in Hours* Mean	Standard Deviation	*Incubation Temperature in Degrees C.‡* Mean	Standard Deviation	*Percent† Normal Fry*	*Length of Fry in mm* Number Measured	Mean	Standard Deviation
B x B	1	21	71	32.3	0.54			71			
	2	91	91	32.4	0.82			91			
	3	51	96	32.9	0.85			96			
	Combined samples	*163*	*90*	*32.5*	*0.83*	*27.0*	*0.42*	90	*25*	*4.69*	*0.076*
R x B	1	65	83	31.8	0.81			78			
	2	41	90	31.6	1.06			90			
	3	78	90	31.9	0.68			87			
	Combined samples	*184*	*88*	*31.8*	*0.83*	*27.0*	*0.40*	85	*25*	*4.63*	*0.069*
G x B	1	43	88	32.4	0.92			88			
	2	44	93	32.4	0.96			93			
	3	62	90	32.2	1.09			90			
	Combined samples	*149*	*91*	*32.3*	*1.01*	*27.0*	*0.40*	*91*	*25*	*4.61*	*0.095*
W x B	1	49	98	27.6	1.49			8			
	2	57	88	28.3	1.77			2			
	3	77	95	27.8	1.89			1			
	Combined samples	*183*	93§	27.9	*1.77*	*26.8*	*0.28*	*3***	*0*		

*R = red-ear sunfish, B = bluegill, G = green sunfish, and W = warmouth.
†Percentage based on eggs in each sample at the time sperm and eggs were mixed.
‡Incubation temperature from time of fertilization to mean time of hatching.
§More than 90 percent of these larvae were morphologically abnormal and all were behaviorally abnormal.
**Appeared morphologically normal, but all were behaviorally abnormal.

Table A20.-Experiment S 7: some aspects of the development of green sunfish zygotes and hybrid zygotes during the first 151 hours of their development. Throughout the experiment hourly temperatures averaged 24.4° C. (75.9° F.), standard deviation 0.46° C. (0.83° F.).

Parents* ♂ x ♀	Sample Number	Number of Eggs	Percent† Hatched	Hatching Time in Hours: Mean	Hatching Time in Hours: Standard Deviation	Incubation Temperature in Degrees C.‡: Mean	Incubation Temperature in Degrees C.‡: Standard Deviation	Percent Normal Fry	Length of Fry in mm: Number Measured	Length of Fry in mm: Mean	Length of Fry in mm: Standard Deviation
G x G	1	49	71	50.4	2.42			69			
	2	43	74	50.8	1.60			74			
	3	64	80	49.2	2.53			76			
	Combined samples	*156*	*76*	*50.0*	*2.37*	*23.8*	*0.27*	*74*	*25*	4.79	0.095
R x G	1	41	85	48.4	1.17			85			
	2	45	89	47.7	1.71			89			
	3	48	83	48.0	1.84			83			
	Combined samples	*134*	*86*	*48.0*	*1.64*	*23.8*	*0.26*	*86*	*25*	4.62	0.097
B x G	1	38	50	51.4	2.71			50			
	2	66	83	49.8	2.19			77			
	3	78	77	48.6	3.00			76			
	Combined samples	*182*	*74*	*49.5*	*2.52*	*23.8*	*0.27*	*71*	*25*	*4.74*	*0.079*
W x G	1	34	76	48.9	2.10			0			
	2	48	83	48.9	2.95			79			
	3	70	87	48.4	2.61			78			
	Combined samples	*152*	*84*	*48.7*	*2.64*	*23.8*	*0.27*	*61*	*25*	*4.60*	*0.132*

= red-ear sunfish, B = bluegill, G = green sunfish, and W = warmouth.
†Percentage based on eggs in each sample at the time sperm and eggs were mixed.
‡Incubation temperature from time of fertilization to mean time of hatching.

Table A21.-Experiment S 8: some aspects of the development of green sunfish zygotes and hybrid zygotes during the first 104 hours of their development. Throughout the experiment hourly temperatures averaged 27.3° C. (81.1° F.), standard deviation 0.66° C. (1.18° F.).

Parents* ♂ x ♀	Sample Number	Number of Eggs	Percent† Hatched	Hatching Time in Hours: Mean	Hatching Time in Hours: Standard Deviation	Incubation Temperature in Degrees C.‡: Mean	Incubation Temperature in Degrees C.‡: Standard Deviation	Percent† Normal Fry	Length of Fry in mm: Number Measured	Length of Fry in mm: Mean	Length of Fry in mm: Standard Deviation
G x G	1	101	65	31.1	2.14			63			
	2	87	83	32.8	1.31			79			
	3	118	81	30.7	2.05			80			
	Combined samples	*306*	*76*	*31.5*	*2.08*	*27.1*	*0.44*	*74*	*25*	*4.72*	0.237
R x G	1	40	65	32.5	0.88			60			
	2	52	92	31.7	0.85			88			
	3	114	82	31.4	0.88			81			
	Combined samples	*206*	*81*	*31.7*	*0.95*	*27.1*	*0.44*	*79*	*25*	*4.84*	*0.091*
B x G	1	64	56	30.8	1.20			56			
	2	68	84	31.8	1.08			84			
	3	60	83	31.5	1.14			82			
	Combined samples	*192*	*74*	*31.4*	*1.20*	*27.0*	*0.42*	*74*	*25*	*4.85*	*0.118*
W x G	1	53	62	29.0	1.83			26			
	2	90	86	30.0	1.88			59			
	3	71	89	30.0	2.02			55			
	Combined samples	*214*	*81*	*29.8*	*1.96*	*27.0*	*0.41*	*50*	*25*	*4.68*	*0.098*

*R = red-ear sunfish, B = bluegill, G = green sunfish, and W = warmouth.
†Percentage based on eggs in each sample at the time sperm and eggs were mixed.
‡Incubation temperature from time of fertilization to mean time of hatching.

Table A22.—Experiment S 9: some aspects of the development of green sunfish zygotes and hybrid zygotes during the first 78 hours of their development. Throughout the experiment hourly temperatures averaged 28.1° C. (82.5° F.), standard deviation 0.51° C. (0.92° F.).

Parents* ♂ x ♀	Sample Number	Number of Eggs	Percent† Hatched	Hatching Time in Hours: Mean	Hatching Time in Hours: Standard Deviation	Incubation Temperature in Degrees C.‡: Mean	Incubation Temperature in Degrees C.‡: Standard Deviation	Percent† Normal Fry	Length of Fry in mm: Number Measured	Length of Fry in mm: Mean	Length of Fry in mm: Standard Deviation
G x G	1	39	74	29.0	1.71			67			
	2	94	86	28.8	1.56			81			
	3	44	82	29.8	1.97			82			
Combined samples		*177*	*82*	*29.1*	*1.75*	*27.6*	*0.32*	78	25	*4.64*	*0.187*
R x G	1	109	77	26.7	1.13			75			
	2	96	79	27.4	1.32			79			
	3	52	75	28.9	0.87			75			
Combined samples		*257*	*77*	*27.4*	*1.45*	*27.6*	*0.31*	*77*	*25*	*4.53*	*0.201*
B x G	1	66	71	28.4	1.05			65			
	2	102	74	28.2	1.75			68			
	3	47	68	27.1	1.38			62			
Combined samples		*215*	*72*	*28.0*	*1.57*	*27.6*	*0.31*	66	*25*	4.69	*0.204*
W x G	1	138	64	28.4	1.41			54			
	2	83	77	29.6	1.58			60			
	3	91	66	29.1	1.02			58			
Combined samples		*312*	*68*	*29.0*	*1.46*	*27.6*	*0.32*	*57*	*25*	*4.53*	*0.182*

*R = red-ear sunfish, B = bluegill, G = green sunfish, and W = warmouth.
†Percentage based on eggs in each sample at the time sperm and eggs were mixed.
‡Incubation temperature from time of fertilization to mean time of hatching.

Table A23.-Experiment S 10: some aspects of the development of warmouth zygotes and hybrid zygotes during the first 115 hours of their development. Throughout the experiment hourly temperatures averaged 27.2° C.(81.0° F.), standard deviation 0.45° C.(0.81° F.).

Parents* ♂ x ♀	Sample Number	Number of Eggs	Percent† Hatched	Hatching Time in Hours: Mean	Hatching Time in Hours: Standard Deviation	Incubation Temperature in Degrees C‡: Mean	Incubation Temperature in Degrees C‡: Standard Deviation	Percent† Normal Fry	Length of Fry in mm: Number Measured	Length of Fry in mm: Mean	Length of Fry in mm: Standard Deviation
W x W	1	89	48	28.8	1.60			44			
	2	50	56	30.1	1.11			46			
	3	40	52	29.6	1.46			48			
Combined samples		179	51	29.4	1.55	27.3	0.33	45	25	4.75	0.176
R x W	1	49	39	29.8	1.03			37			
	2	81	53	29.0	1.26			48			
	3	45	44	29.8	1.41			42			
Combined samples		175	47	29.4	1.31	27.3	0.33	43	25	4.63	0.139
B x W	1	72	54	30.5	1.24			42			
	2	60	52	31.2	1.36			45			
	3	65	54	30.7	1.21			48			
Combined samples		197	53	30.8	1.29	27.3	0.33	45	25	4.74	0.180
G x W	1	57	51	30.4	1.66			44			
	2	44	54	31.0	1.26			50			
	3	45	60	31.3	0.92			9			
Combined samples		146	55	30.9	1.37	27.3	0.33	35	25	4.76	0.212

*R = red-ear sunfish, B = bluegill, G = green sunfish, and W = warmouth.
†Percentage based on eggs in each sample at the time sperm and eggs were mixed.
‡Incubation temperature from time of fertilization to mean time of hatching.

Table A24.-Experiment 11: some aspects of the development of warmouth zygotes and hybrid zygotes during the first 78 hours of their development. Throughout the experiment hourly temperatures averaged 28.1° C. (82.5° F.), standard deviation 0.51° C. (0.92° F.).

Parents* ♂ x ♀	Sample Number	Number of Eggs	Percent† Hatched	Hatching Time in Hours: Mean	Hatching Time in Hours: Standard Deviation	Incubation Temperature in Degrees C.‡: Mean	Incubation Temperature in Degrees C.‡: Standard Deviation	Percent† Normal Fry	Length of Fry in mm: Number Measured	Length of Fry in mm: Mean	Length of Fry in mm: Standard Deviation
W x W	1	48	75	28.4	1.56			62			
	2	38	55	28.9	1.62			45			
	3	30	77	29.6	1.17			60			
	Combined samples	*116*	*69*	*28.9*	*1.56*	*27.6*	*0.31*	*56*	*25*	*4.72*	*0.204*
R x W	1	59	83	29.1	0.63			73			
	2	30	77	29.6	0.90			70			
	3	53	79	29.0	1.37			62			
	Combined samples	*142*	*80*	*29.2*	*1.03*	*27.6*	*0.31*	*68*	*25*	*4.60*	*0.199*
B x W	1	26	73	28.9	1.09			62			
	2	33	61	29.0	1.53			48			
	3	55	69	28.9	1.14			53			
	Combined samples	*114*	68	*28.9*	*1.24*	*27.6*	*0.31*	*54*	*25*	*4.77*	*0.141*
G x W	1	33	67	29.6	1.12			52			
	2	37	59	29.8	0.91			51			
	3	60	80	29.7	0.98			73			
	Combined samples	*130*	*71*	*29.7*	*1.00*	*27.6*	*0.32*	*62*	*25*	*4.90*	*0.203*

*R = red-ear sunfish, B = bluegill, G = green sunfish, and W warmouth.
†Percentage based on eggs in each sample at the time sperm and eggs were mixed.
‡Incubation temperature from time of fertilization to mean time of hatching.

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