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Observations on reproduction, isozyme patterns, and meristics in mosquitofish (Pisces: Poeciliidae) from West Virginia

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Observations on reproduction, isozyme patterns, and meristics in mosquitofish (Pisces: Poeciliidae) from West Virginia

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ABSTRACT

Prior to 1988, two subspecies of the mosquitofish *Gambusia affinis* (*G. a. affinis* and *G. a. holbrooki*) were recognized along the Gulf and Atlantic Coasts of the United States. Based on electrophoretic analysis, subsequent investigators recognized *G. holbrooki* and *G. affinis* as separate species. Populations in drainages west of Mobile Bay were considered *G. affinis*, and those east of this divide being *G. holbrooki.* The only mosquitofish population in West Virginia is found in the Meadow River wetlands (1392 ha). This wetland complex is located at the western end of Greenbrier County in the Allegheny Mountain Province. Heart, skeletal muscle, and brain tissues, malate dehydrogenase isozyme patterns, along with morphometric characteristics, (e.g. dorsal and fin rays, etc.), were used to determine that the West Virginia population was *G. affinis.* The average PI value for the West Virginia and Kentucky populations was 1.69 and the average PI value for the Virginia population was 2.19. Females from the Meadow River wetlands became sexually mature at 32 mm in length. The population of mosquitofish in the wetlands began its reproductive period on May 24 and ended between September 7 and September 29, which is a period of approximately 15 weeks. During this time, a total of 5 broods were liberated. The smallest mature male was 22 mm long (range 22-38 mm) and the smallest mature female was 32 mm long (range 32-51 mm). The number of individuals in each brood was dependent on the total

length of the female. The relationship between the total length of the female and the number of embryos was shown by the equation $Y=-40.46+(2.009X)$ and $r=0.648$ which is significant at the 0.05 confidence limit. The percentage of females that were gravid throughout the summer was dependent on the water temperature (21-30° C).

Introduction

The name *Gambusia* is derived from the Cuban term gambusino signifying "nothing" (Jenkins, 1994). So when one fishes for gambusino one fishes for nothing. To a Cuban fisherman, trying to feed his family or make a living, to catch *Gambusia* really is to catch nothing of importance. However, to a biologist, *Gambusia*, the mosquitofish, is a very interesting specimen to study because of its relatively unique reproductive life history.

Mosquitofish are members of the live bearing family *Poeciliidae* (Jenkins,1994). They are ovoviviparous, which gives embryos the advantage of developing within the safety of the mother's body and using yolk as nourishment until birth. *Gambusia* is the only live bearing fish in many states such as Kentucky, Missouri, and West Virginia. One interesting reproductive feature that mosquitofish possess is the gonopodium. This is a specialized anal fin on males which allows internal fertilization of the females.

Mosquitofish have been introduced into many lakes and ponds as a natural control of mosquito populations. In many cases these introductions were an attempt to control malaria. During World War II, allied forces introduced mosquitofish to various islands in the South Pacific, in an attempt to control the disease (Krumholz, 1948).

Prior to 1988, two subspecies of *Gambusia affinis* (*G. a. affinis* and

G. a. holbrooki) were recognized along the Gulf and Atlantic Coasts of the United States. The western subspecies (*G. a. affinis*) was found from Alabama, throughout the Mississippi drainage, into Texas and eastern Mexico (Revis, 1963). The eastern subspecies (*G. a. holbrooki*) ranges from central Alabama east into Florida and along the Atlantic coast drainage to New Jersey (Revis, 1963). Wooten et al. (1988) resolved 13 polymorphic loci in *Gambusia* populations from 76 locations in 19 drainages in the southeastern United States. Populations from eastern drainage had significantly higher levels of heterozygosity (H=0.113) than those in western drainage (H=0.055). Black and Howell (1979) showed that females from all populations of *G. a. affinis* possessed a large heteromorphic sex chromosome pair (WZ) , while those of *G. a. holbrooki* lacked WZ chromosomes. No males of either subspecies had heteromorphic sex chromosome pairs. Based on the above studies and morphological differentiations, Wooten et al. (1988) recognized *G. holbrooki* and *G. affinis* as separate species. Populations in drainage west of Mobile Bay were considered *G. affinis*, and those east of this divide being *G. holbrooki*.

The major objectives of this investigation were: (1) to elucidate the reproductive biology of the *Gambusia* population in the Meadow River wetlands, and (2) to compare meristic and electrophoretic characters among the populations of mosquitofish in the Meadow River wetlands with two populations of mosquitofish, one from the Atlantic coast drainage (Virginia), *Gambusia holbrooki*, and one from the Mississippi River drainage (Kentucky), *Gambusia affinis*. This comparison was conducted to determine if the population in West Virginia is *Gambusia affinis* or *Gambusia holbrooki*. During this study the following reproductive, meristic, and electrophoretic parameters were examined:

Reproductive Biology

The different aspects of the reproductive biology were: (1) length of the reproductive period in males and females, (2) size and age of males and females at first maturity, (3) number of broods to which females give birth during one season, and (4) number of individuals in each brood.

Meristics

In their original taxonomic designation that separated *G. affinis affinis* from *G. affinis holbrooki,* Wooten et al. (1988) used some distinguishing morphological characteristics. These researchers reported that *G. affinis* has 7 dorsal fin rays and 9 anal fin rays while *G. holbrooki* has 8 dorsal fin rays and 10 anal fin rays. They also noted that the *G. affinis* has none. Meristics were used to help determine the correct taxonomic designation of the West Virginia population of mosquitofish.

Electrophoretic Comparison

Using electrophoretic technique, proteins are separated by electrical charges. In this thesis, isoelectric focusing (IEF) techniques, which allow researchers to separate isozymes, was used. Isozymes are functionally similar forms of enzymes, including all polymers of subunits produced by different gene loci or by different alleles at the same locus (Markert and Moller, 1959). They have different charges and they also differ in pH. This allows them to be separated using IEF systems. Wooten et al. (1988) reported that many different isozymes, such as malate dehydrogenase and glycerol-3-phosphate dehydrogenase, showed differences between the two species. The isozyme malate dehydrogenase was the isozyme used in this research to distinguish between *G. affinis* and *G. holbrooki.*

Description of Study Area

The Meadow River wetlands is found in the western tip of Greenbrier County, which is part of the Allegheny physiographic region in West Virginia. The Meadow River wetlands is the second largest wetland complex within the state of West Virginia comprising approximately 1392 ha of swamp and wet meadow (Brant, 1988). The elevation of the wetland complex is approximately 2400 feet (732 m).

The most important geological formation to the Meadow River wetlands complex is the Mauch Chunk series from the upper division of the Mississippian in West Virginia (Brant, 1988). The Hinton group of the Mauch Chunk series is a limestone, sandstone, and shale complex that controls the mineralogy of the wetland. This formation is important because all springs that feed the complex come from the Hinton Group of the Mauch Chunk series.

Mosquitofish were collected from two sites. The first is located on Tommy Hall Road, which is approximately 15 kilometers east on US RT. 60 from the Sam Black Church exit on I-64. This is a swamp-like area with submerged trees and aquatic vegetation, which creates an ideal habitat for mosquitofish. The main channel of the Meadow River is adjacent to this collection area.

The second collection area is accessible from Rt. 3, 1 kilometer east from the Sam Black Church exit. This collection site can be characterized as a small pond with heavy aquatic vegetation and massive algal blooms in the summer. This pond is located beside a larger, man-made pond, which is fed by a drainage ditch adjacent to Rt.. 3. The second collection area produced the best results because a large number of fish were confined to a small area.

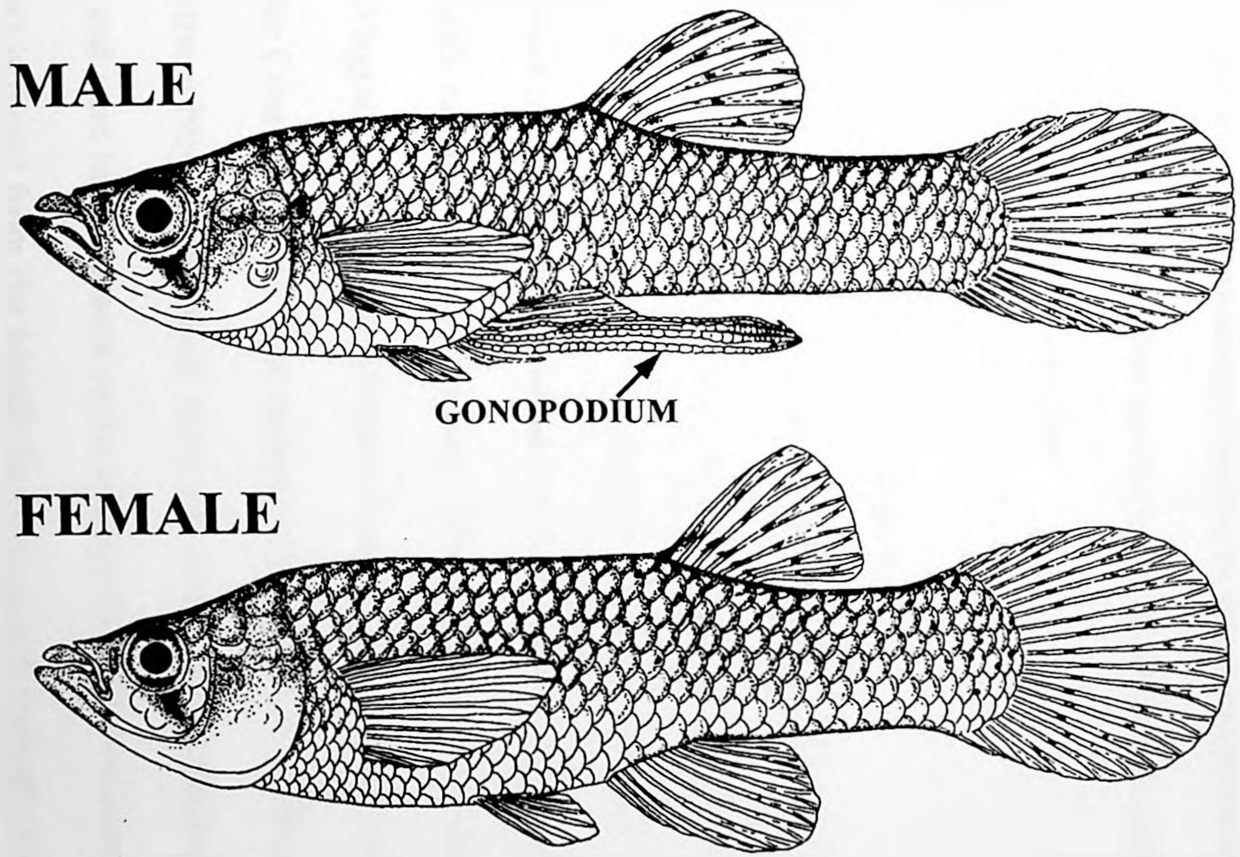
Generic Description

Gambusia, like all live bearing fish, are relatively small (Fig. 1). Adult females are usually larger than adult males averaging 20-45mm total length. Adult males typically average between 22-33mm total length. *Gambusia* is a top minnow characterized by a supraterminal mouth, posteriorly placed dorsal fin, large cycloid scales, and a dorsoventrally flattened head. Adults are typically olive in color with round, speckled fins. Usually a V-shaped dusky bar is present below the eyes. The adults are sexually dimorphic, males having a specialized anal fin, the gonopodium, which functions in sperm transfer.

Habitat

The typical habitat for mosquitofish is any slow moving or still body of water. Backwaters and adjacent oxbows of warm sluggish, lowland streams are some of the ideal places to find mosquitofish (Pflieger, 1975). Mosquitofish are also found in swamps, ponds and even roadside ditches. They can also be found along the shorelines of pond, lakes, and reservoirs (Jenkins and Burkhead, 1994). Mosquitofish were most abundant where the water is clear and aquatic vegetation or other cover are available. Also, adults prefer the highest temperature that they can find below 33° C (Jenkins and Burkhead, 1994).

Figure 1. Male and female mosquitofish.


MALE
GONOPODIUM
FEMALE

Although mosquitofish prefer these conditions, they can tolerate very inhospitable environments. For example, they have been found in warm and sulphur springs, muddy and polluted water, rather acidic to alkaline conditions, and even near anaerobic conditions where dissolved oxygen is less than 1mg/L (Jenkins, 1948). One species has been known to acclimate to full seawater and higher salt concentrations.

Distribution

This project deals with two species of mosquitofish, *Gambusia affinis* and *Gambusia holbrooki.* The Mobile Bay area, is a zone of semispecific integration between the two species, and is considered the divide between the two species. Mosquitofish to the east of this divide are identified as *G. holbrooki,* and the mosquitofish ranging west are *G. affinis.* The only known population to occur in West Virginia occurs in the Meadow River wetlands in the western tip of Greenbrier County (Fig. 2). The eastern mosquitofish (*G. holbrooki*) ranges from southern New Jersey, south along the Atlantic coast drainage into Florida, and west into Alabama to the Mobile Bay. The western mosquitofish (*G. affinis*) is found from the Mobile Bay area, north through the Mississippi River Valley into some of the Great Lake states, such as Illinois and Indiana. Its range also extends west into Texas and eastern Mexico (Fig. 3).

Figure 2. Distribution of mosquitofish in West Virginia.

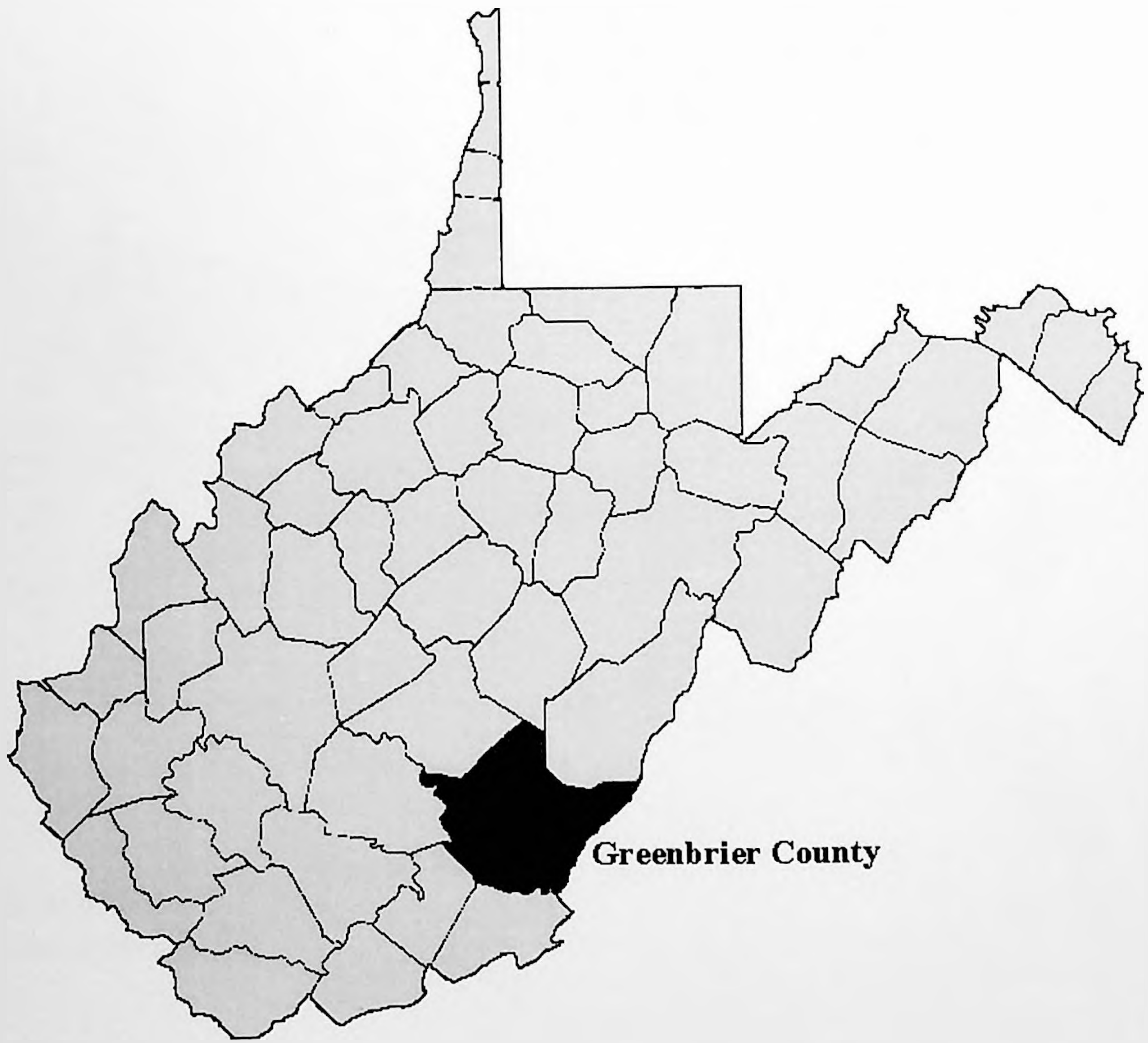
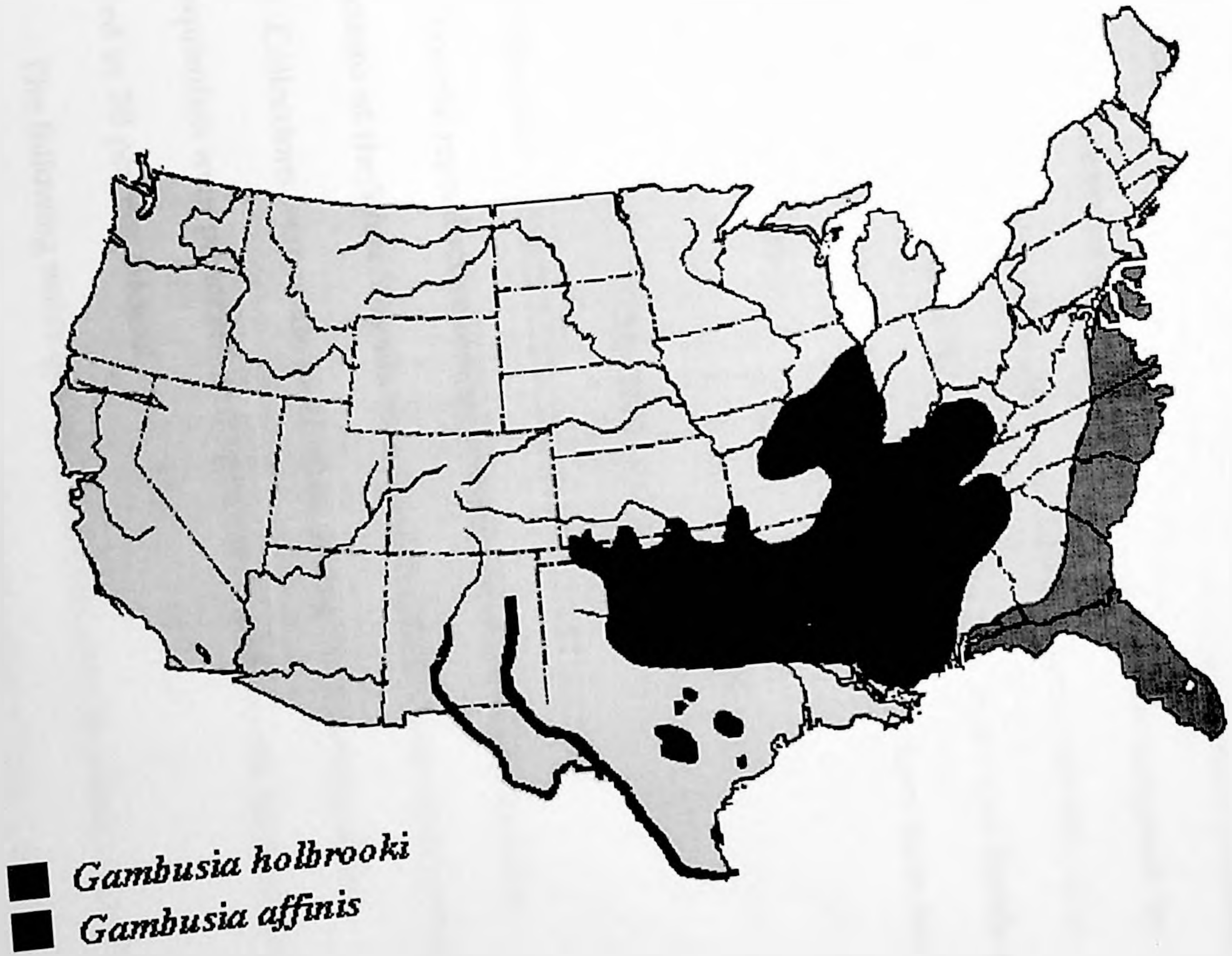

Greenbrier County

Figure 3. Distribution of *Gambusia affinis* and *Gambusia affinis* in the United States.


Gambusia holbrooki
Gambusia affinis

Taxonomy

Mosquitofish are members of the live bearing family Poeciliidae which includes about 45 species in temperate and tropical America (Jenkins, 1994). After the introduction of American mosquitofish into Europe, some confusion arose about the systematics of the fish. Two subspecies were designated by Lindberg (1934), *Gambusia affinis affinis* and *Gambusia affinis holbrooki*. *G. a. holbrooki* ranges along the east coast drainages from New Jersey into Florida and Alabama, while *G. a. affinis* is found west of the Mississippi River basin into Texas and eastern Mexico.

Materials and Methods

Field Collections

For the reproductive biology component of the study, monthly collections of the West Virginia mosquitofish (20-30) were made with dip nets. Collections were made more often during the spawning season. Mosquitofish were preserved in 10 percent formalin in the field and later stored in 70 percent ethanol.

The following water quality parameters were measured in the field: dissolved oxygen (mg/L), pH, total alkalinity (mg/L $CaCO_3$), and carbon dioxide (mg/L). Temperatures (C°) were recorded with a maximum-minimum thermometer.

Mosquitofish (30-40) were collected with dip nets from the following locations: (1) Meadow River wetlands, (2) Atlantic coast drainage (Lynchburg, Virginia), and (3) Gulf drainage (Kentucky). They were stored on dry ice and returned to the laboratory for electrophoretic analyses.

Electrophoresis

Skeletal muscle, heart, and brain tissue were excised from each fish and homogenized in Trizima buffer (pH 8.0). Extracts were centrifuged at 12,000 rpm for 25 minutes using the Sorvall superspeed RC2-B automatic refrigerated centrifuge. The supernate was transferred to a new centrifuge tube using a pipet and stored in a -80° C freezer until electrophoretic analysis.

Isozyme patterns of thirty individuals from each population were determined for malate dehydrogenase using the Pharmacia Phast System. Isozymes were separated in precast polyacrylamide gels with a pH gradient of 3-9. Gels were prefocused at 2000 Volts (V), 2.5 milliamps (mA), 3.5 Watts (W) at 15°C for 75 Volthours (Vh). Samples were applied to the gel while the voltage was less than 200 to avoid streaking. The proteins were focused for 410 Vh. Gels were then stained for one hour using a mixture of 5 ml of malate substrate (ph 8.0), 10 ml of tris buffer (pH 8.0), 35 ml distilled water, 15 mg nitro blue tetrazolium (NBT), 15 mg nicotinamide adenine dinucleotide (NAD), and 1 mg of phenazine methosulfate (PMS) (Fisheries Management, 1990).

Once the gels were fixed in a 5% glycerol solution for one hour and then allowed to dry, they were scanned using Adobe software and a Hewlett Packard scanner. Using this program, migration distances of the MDH-A allele were measured in pixels for each individual fish. Using an isoelectric protein standard and the migration distances, an isoelectric point (PI) value was calculated for each allele.

Reproductive Biology

The total length of each individual fish was measured to the nearest 0.01 mm using vernier calipers. Only females were dissected. The egg pouch was removed from each female and the eggs or the embryos were counted and measured. The eggs and the embryos (total length) were measured to the nearest 0.1 mm using a stage micrometer.

Meristics

For the meristic comparison, 15 males and 15 females from each of the three populations were examined. The anal fin rays of the females and the dorsal fin rays of the males and the females were counted with the aid of a dissecting microscope. To determine if teeth were present on the third anal ray of the gonopodium, the modified anal fin of the male was removed and observed under a compound microscope.

Results

Reproductive Biology

The number of gravid females from each collection is summarized in Table 1. From this table the size of females at first maturity can be determined. Size range of all gravid females is shown starting with the smallest gravid female captured (32 mm). Since the smallest gravid female collected was 32 mm, this indicates that the female mosquitofish in the Meadow River wetlands reached maturity at 32 mm in length.

The length of the female reproductive period can be ascertained from the measurements in Table 1. The first gravid females were collected on May 24 and the last gravid females were collected on September 7. This is a total of 15 weeks for the reproductive period of the females in the Meadow River wetlands.

Males tend to grow very little or stop growing all together once the gonopodium has fully developed. So for males, the gonopodium is used as an indicator of maturity. The sizes of males with mature gonopodia ranged from 22 to 38 mm. Since the smallest males captured with a mature gonopodium was 22 mm, the males in the Meadow River wetlands reached maturity at a

Table 1. Number of gravid females from each collection (n=total number of females collected).

Total Length (mm)	April 19 n=14	May 11 n=11	May 24 n=14	June 5 n=17	June 20 n=19	July 7 n=19	July 27 n=10	August 10 n=5	August 23 n=13	September 7 n=16	September 29 n=15	October 11 n=18
32	0	0	0	0	0	1	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	1	1	0	0	0	0	0	0
35	0	0	0	0	1	4	0	0	0	0	0	0
36	0	0	0	1	1	3	0	0	0	0	0	0
37	0	0	0	0	3	0	0	0	1	0	0	0
38	0	0	0	0	5	0	5	1	0	0	0	0
39	0	0	0	0	4	1	3	0	1	0	0	0
40	0	0	0	1	1	1	1	0	0	0	0	0
41	0	0	0	0	0	1	0	0	0	0	0	0
42	0	0	0	0	2	2	0	0	2	3	0	0
43	0	0	0	0	0	1	0	0	0	0	0	0
44	0	0	1	0	0	0	1	1	1	0	0	0
45	0	0	1	0	0	2	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	1	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	1	0	0	0	1	0	0
51	0	0	0	0	0	0	0	0	0	1	0	0
Total	0	0	2	2	18	19	10	2	5	5	0	0

length of 22 mm.

Krumholz (1948) reported that the average gestation period for mosquitofish is 24.9 days. As stated previously, in the Meadow River population, the first gravid female was collected on May 24 and the last on September 7 (Table 1). Also, there were many juveniles taken in the September 29 collection. Taking in consideration the gestation period of the September 7 collection, this interval of time indicated that the Meadow River mosquitofish had 5 broods during the 1996 reproductive period.

The number of individuals in each brood was dependent on the size of the female. The relationship between the total length of the females and the number of embryos was shown by the equation $Y=-40.46+(0.2009\ X)$, where X=total length and Y=number of embryos (Fig. 4). A coefficient of correlation ($r=0.648$) indicated a significant fit of the data (0.05 confidence limit) between the total length of the female and the number of individuals in each brood.

The reproductive process of the mosquitofish in the Meadow River wetlands was dependent on the water temperature. The percentage of gravid females was directly correlated to temperature (Fig. 5). Based on the early collections in April and early May, eggs became mature as the water temperature increased. Throughout the summer as water temperature

Figure 4. Linear regression analysis of total length and number of embryos in mosquitofish from the Meadow River wetlands.

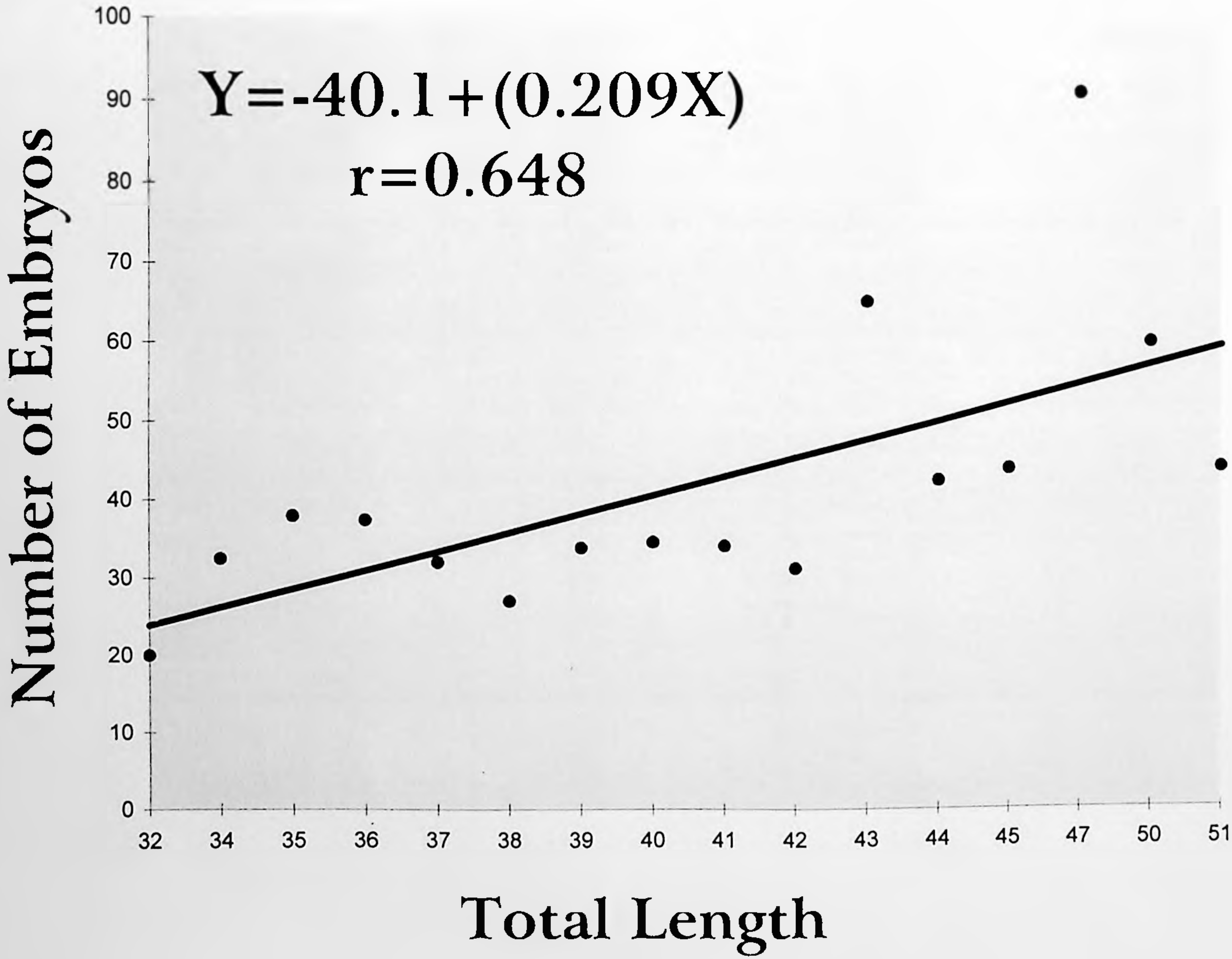
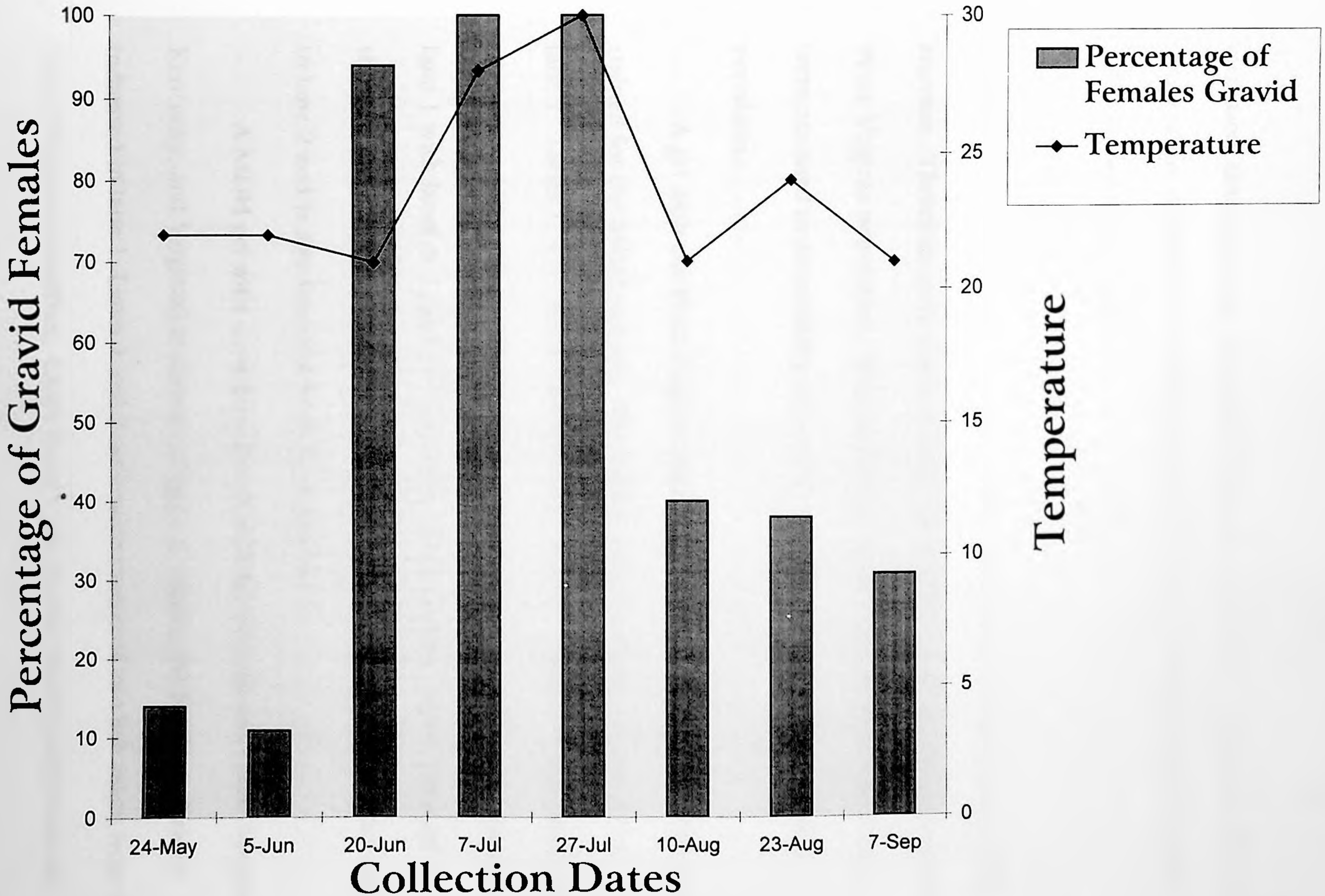

Y=-40.1+(0.209X)
r=0.648
Number of Embryos
100
90
80
70
60
50
40
30
20
10
0
32
34
35
36
37
38
39
40
41
42
43
44
45
47
50
51
Total Length

Figure 5. Collection dates, water temperature, and percentage of gravid females from the Meadow River wetlands.


Percentage of Gravid Females
Collection Dates
Temperature
Percentage of Females Gravid
Temperature
24-May
5-Jun
20-Jun
7-Jul
27-Jul
10-Aug
23-Aug
7-Sep
0
10
20
30
40
50
60
70
80
90
100
0
5
10
15
20
25
30

increased, the percentage of gravid females also increased. In August, when the temperature dropped 9°C in only two weeks, the percentage of gravid females also dropped dramatically.

Electrophoresis

Isozymes from muscle tissues resolved well when stained for the MDH enzyme. Therefore, only isozymes from muscle tissue was used to speciate the West Virginia population. Brain and heart tissues did not resolve well and were not used in determining the species designation of the West Virginia population.

A gel with the West Virginia population is shown in figure 6. This gel is stained for the MDH enzyme. The MDH-A muscle allele is indicated in lane 1. Lanes 1, 4, 7, and 10 are all muscle tissues. Figure 7 is a gel MDH gel of the Kentucky population. Again, the MDH-A muscle allele is indicated in lane 1 with lanes 4, 7, and 10 also being muscle tissues. Figure 8 is a gel showing the Virginia population. The MDH-A allele on this gel appears first in lane 2 and is also found in lanes 5, 8, and 11.

A MDH gel with a combination of all three populations (West Virginia, Kentucky, and Virginia) is shown in Figure 9. Again, the MDH-A allele is indicated in lane 1. Lanes 1 and 3 are muscle tissues of two fish taken from the West Virginia population. Lanes 5 and 7 are muscle tissues taken from the

Figure 6. Isoelectric gel of the West Virginia population stained for the MDH enzyme.

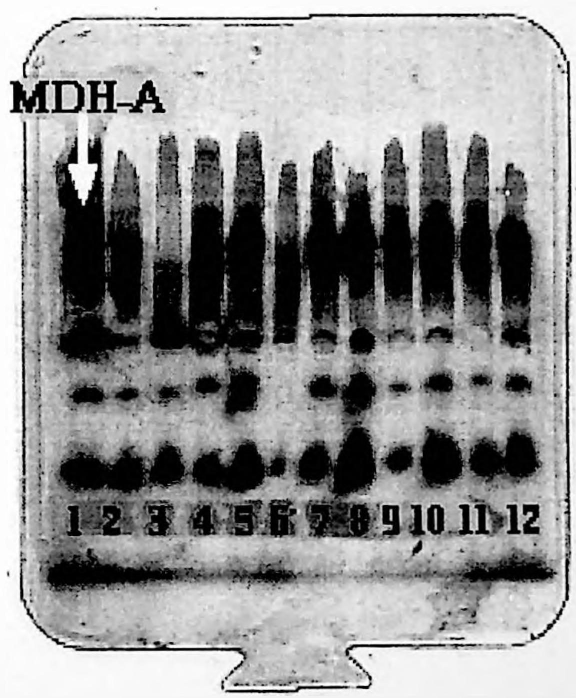

MDH-A
1 2 3 4 5 6 7 8 9 10 11 12

Figure 7. Isoelectric gel of the Kentucky population stained for the MDH enzyme.

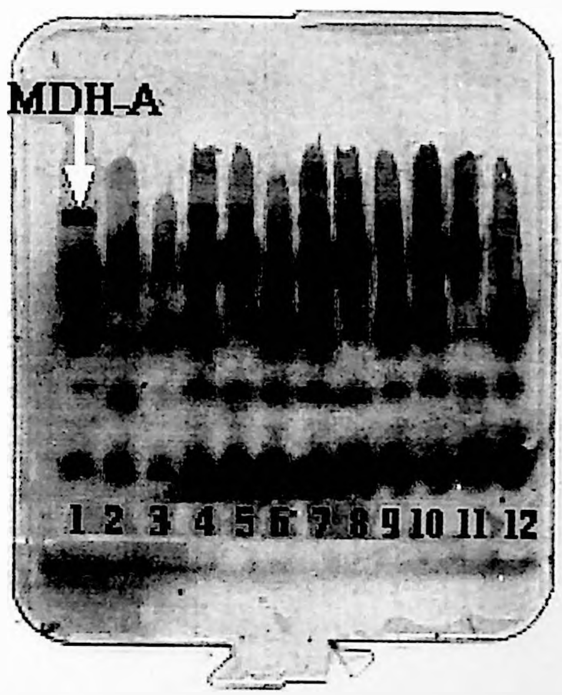

MDH-A
1 2 3 4 5 6 7 8 9 10 11 12

Figure 8. Isoelectric gel of the Virginia population stained for the MDH enzyme.

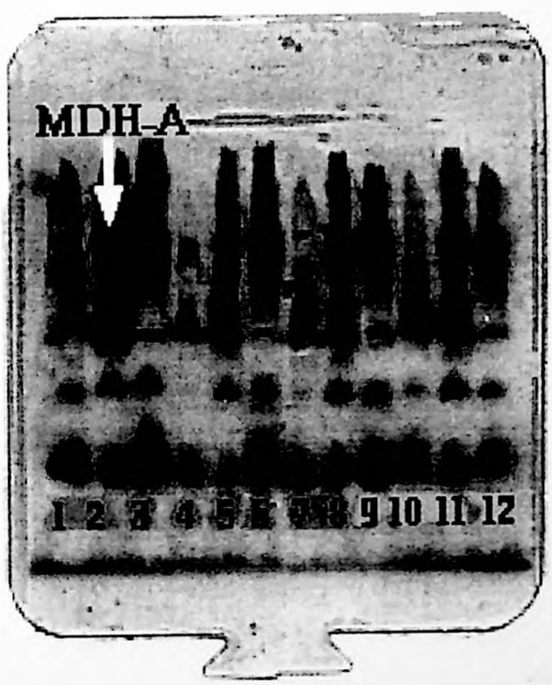
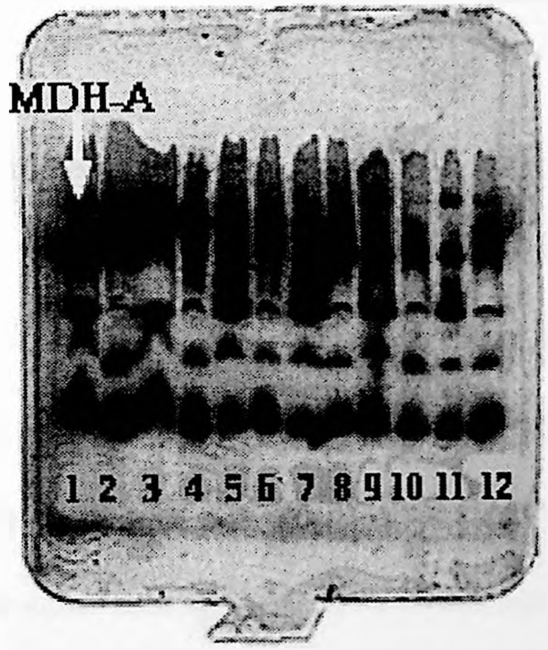

MDH-A
1 2 3 4 5 6 7 8 9 10 11 12

Figure 9. Isoelectric gel of the West Virginia, Kentucky, and Virginia populations stained for the MDH enzyme.


MDH-A
1 2 3 4 5 6 7 8 9 10 11 12

Virginia population and lanes 9 and 11 are muscle tissues taken from two fish from the Kentucky population. The PI values for each of these MDH-A muscle alleles are given in Figure 10. The MDH-A allele could be resolved for thirteen fish from each population. The average PI value for each the West Virginia and Kentucky populations was 1.7 and the average PI value for the Virginia population was 2.2. These data indicate that the Kentucky and the West Virginia populations are the same species of *Gambusia*.

Meristics

The meristic analysis performed on 30 fish, 15 females and 15 males, from the West Virginia, Kentucky, and Virginia populations is shown in Table 2. All thirty fish in both the West Virginia and Kentucky populations had dorsal fin ray counts of 7, while all 30 fish from the Virginia population had 8 dorsal rays. All of the 15 females from the West Virginia and Kentucky populations were the same having 9 anal rays, while the 15 females from the Virginia population had 10 anal rays. The 15 males from the West Virginia and Kentucky populations had no teeth present on the third anal ray of the gonopodium; however, in all 15 males from the Virginia population, teeth were found on the third anal ray of the gonopodium.

Figure 10. PI values for the MDH-A allele from the West Virginia, Kentucky, and Virginia populations.

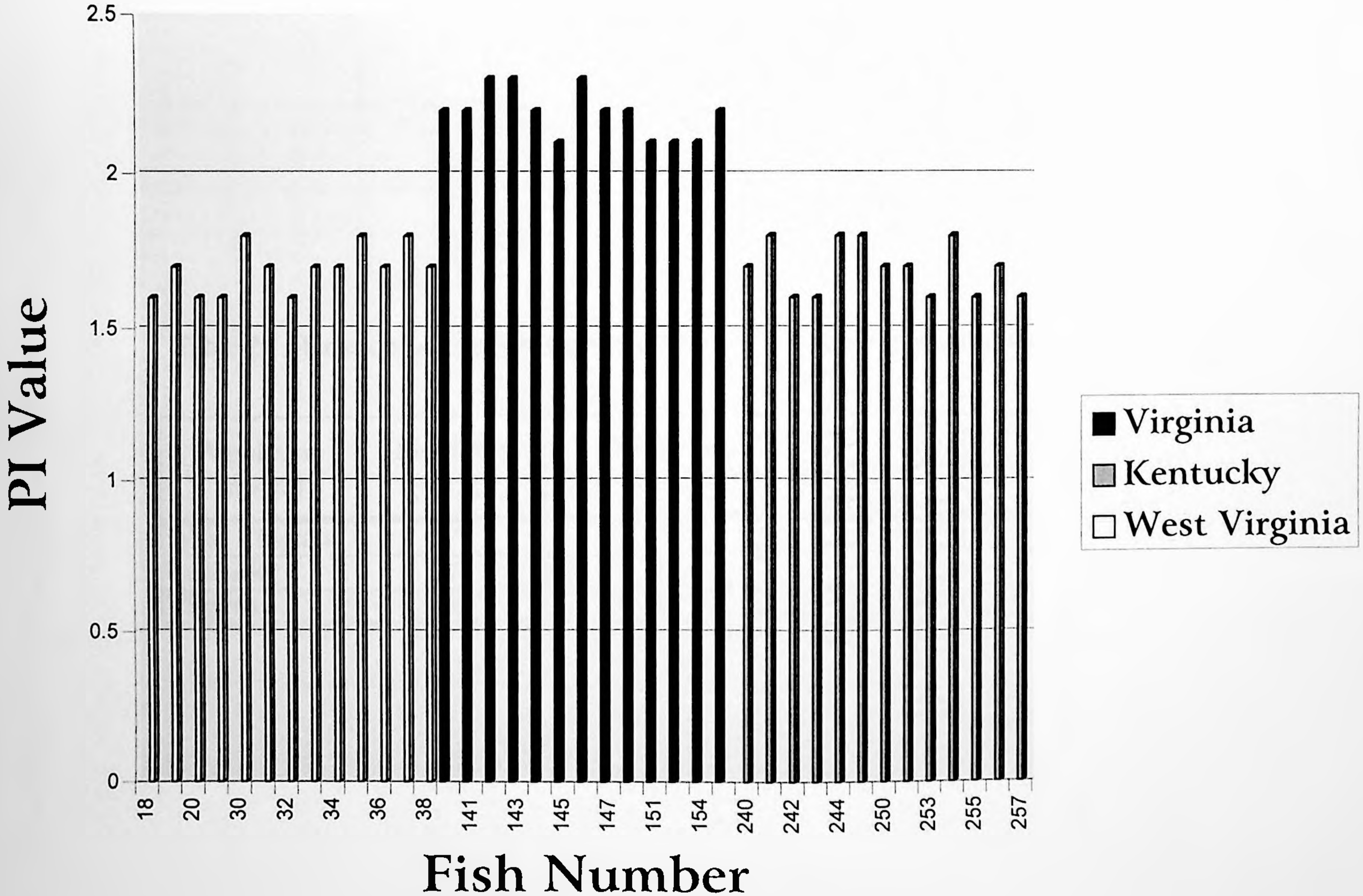

PI Value
2.5
2
1.5
1
0.5
0
18
20
30
32
34
36
38
141
143
145
147
151
154
240
242
244
250
253
255
257
Fish Number
Virginia
Kentucky
West Virginia

Table 2. Meristic study comparing three different populations of mosquitofish.

Population (n=30)	Dorsal Rays (n=30)	Anal Rays (Females: n=15)	Teeth Present on 3rd Ray of Gonopodium (Males: n=15)
West Viriginia	7	9	No
Kentucky	7	9	No
Virginia	8	10	Yes

Discussion and Conclusions

Reproductive Biology

The females from the Meadow River wetlands reached maturity at a minimum 32 mm in length. Krumholz (1948) studied three different populations of mosquitofish in Illinois and noted females reached maturity at 31 mm, 26 mm, and 24 mm.

The population of mosquitofish in the Meadow River wetlands began its reproductive period on May 24 and ended in late September, which is a period of 15 weeks. Again, this is very similar to Krumholz (1948), who stated that the populations of mosquitofish in Illinois has a reproductive period of 14-15 weeks.

Krumholz (1948) reported that the smallest mature male from the three populations that he studied in Illinois was 21 mm long. This was almost the exact same size of the smallest mature male (22 mm) that was taken from the Meadow River wetlands.

The Meadow River population had 5 broods through the 1996 reproductive period, which, again, was similar to the populations studied by Krumholz (1948). It should be noted that not all females liberate 5 broods. Females that mature during their first summer of life liberate only 2-3 broods, while the females that do not mature until their second summer enjoy a longer reproductive period and therefore produce possibly 4-5 broods (Krumholz, 1948).

The number of individuals in each brood was dependent on the total length of the females in the Meadow River population. Again, Krumholz (1948) reported the same phenomenon in the Illinois populations. This relationship was probably due to the physiological limitations of the mosquitofish; the smaller the fish the less room for a larger brood.

Electrophoresis

Wooten et al. (1988) stated that *G. affinis affinis* and *G. affinis holbrooki* should be considered two different species based on electrophoretic data. Differences were found between the two species using a number of different enzyme systems such as malate dehydrogenase and glycerol-3-phosphate dehydrogenase.

Malate dehydrogenase was used to determine if the *Gambusia* population in West Virginia was *G. affinis* (Kentucky) or *G. holbrooki* (Virginia). Mean PI values for West Virginia and the Kentucky populations were the same while the average PI value for the Virginia population was higher indicating that the West Virginia population is the same as the Kentucky population, *G. affinis*.

Meristics

Wooten et al. (1988) also used some distinguishing morphological characteristics in their original taxonomic designation separating *G. affinis affinis* from *G. affinis holbrooki.* These researchers reported that *G. affinis* has 7 dorsal fin rays and 9 anal fin rays while *G. holbrooki* has 8 dorsal fin rays and 10 anal fin rays. They also noted that *G. affinis* males do not have teeth on the third anal ray of the gonopodium and the *G. holbrooki* males have teeth present on the third anal ray of the gonopodium.

Counts were made from three populations, one from the Meadow River population (West Virginia), one from a known *G. holbrooki* population (Virginia), and one from a known *G. affinis* population (Kentucky). The results of the fin ray counts from the Virginia and Kentucky populations were consistent with the fin ray counts that Wooten et al. (1988). The fin ray counts from the unknown West Virginia population is the same as the Kentucky population, having 7 dorsal fin rays, 9 anal fin rays, and having no teeth present on the third anal ray of the gonopodium. Therefore, based on both electrophoretic and meristic data, the correct species designation for population of *Gambusia* in the Meadow River is *Gambusia affinis*.

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APPPENDIX

April 19, 1996

Fish No.	Total Length (mm)	Immature Eggs
1	37.2	162
2	33.05	58
3	31.56	28
4	36.85	75
5	27.55	53
6	33.1	70
7	34.2	68
8	23.35	37
9	32.13	98
10	25.37	27
11	32.3	91
12	29	89
13	28.8	52
14	27.95	63
15	26.5	MALE
16	26.3	MALE
17	30.1	MALE
18	23.65	MALE
19	29	MALE
20	27.05	MALE
21	27.28	MALE
22	27.1	MALE
23	24.83	MALE

May 11, 1996

Fish No.	Total Length (mm)	Immature Eggs	Mature Eggs
1	32.6	0	17
2	47.3	0	110
3	36.14	0	31
4	46.1	0	78
5	28.35	39	0
6	31.7	0	13
7	31.35	0	12
8	33.55	0	33
9	32.9	0	10
10	30.13	0	10
11	46.75	0	70
12	29.28	MALE	0
13	25.14	MALE	0
14	24.05	MALE	0
15	27.2	MALE	0
16	24.8	MALE	0
17	23.57	MALE	0
18	20.33	JUVENILES	0
19	22.55	JUVENILES	0
20	22	JUVENILES	0
21	24.18	JUVENILES	0
22	23.75	JUVENILES	0
23	23.55	JUVENILES	0
24	20.93	JUVENILES	0
25	21.53	JUVENILES	0
26	22.4	JUVENILES	0
27	21.15	JUVENILES	0
28	21.9	JUVENILES	0
29	18.7	JUVENILES	0
30	23.57	JUVENILES	0
31	24.1	JUVENILES	0
32	20.13	JUVENILES	0
33	19.95	JUVENILES	0
34	22.13	JUVENILES	0
35	23.97	JUVENILES	0
36	23.8	JUVENILES	0
37	18.07	JUVENILES	0

May 24, 1996

Fish No.	Total Length (mm)	Mature Eggs	Embryos
1	31.75	20	0
2	31.3	25	0
3	27	5	0
4	28.65	2	0
5	44.6	0	83
6	45.7	0	56
7	28.3	MALE	0
8	25.35	MALE	0
9	23.15	JUVENILE	0
10	25.5	JUVENILE	0
11	23.1	JUVENILE	0
12	22.5	JUVENILE	0
13	21.3	JUVENILE	0
14	24	JUVENILE	0
15	21.2	JUVENILE	0
16	25.35	JUVENILE	0
17	25.35	JUVENILE	0
18	24.7	JUVENILE	0
19	26.65	JUVENILE	0
20	27.35	JUVENILE	0
21	26.05	JUVENILE	0
22	26.25	JUVENILE	0
23	25.05	JUVENILE	0
24	26.35	JUVENILE	0
25	23.35	JUVENILE	0
26	23.3	JUVENILE	0
27	25.75	JUVENILE	0
28	25.5	JUVENILE	0
29	24.4	JUVENILE	0
30	24.05	JUVENILE	0
31	25.2	JUVENILE	0
32	24.85	JUVENILE	0

June 5, 1996

Fish No.	Total Length (mm)	Mature Eggs	Embryos
1	38.13	36	0
2	36.33	29	13
3	40.38	0	49
4	35	32	0
5	34.46	45	0
6	36.25	44	0
7	36.45	35	0
8	32.37	14	6
9	37.95	40	0
10	34.9	38	0
11	32.3	34	0
12	33.5	30	0
13	30.3	13	0
14	28.5	2	0
15	30.43	8	0
16	31.5	31	0
17	29.65	8	0
18	23.63	MALE	0
19	26.68	MALE	0
20	24.8	MALE	0
21	28.23	MALE	0
22	28.5	MALE	0
23	27.55	MALE	0
24	25.5	MALE	0
25	24.65	MALE	0
26	26.2	MALE	0
27	25.25	MALE	0
28	24.5	MALE	0
29	23.6	JUVENILE	0
30	22.7	JUVENILE	0
31	27.65	JUVENILE	0

June 20, 1996

Fish No.	Total Length (mm)	Mature Eggs	Embryos
1	49.53	104	0
2	37.9	0	26
3	38.4	0	37
4	39.7	0	43
5	37.13	0	39
6	42.33	0	46
7	39	0	49
8	39.63	0	55
9	38.8	0	41
10	39.77	13	32
11	42	0	38
12	40.9	16	35
13	38.95	0	46
14	38.45	0	40
15	35.43	49	0
16	36.95	0	26
17	37.9	0	51
18	38.43	0	40
19	34.77	0	33
20	25.55	MALE	0
21	34.07	MALE	0
22	28.65	MALE	0
23	26.55	MALE	0
24	26.33	MALE	0
25	24.57	MALE	0
26	23.3	MALE	0
27	26	MALE	0
28	27.15	MALE	0
29	24.6	MALE	0

July 7, 1996

Fish No.	Total Length (mm)	Mature Eggs	Embryos
1	45.15	0	65
2	50.05	0	108
3	45.78	0	11
4	47.85	0	91
5	35.43	0	46
6	43.65	0	65
7	36.13	0	46
8	42.68	0	49
9	36.68	0	41
10	42.2	0	65
11	38.1	0	34
12	40.25	38	0
13	34.15	0	32
14	35.63	0	32
15	39.88	33	0
16	32.7	0	20
17	41.35	0	34
18	35.9	0	36
19	36.33	0	37
20	28.75	MALE	0
21	28.73	JUVENILE	0
22	14.85	JUVENILE	0
23	16.6	JUVENILE	0
24	15.4	JUVENILE	0
25	18.05	JUVENILE	0
26	20.03	JUVENILE	0
27	17.83	JUVENILE	0

July 20, 1996

Fish No.	Total Length (mm)	Mature Eggs
1	27.68	JUVENILE
2	16.6	JUVENILE
3	21.4	JUVENILE
4	11.6	JUVENILE
5	18.4	JUVENILE
6	14.5	JUVENILE
7	20	JUVENILE
8	15.25	JUVENILE
9	15.13	JUVENILE
10	14.15	JUVENILE
11	20.7	JUVENILE
12	18.06	JUVENILE
13	18.63	JUVENILE
14	18.3	JUVENILE
15	16.48	JUVENILE
16	18.4	JUVENILE
17	19.1	JUVENILE
18	10	JUVENILE
19	14.63	JUVENILE
20	8.95	JUVENILE
21	17.5	JUVENILE
22	11.38	JUVENILE
23	17.1	JUVENILE
24	16.65	JUVENILE
25	18	JUVENILE
26	16.8	JUVENILE
27	11.3	JUVENILE
28	16.05	JUVENILE
29	15.8	JUVENILE
30	15.04	JUVENILE
31	16.7	JUVENILE
32	16.05	JUVENILE
33	13.3	JUVENILE
34	12.3	JUVENILE
35	22.45	JUVENILE
36	20.09	JUVENILE
37	23.58	JUVENILE

July 27, 1996

Fish No.	Total Length (mm)	Embryos
1	39.43	32
2	44.03	49
3	40.1	20
4	38.23	21
5	38.78	17
6	38.4	21
7	39.1	19
8	39.95	26
9	38.75	31
10	38.3	15
11	24.48	JUVENILE
12	20	JUVENILE
13	19.75	JUVENILE
14	14.98	JUVENILE
15	18	JUVENILE
16	20.15	JUVENILE
17	21.98	JUVENILE
18	14.2	JUVENILE
19	16.98	JUVENILE
20	18.06	JUVENILE
21	23.02	JUVENILE
22	19.95	JUVENILE
23	13.68	JUVENILE
24	17.3	JUVENILE
25	16	JUVENILE
26	15.63	JUVENILE
27	21	JUVENILE
28	15.28	JUVENILE
29	17.13	JUVENILE

August 10, 1996

Fish No.	Total Length (mm)	Mature Eggs	Embryos
1	42.58	20	0
2	44.5	0	16
3	38.4	0	8
4	29.3	20	0
5	38.1	MALE	MALE
6	30.34	MALE	MALE
7	31.14	MALE	MALE
8	26.37	MALE	MALE
9	26.57	MALE	MALE
10	25.9	MALE	MALE
11	19.2	JUVENILE	0
12	20.5	JUVENILE	0
13	19.13	JUVENILE	0
14	22.15	JUVENILE	0
15	21.65	JUVENILE	0
16	24.33	JUVENILE	0
17	21.58	JUVENILE	0
18	17.19	JUVENILE	0
19	10.11	JUVENILE	0
20	10.9	JUVENILE	0
21	16.1	JUVENILE	0
22	23.4	JUVENILE	0
23	20.65	JUVENILE	0
24	23	JUVENILE	0
25	15.7	JUVENILE	0
26	24.6	JUVENILE	0
27	23.98	JUVENILE	0
28	18.07	JUVENILE	0
29	20.63	JUVENILE	0
30	20.03	JUVENILE	0
31	20.75	JUVENILE	0

August 23, 1996

Fish No.	Total Length (mm)	Mature Eggs	Embryos
1	44.8	0	22
2	42.3	0	22
3	39.1	0	11
4	42.35	0	4
5	37.9	4	0
6	37.6	0	12
7	36.65	0	0
8	38.95	0	9
9	22.35	MALE	MALE
10	23.58	MALE	MALE
11	31.3	MALE	MALE
12	24.87	MALE	MALE
13	24.2	MALE	MALE
14	22.66	MALE	MALE
15	25.05	MALE	MALE
16	22.17	MALE	MALE
17	21.7	MALE	MALE
18	23.13	MALE	MALE
19	14.27	JUVENILE	0
20	19.53	JUVENILE	0
21	20.95	JUVENILE	0
22	18.4	JUVENILE	0
23	21.1	JUVENILE	0
24	21.2	JUVENILE	0
25	22.1	JUVENILE	0
26	21.5	JUVENILE	0
27	26.95	JUVENILE	0
28	26.86	JUVENILE	0
29	24.1	JUVENILE	0
30	24.98	JUVENILE	0
31	27.1	JUVENILE	0

September 7, 1996

Fish No.	Total Length (mm)	Mature Eggs	Embryos
1	49.3	1	0
2	51.4	0	44
3	42.85	0	16
4	50.05	0	12
5	42.35	0	0
6	42.45	0	9
7	23.15	0	0
8	25	0	0
9	25.09	0	0
10	25.53	0	0
11	26.62	0	0
12	27.5	0	0
13	29.3	0	0
14	29.65	0	0
15	30.4	0	0
16	30.46	0	0
17	31.1	0	0
18	30	MALE	0
19	24.75	MALE	0
20	26.75	MALE	0
21	26.74	MALE	0
22	24.1	MALE	0
23	32.8	MALE	0
24	25.87	MALE	0
25	30.79	MALE	0
26	25.05	MALE	0
27	26.07	MALE	0
28	30.78	MALE	0
29	25.25	MALE	0
30	21.75	MALE	0
31	24.15	MALE	0
32	24.3	MALE	0
33	13.52	JUVENILE	0
34	14.16	JUVENILE	0
35	15	JUVENILE	0
36	16.58	JUVENILE	0
37	16.92	JUVENILE	0

September 29, 1996

Fish No.	Total Length (mm)	Mature Eggs	Embryos
1	23.2	0	0
2	23.25	0	0
3	23.65	0	0
4	23.68	0	0
5	24.75	0	0
6	25.2	0	0
7	25.47	0	0
8	26.12	0	0
9	26.5	0	0
10	27.24	0	0
11	27.5	0	0
12	28.16	0	0
13	28.7	0	0
14	28.9	0	0
15	30.1	0	0
16	30.86	0	0
17	27.73	MALE	0
18	26.3	MALE	0
19	26.55	MALE	0
20	30.35	MALE	0
21	33.13	MALE	0
22	24.4	MALE	0
23	23.65	MALE	0
24	11.95	JUVENILE	0
25	16.93	JUVENILE	0
26	20.57	JUVENILE	0
27	22	JUVENILE	0

October 11, 1996

Fish No.	Total Length (mm)	Mature Eggs
1	43.7	0
2	46.05	0
3	34.93	0
4	31.4	0
5	31.95	0
6	31.13	0
7	30.55	0
8	34.6	0
9	25.6	0
10	30.37	0
11	24.3	0
12	23.5	0
13	22	0
14	23.54	0
15	23.38	0
16	25.65	0
17	24.45	0
18	25.85	0
19	27.6	MALE
20	24.63	MALE
21	23.81	MALE
22	25.1	MALE
23	33.1	MALE
24	27.2	MALE