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ANTIPREDATIVE FUNCTION OF THE GELATINOUS COATING OF THE EGGS OF THE FROG, <u>RANA PIPIENS</u>

ΒY

WALTER STANLEY JENNINGS, JR.

A THESIS

SUBMITTED TO THE GRADUATE FACULTY OF THE UNIVERSITY OF RICHMOND IN CANDIDACY FOR THE DEGREE OF

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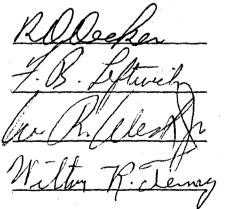
ANTIPREDATIVE FUNCTION OF THE GELATINOUS COATING OF THE EGGS OF THE FROG, <u>RANA PIPIENS</u>

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1

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ABSTRACT

The eggs of Rana pipiens, the southern leopard frog, were offered in several forms to Lepomis macrochirus, the bluegill. The fish were offered random trial discrimination tests while in groups of one or five in 40 l aquaria. L. macrochirus learned to make distinct discriminations and rejected fertilized and unfertilized egg masses, boiled egg masses, dried frog eggs and gelatin made with homogenated frog eggs. In contrast, Shrimp-el-etts, Shrimp-el-etts dyed black, gelatin made with peptone, and ovarian eggs were accepted readily. The results suggest a protective function for the gelatinous coat: both physical and chemical. The chemical is evidently added in the oviduct with the gelatinous coat, and is not affected by fertilization, desiccation or temperatures up to 100 C. Comparisons of these experiments with other studies suggest that the gelatinous coat is even more effective in discouraging predation by vertebrates in the field than in the laboratory.

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INTRODUCTION

This study was undertaken to elucidate the characteristics of eggs of <u>Rana pipiens</u> that discourage predation by fishes.

The aquatic egg masses of amphibians, which are often found in the shallow water near the shore of lakes or streams, would appear to be particularly vulnerable to predation from both aquatic and terrestrial animals. However, studies of amphibian egg mortality have not shown this to be the case. Anderson et al (1971) reported that most of the embryonic mortality of <u>Ambystoma</u> <u>tigrinum</u> in a New Jersey pond did not occur in the egg, but in the larval stage. Similarly, in a study of survival rates of the different life stages of <u>R</u>. <u>aurora</u> and <u>R</u>. <u>pretiosa</u> in British Columbia (Licht, 1974), the tadpoles were much more susceptible to predation than were the eggs.

Several studies have involved offering eggs of ranid species to potential predators. Licht (1969) offered the ovarian eggs of four ranid and one hylid species to the larvae of the northwestern salamander (<u>Ambystoma gracile</u>), the three spined stickleback (<u>Gasterosteus aculeatus</u>) and the cutthroat trout (<u>Salmo clarkii</u>). Ovarian eggs of all species were consumed freely by all predators. Walters (1975) offered the fertilized eggs and larvae of four ranid and one hylid species to the

larvae of three species of salamanders and found that a smaller number of ranid eggs were eaten per predator per day than either ranid or hylid tadpoles or hylid eggs. Most recently, Werschkul and Christensen (1977) offered the eggs and tadpoles of <u>Rana sphenocephala</u> and <u>Rana areolata</u> to the bluegill, <u>Lepomis macrochirus</u>, and found that the presence of the gelatinous coating was probably an important factor in discouraging predation.

There is ample evidence from the literature that the eggs of toads, genus <u>Bufo</u>, are toxic to vertebrates. Toxicity from injection (Licht, 1968) and from ingestion (Licht, 1967; Licht, 1968; Licht, 1969) of toad eggs have been documented. Toad eggs display a toxicity similar to that of adult tissues (Wright and Wright, 1949).

Except for the recent paper by Werschkul and Christensen (1977), a thorough review of the literature revealed no evidence of a protective function for the gelatinous coating of ranid eggs. This study was undertaken to determine if fishes will eat the eggs of <u>R</u>. <u>pipiens</u>, the northern leopard frog; if not, what qualities of the eggs make them unpalatable. <u>Lepomis macrochirus</u> was chosen as the experimental predator because of its ubiquitous feeding habits in fresh water (Flemer and Woolcott, 1966; Sadzikowski and Wallace, 1976) and its ability to adjust to aquarium life.

MATERIALS AND METHODS

Lepomis macrochirus were collected from Herring Creek in Charles City County, Virginia, and Westhampton Lake in Richmond, Virginia. The fish were kept in 40 l and 80 l aquaria, in water temperature ranging from 17 to 19 C. Adult female <u>Rana pipiens</u> were obtained from Mogul-Ed and caused to ovulate by the method of Rugh (1952). Frog eggs were fertilized according to the method of Hacker (1968). Fertilized eggs were used within three days, unfertilized ones were either used immediately or stored at 5 C until utilized. If eggs began to dissociate from the mass or their gelatinous coat became cloudy, they were discarded. The following eight experiments were performed:

Experiment I -- The objective of this experiment was to determine the palatability of frog eggs in several forms and different mass sizes. Thirty six <u>L</u>. <u>macrochirus</u> (1.5 - 7 cm s.l.) were allowed to acclimate to the aquarium environment until they would accept either pellets or flakes of commercial fish food. Acclimation time varied from two to eight weeks, the largest animals took longest. The experiment was performed with one <u>L</u>. <u>macrochirus</u> in a 4 1 tank or five <u>L</u>. <u>macrochirus</u> in a 40 1 tank. Immediately preceeding each series of trials a food pellet was dropped into the experimental tank. The experiment was continued only if the pellet was

consumed before it reached the bottom. The following types of food were offered:

- Shrimp-el-etts -- small pellets of commercial fish food of approximately the same size as the frog eggs to be used.
- Black pellets -- Shrimp-el-etts dyed black with food coloring to more closely resemble the frog eggs.
- 3) Tetra-min staple fish food -- a flaky, floating fish food that superficially resembles dried frog eggs.
- 4) Fertilized frog eggs -- <u>R</u>. <u>pipiens</u> eggs expressed from a gravid female and then fertilized.
- 5) Unfertilized frog eggs -- <u>R</u>. <u>pipiens</u> eggs expressed from a gravid female and either kept at room temperature and used within 48 hours or refrigerated at 5 C until needed.
- 6) Dried frog eggs -- unfertilized frog eggs which were placed in a drying chamber at 45 C until dry and then cut into flakes of varying sizes.

The foods were administered at the surface of the water with a pair of forceps. Each trial lasted 20 minutes or until the food was consumed. If frog eggs were used, the number of eggs in the mass was recorded. Responses to foods were recorded in three categories as follows: Orientation toward food - fish appears to be aware of food and orients its body toward it; does not attempt to feed.

Bite - fish attempts to ingest food; either does not or regurgitates within 30 seconds.

Food consumption - fish ingests food; does not regurgitate within 30 seconds.

If the fish did not make any observable responses to the stimulus within 20 minutes, the trial was disregarded. During all 489 trials, fish were continously monitered for signs of distress, e.g. listing, hyperactivity or hypoactivity.

Experiment II -- This experiment was designed to illustrate changes in the behavior (i.e. learning) of L. macrochirus with previous exposure to eggs for short periods of time. Ten L. macrochirus were divided into equal groups matched according to relative size and placed in two 40 l tanks. Foods were offered the two groups in such a way as to control for the decrease in feeding behavior as a result of hunger satiation. 0n day one, tank one received dried frog eggs until they were refused for three consecutive trials. Then pellets of fish food were offered until they were refused. Tank two received only pellets. On days two and three, both tanks received first dried eggs until they were refused, then pellets until they were refused. Numbers of eggs

and pellets were recorded and behaviors were subjectively ranked as in Experiment I.

<u>Experiment III</u> -- The character and strength of the possible learned avoidance was further tested. Five fish (4 - 6cm s.l.) in a 40 l tank were allowed to respond to 36 random visual discriminations. Tetra-min staple fish food or dried frog eggs were held with forceps 1 cm above the surface of the water for 30 seconds. The only response recorded was whether a fish touched or bit the potential food in the forceps.

Experiment IV -- An attempt was made to simulate frog eggs. A solution was prepared as follows:

1g Knox gelatin

1.5g bacto-peptone

40ml water at 45 C

green and red food coloring added in equal amounts until the solution was black in appearance

This solution was then cooled until it gelled. A 50 trial discrimination test was then administered to five fish (4 - 6cm s.l.) in each of two 40 l tanks. Unfertilized frog eggs, dried frog eggs and small pieces of the black gelatin were offered in random sequences. The fish were allowed 10 minutes to respond in each trial and the responses were subjectively ranked as in Experiment I.

Experiment V -- This experiment was designed to minimize

the effects of the physical barrier that is presented by the gelatinous coating. Unfertilized frog egg masses were homogenized for 30 minutes in a blender with 20ml water. Equal amounts of green and red food coloring were added until one drop of the solution was opaque. One gram of gelatin was added, the solution was warmed to 45 C and then allowed to gel. Black gelatin with peptone was also prepared (see Experiment III). A 50 trial discrimination test was then administered to five fish (4 - 6cm s.l.) in a 40 l tank. Gelatin with peptone and gelatin with frog egg homogenate were offered in a random sequence. The fish were allowed 10 minutes to respond in each trial, and the responses were subjectively ranked as in Experiment I.

<u>Experiment VI</u> -- This experiment was designed to assay possible toxicity in the frog egg by administration of stomach loads. Twenty four fish (3 - 6cm s.l.) were separated into two groups closely matched with respect to size of the fish. All fish were given two stomach loads spaced 24 hours apart, and the groups were kept in separate tanks. The experimental group received homogenized unfertilized frog eggs. The control group received homogenized black gelatin as described in Experiment III. Stomach loads were administered with a 20ml syringe through a size 8 French Infant feeding tube. The amount of fluid per stomach load for each fish was determined

from a previously prepared nomograph (standard length versus stomach load which had been arrived at empirically). Thirty minutes after the injection, any residue of regurgitated material was collected from each tank and its volume recorded. Fish were observed for two hours and thereafter daily for signs of distress. Experiment VII -- Whether an unpalatable substance was contained in the entire egg or only in the gelatinous coat was tested in this experiment. Adult female R. pipiens were pithed and the ovaries were examined to determine whether eggs were present. If yolking had occurred, the ovaries were excised and refrigerated at 5 C. A 50 trial discrimination was then administered to five fish (4 - 6cm s.l.) in a 40 l tank. Ovarian eggs (no gelatinous coating) and unfertilized frog eggs were offered in random sequence. The fish were allowed 10 minutes to respond in each trial and the responses were subjectively ranked as in Experiment I. Experiment VIII -- This experiment was designed to show whether the unpalatable substance was a heat labile protein. Seventeen milliliters of unfertilized frog eggs were boiled for 10 minutes in 17 ml water. A 50 trial discrimination test was then administered to 10 fish in two 40 l tanks. Normal unfertilized frog eggs and boiled frog eggs were offered in random sequence. The fish were allowed 10 minutes to respond in each trial

and the responses were subjectively ranked as in Experiment I.

RESULTS

Experiment I. The responses of <u>Lepomis macrochi</u>-<u>rus</u> to foods are shown in Table I. Fertilized and unfertilized frog eggs were eaten with the same relative frequency ($X^2 = 1.65$; P>0.4). Pellets and black pellets were also eaten with the same relative frequency ($X^2 = 1.42$; P>0.4). Dried frog eggs were eaten more often than fertilized and unfertilized eggs ($X^2 = 33.81$; P<0.001). Pellets and black pellets were eaten more often than dried eggs ($X^2 = 116.3$; P<0.001) or fertilized and unfertilized eggs ($X^2 = 289.6$; P<0.001).

The most complete feeding responses observed in each trial are shown in Table II. There was no significant difference between fertilized and unfertilized eggs ($X^2 = 3.85$; P>0.05) or between pellets and black pellets ($X^2 = 0.120$; P>0.7). Dried eggs were eaten more often than fertilized and unfertilized eggs ($X^2 =$ 19.48; P<0.001). Pellets and black pellets were eaten more often than dried eggs ($X^2 = 43.17$; P<0.001) or fertilized and unfertilized eggs ($X^2 = 118.30$; P<0.001).

The effect of the size of the frog egg mass on the ingestion is shown in Table III. As the size of the egg mass increases the percent of mass eaten decreases, and the average number of bites needed for ingestion of each mass increases. No masses with more than 20 eggs were ever eaten, in spite of the fact that a 20

egg mass is smaller in all dimensions than minnows that were easily ingested. Lepomis macrochirus that ingested the larger masses did so only with great difficulty and were much less likely to even try them. On the days following a series of trials using frog eggs, a number of eggs and numerous small pieces of the jelly usually were found on the bottom of the experimental tank. Approximately 50% of the eggs that had been ingested on the previous day could be accounted for in this manner. Fragmentation of jelly cases and the manner in which the tank was being filtered made it difficult to accurately measure the amounts regurgitated overnight. The frog eggs with jelly cases still intact appeared not to have been affected by the fishes' digestive processes, except that most were single eggs no longer in masses.

Experiment II. Evidence for learned avoidance of dried frog eggs by <u>L</u>. <u>macrochirus</u> is shown in Table IV. Lepomis macrochirus in tank I consumed 24 masses of dried frog eggs on day one, but none on days two or three. Those of tank II were not offered frog eggs on day one; however, they consumed ten masses on day two and none on day three. Roughly equal numbers of pellets were eaten by both groups and pellets were eaten on all three days in both tanks.

Experiment III. <u>Lepomis macrochirus</u> showed a marked preference for the flaked fish food over the

dried frog eggs when they were held over the tank $(X^2 = 36; P < 0.001)$. In all 21 trials in which flaked fish food was offered, the <u>L. macrochirus</u> broke the surface to take it from the forceps. In 15 trials with dried frog eggs the <u>L. macrochirus</u> never broke the surface or touched the eggs.

Experiment IV. <u>Lepomis macrochirus</u> displayed a marked preference for the black gelatin over the two forms of frog eggs (X^2 = 29.46; P<0.001) as shown in Table V. The black gelatin was eaten in 19 of 24 trials.

Experiment V. The responses of <u>L</u>. <u>macrochirus</u> to two gelatin mixtures are shown in Table VI. A clear preference was displayed ($X^2 = 33.44$; P<0.001). The black gelatin with peptone was eaten in 26 of 27 trials. The gelatin with frog egg homogenate was eaten in only four of 24 trials. The four trials in which the frog egg homogenate was eaten were the first four times in which it was presented.

Experiment VI. None of the 24 force fed <u>L</u>. <u>macro-</u> <u>chirus</u> evidenced any toxic effects as a result of the force feedings. One <u>L</u>. <u>macrochirus</u> in the control group died four days after the experiment began (possibly injured). During administration of the stomach loads it was observed that gelatin was accepted comfortably, but a comparable amount of homogenized frog egg mass backed up and began to exude from the mouth of the animal. When returned to the tanks, the <u>L</u>. <u>macrochirus</u> that received the homogenized frog egg masses appeared to regurgitate the bulk of their stomach load. Seventeen milliliters of frog egg homogenate were injected. Thirty minutes after the injections, 13.5 ml of frog egg homogenate was collected from the bottom of the tank. The amount of gelatin on the bottom of the control tank after 30 minutes was negligible.

Experiment VII. The responses of <u>L</u>. <u>macrochirus</u> to ovarian (no gelatinous coating) and unfertilized eggs are shown in Table VII. The fish displayed a marked preference ($X^2 = 42.15$; P<0.001) for ovarian eggs. Ovarian eggs were consumed voraciously in all 24 trials in which they were offered. Unfertilized frog egg masses were eaten in only one of 22 trials.

Experiment VIII. The <u>L</u>. <u>macrochirus</u> did not discriminate ($X^2 = 2.01$; P>0.3) between boiled and unboiled unfertilized frog eggs, as shown in Table VIII. Boiled unfertilized frog egg masses were eaten in one of 26 trials in which they were offered. Unfertilized frog egg masses were eaten in one of 24 trials.

DISCUSSION

That ovarian eggs were preferred to unfertilized egg masses can be explained by ascribing a protective function to the gelatinous covering of <u>Rana pipiens</u> egg masses. The protection provided by the gelatinous coat appears to be separable into two distinct categories: 1) a physical protection is suggested by the increased difficulty of ingestion of larger egg masses; 2) the avoidance of gelatin with frog egg homogenate is probably due to a chemical in the egg mass. This is supported also by the preference of Shrimp-eletts over dried frog eggs and that a significant percentage of the eggs ingested were regurgitated.

The gelatinous coat of the eggs of <u>R</u>. <u>pipiens</u>, an oviducal secretion, contains three microscopically distinguishable layers (Pereda, 1970a), which can be further divided by cytochemical analysis into five or six layers (Steinke and Benson, 1970). Using radioactive tracers, Pereda (1970b) found that the three gelatinous layers were produced by different and specific regions of the oviduct.

The gelatinous oviducal secretions have undergone biochemical and immunological analysis (Lee, 1967). The gelatinous coating of <u>R</u>. <u>pipiens</u> eggs contains five distinct antigens (Shivers, 1962). Sulphated and nonsulphated mucopolysaccharides have been found in different concentrations in the different layers (Steinke and Benson, 1970; Pereda, 1970b). No toxins have been described to date.

In the present study, ovarian eggs of <u>R</u>. <u>pipiens</u> were preferred to eggs with a gelatinous coat indicating that the chemical barrier is probably added with the gelatinous coat. It is not possible to tell from these data whether the chemical diffuses to the oocyte from the gelatinous coat after its application. Fertilization has no apparent affect on the potency of the chemical. It appears to be a stable compound because it is still effective after desiccation of the eggs or subjection to a temperature of 100 C.

There is an apparent dichotomy between previous results in laboratory and field studies concerning eggs of amphibians. Licht (1974) found an embryonic survival of over 90 percent for <u>R</u>. <u>aurora</u> and over 70 percent for <u>R</u>. <u>pretiosa</u> in British Columbia. The loss was attributed to desiccation; there was no implication of any vertebrate predation on the eggs. Similarly, Anderson et al. (1971) found that only climatological factors were responsible for significant embryonic death in New Jersey <u>Ambystoma tigrinum</u>. In contrast, Licht (1969), Walters (1975), Werschkul and Christensen (1977) and the present study all show significant amounts of frog egg ingestion. A possible explanation is offered by the

present study, however.

The largest mass consumed during all experiments contained nineteen eggs. Forty eight percent of all masses eaten contained one egg and 78 percent contained one to three eggs. Because R. pipiens eggs are laid in masses of several hundred it is unlikely that L. macrochirus would normally encounter masses of such small size. Also, it was observed that L. macrochirus would consume frog eggs for only a short period of time. A short time after their initial acceptance of commercial fish foods the fish would at least nibble at anything that was introduced into the tank, including fingers, tips of pens and paper. If frog eggs were introduced at this time they were often ingested, but not without considerable effort. The mass appeared so sticky and/or unpalatable that the fish was unable to either ingest or expell it, and seemed to be attempting to move the mass from the oral cavity in any direction. Thus ingestion often appeared to be accidental or of secondary importance.

If trials were repeated for these fish for from four to ten days the number of ingestions of frog eggs per day fell to zero. It is possible that ingestion of frog eggs was a result of extreme hunger and the unnatural experimental environment, and thus an artifact that has no relation to the true ecological relation-

ships between <u>R</u>. <u>pipiens</u> eggs and <u>L</u>. <u>macrochirus</u>. It is clear, however, that the gelatinous coat of <u>R</u>. <u>pipiens</u> is a very effective protective device for the embryo.

The failure of Licht (1969) to observe rejection of eggs of <u>Rana</u> sp. by several potential predators was due to his use of ovarian eggs, which have no gelatinous coating. Ovarian eggs of <u>Bufo</u> sp. display a toxicity similar to that of adult tissues (Licht, 1969). Because no symptoms characteristic of poisoning by the "bufotoxin" have been observed from ingestion of eggs of <u>Rana</u> sp., the chemicals involved in the two genera are probably not the same.

Werschkul and Christensen (1977) did not observe the full extent of rejection of <u>R</u>. <u>pipiens</u> eggs by <u>L</u>. <u>macrochirus</u> because each fish was used only once. The present study found the first trial to be the one most likely to have frog eggs eaten by the fish. Their findings that eggs with jelly coat removed were eaten less often than tadpoles is probably due to an incomplete removal of the jelly coat. In the present study, ovarian eggs were substituted for removal of the jelly coat because of the difficulty involved with complete removal. Werschkul and Christensen did not elucidate what techniques were used.

In summary, administration of the eggs of <u>R</u>. <u>pipiens</u> in various forms to <u>L</u>. <u>macrochirus</u> demonstrates that the

gelatinous coat provides significant protection for the eggs. This protection appears to be a result of two distinct qualities, one physical and the other chemical. The unpalatable chemical first appears with the secretion of the gelatinous coat, and its effectiveness is not altered by fertilization, desiccation or temperatures up to 100 C. That egg masses were eaten at all is probably an artifact of the experimental conditions.

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Table I. Total number of observed behaviors of <u>Lepomis</u> <u>macrochirus</u> in response to foods.

| | Onionto | | | | |
|-----------------------------------|---------|------------------|------|-------|----------------------|
| Food | toward | ations 1 food | • | Bites | Food consumptions |
| Fertilized frog eggs | | 30 | . 7. | 205 | 43 |
| Unfertilize frog eggs | ed | 28 | | 167 | 26 |
| Dried unfertilize frog eggs | ed | 35 | | 144 | 79 |
| Shrimp-el-e fish food | etts | 2 | | 27 | 134 |
| Shrimp-el-e dyed black | etts | 0 | | 8 | 25 |

Table II. Number of most complete feeding behaviors of <u>Lepomis macrochirus</u> during each trial in response to five foods.

| | Observed | Behavior | |
|------------------------------------|----------------------------|----------|----------------------|
| | rientations coward food | Bites | Food consumptions |
| Fertilized frog eggs | 24 | 34 | 43 |
| Unfertilized frog eggs | 20 | 40 | 26 |
| Dried unfertilized frog eggs | 17 | 31 | 79 |
| Shrimp-el-et- fish food | ts O | 13 | 134 |
| Shrimp-el-et dyed black | ts O | 2 | 25 |

| Number of eggs in mass | Total bites | Total egg masses consumed | Total masses offered | Total broken | Percent masses eaten | Number of bites to $consume(\bar{x})$ |
|------------------------------|----------------|---------------------------------|----------------------------|-----------------|----------------------------|---------------------------------------|
| 1-3 | 180 | 54 | 87 | 4 | 62.1 | 3.33 |
| 4-9 | 76 | 11 | 39 | 7 | 28.2 | 6.91 |
| 10-20 | 54 | 4 | 18 | 1 | 22.2 | 10.80 |
| 20-80 | 44 | 0 | 12 | 2 | 0.0 | 00 |

Table III. The effects of frog egg mass size on consumption by Lepomis macrochirus.

Table IV. The effects of dried frog egg masses on their subsequent ingestion by <u>Lepomis macrochirus</u>.

| · · · · · · · · · · · · · · · · · · · | | | <u> </u> |
|---------------------------------------|---|-----------|------------|
| | | Tank 1 | Tank 2 |
| | Total masses offered | 37 | - . |
| D 1 | Total bitten | 13 | |
| <u>Day 1</u> | Total consumed | 24 | . – |
| · · · · · · · · · · · · · · · · · · · | Total Shrimp-el-etts consumed at end | 3 | 19 |
| | | | |
| • | Total masses offered | 4 | 15 |
| | Total bitten | 4 | 3 |
| <u>Day 2</u> | Total consumed | 0 | 10 |
| | Total Shrimp-el-etts consumed at end | 41 | 20 |
| | | | |
| | Total masses offered | 3 | 3 |
| | Total bitten | 2 | 3 |
| <u>Day 3</u> | Total consumed | 0 | 0 |
| | Total Shrimp-el-etts consumed at end | 10 | 10 |
| | | • · · · · | |

. .

Table V. Comparison of observed responses of <u>Lepomis</u> <u>macrochirus</u> to three foods.

| • • • • • • • • • • • • • • • • • • • | Observed Re | esponses | |
|---------------------------------------|-----------------------------|----------|----------------------|
| Food | Orientations toward food | Bites | Food consumptions |
| Dried frog eggs | 7 | 3 | 2 |
| Unfertilized frog eggs | 7 | 5 | 0 |
| Black gelatin with peptone | e O | 5 | 19 |

Table VI. Comparison of observed responses of <u>Lepomis</u> <u>macrochirus</u> to two different gelatine mixtures.

| | | the second s | |
|---|-----------------------------|--|----------------------|
| | Observed Re | esponses | |
| Gelatine mixture | Orientations toward food | Bites | Food consumptions |
| Gelatine with frog egg homogenate | 10 | 10 | 4 |
| Gelatine with peptone | 0 | 1 | 26 |

Table VII. Comparison of observed responses of <u>Lepomis</u> <u>macrochirus</u> to the ovarian and unfertilized eggs of <u>Rana pipiens</u>.

| | Observed | Responses | |
|---------------------------|-----------------------------|-----------|----------------------|
| Type of egg | Orientations toward food | Bites | Food consumptions |
| Ovarian eggs | 0 | 0 | 24 |
| Unfertilized frog eggs | 10 | 11 | 1 |
| | | | |

Table VIII. Comparison of observed responses of <u>Lepomis</u> <u>macrochirus</u> to boiled and unboiled unfertilized eggs of <u>Rana pipiens</u>.

| | Observed Re | esponses | |
|-------------------------------------|-----------------------------|----------|----------------------|
| Type of egg | Orientations toward food | Bites | Food consumptions |
| Unfertilized frog eggs | 19 | 4 | 1 |
| Boiled unfertilized frog eggs | 16 | 9 | 1 |

Walter Stanley Jennings, Jr. was born in Norfolk, Virginia, on May 18, 1952. He lived in what is now Chesapeake, Virginia, while attending elementary and high school in Norfolk, Virginia. He graduated from Carolton Oaks School in June 1970. In September, 1970 he enrolled at the University of Virginia, majoring in biology and psychology, and was graduated in May, 1974 with a Bachelor of Arts degree.

He enrolled as a special student in September, 1974 at William and Mary College but withdrew in October, 1974 without penalty to devote full time to a research problem in clinical neurosurgery under Dr. D. P. Becker at Medical College of Virginia in Richmond, Virginia. He left this work in August, 1975, to enter the University of Richmond as a full time graduate student in biology and is a candidate for the Master of Science degree in May 1978.

He was a member of Beta Beta Beta honorary biological society at the University of Richmond.

He is married to the former Judith Ann Sorensen of Kansas City, Missouri (on May 7, 1977).

He has been accepted at the University of Virginia Medical School where he is working toward the M.D. degree.