US ISSN: 0025-4231

BULLETIN OF THE

Maryland Herpetological Society

DEPARTMENT OF HERPETOLOGY

THE NATURAL HISTORY SOCIETY OF MARYLAND, INC.

MDHS .... A FOUNDER MEMBER OF THE EASTERN SEABOARD HERPETOLOGICAL LEAGUE

JULY-DECEMBER 2014 VOLUME 50 NUMBERS 3-4

BULLETIN OF THE MARYLAND HERPETOLOGICAL SOCIETY

Volume 50 Numbers 3-4 July-December 2014

CONTENTS

BULLETIN OF THE

mdhs

Volume 50 Numbers 3-4 July-December 2014

The Maryland Herpetological Society
Department of Herpetology, Natural History Society of Maryland, Inc.

Library of Congress Catalog Card Number: 76-93458

Membership Rates

Membership in the Maryland Herpetological Society is $25.00 per year and includes the Bulletin of the Maryland Herpetological Society. Foreign is $35.00 per year. Make all checks payable to the Natural History Society of Maryland, Inc.

Meetings

Meetings are held monthly and will be announced in the “Maryland Herpetological Society” newsletter and on the website, www.marylandnature.org.

A Study of the Effects of Water Quality on the Success of Hatching Rates of Egg Masses of *Ambystoma tigrnum tigrinum* and a Volumetric Method of Estimating Egg Number in Each Mass

Susan A. Stanzione

Abstract.

The purpose of this study was twofold: to determine if a volumetric examination of *Ambystoma tigrnum tigrinum* egg masses could replace the tedious task of counting and averaging; and to compare the hatching rates of *Ambystoma tigrnum tigrinum* in a successful breeding pond, and *of A. t. trigrinum* egg masses translocated to a marginal breeding pond and a pond that had never had a breeding population.

The results of the volumetric study were statistically significant at a 70% confidence level, but further study with larger numbers seems warranted.

The hatching rates for the historically successful breeding pond (Massey) and the marginal pond (TP-3) were within 8.3 percentage points, while that of the pond without a population (TP-1) was significantly lower. This seems to be related to the agrichemicals most probably used on adjacent cropfields and the topography which in one instance (Massey) allows the pollutants to be carried away from the pond without negative effect and, in the other, channels the flow almot directly into the pond. Additional study to include other breeding ponds seems warranted.

Acknowledgements.

Many people deserve more than a little thanks for their contribution to this effort. Dave Martin provided invaluable information on agrichemicals and their effect on the environment. Ann Baldwin graciously forwarded the USGS ground water case study on pesticides on the Delmarva. Chuck Henney lent the snorkeling equipment for the foray to recover egg masses from the stakes submerged by record setting spring rains. Dennis Arenson supplied statistical support and interpretation that changed my view on what was and was not important. By sharing data from her ongoing research, Chris Maex revealed much about the fate of the larvae, especially those translocated and released into the TP ponds. Eleanor High and Dorothy McDermot gave technical support as well as understanding when plans were changed because of "the paper." Marsha Mallonee served as able field assistant despite temperature and temper swings. My nephew Dan, gleefully shared his computer expertise to make a daunting task seem like child's play. Christine's final proofreading was invaluable.

None of this would have come to fruition were it not for the guidance, support and assistance of Dr. Charles J. Stine. For his encyclopedic knowledge about *Ambystoma tigrnum tigrinum* and his willingness to share this with a student; for his example of passionate interest in the environment and of conviction that one person, committed, can make a difference. Thanks, Dr. Stine.

As always, there is that one person who is with you from the beginning to the end. From those first inept attempts to gently lift a gelatinous mass intact through the ice on a frozen pond to

[1] A graduate project submitted to the Johns Hopkins University in conformity with the requirements for the degree of Master of Science in Interdisciplinary Science Studies. Baltimore, Maryland, Summer 1993, Dr. Charles J. Stine, School of Continuing Studies/Division of Liberal Arts.

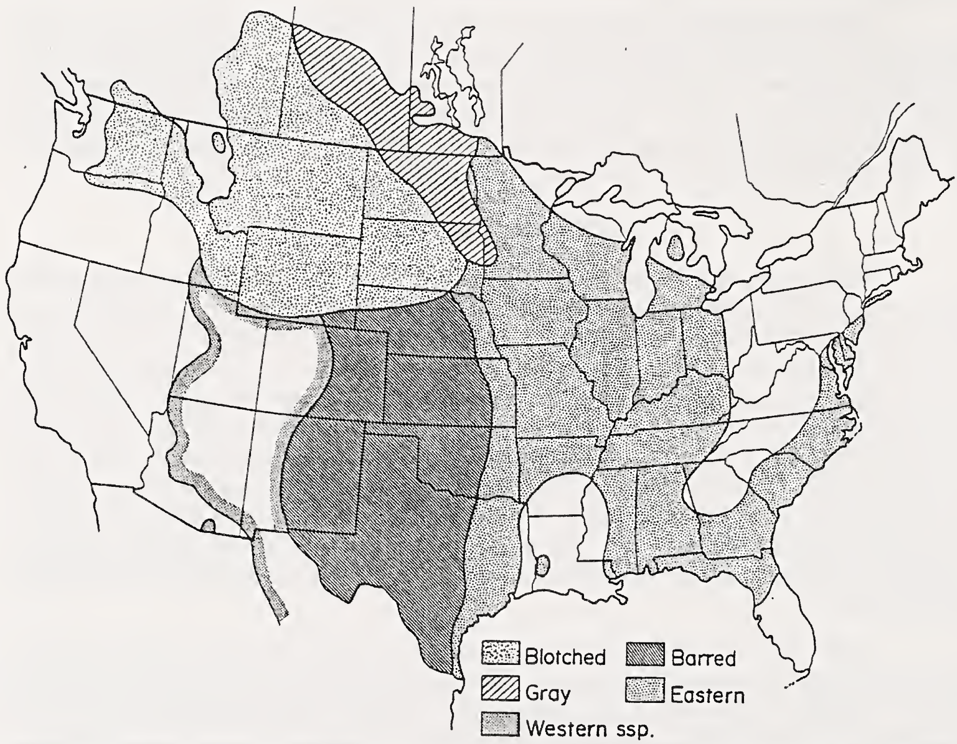
the correcting, editing and final typing of this paper, Patricia, was a constant companion and partner in this endeavor. For her wisdom and supreme patience, I am truly grateful.

Introduction.

The tiger salamander, *Ambystoma tigrnum,* is the most widely distributed species of salamander in the world, with seven recognized subspecies (Sever, 1978). It is distributed from southern central Canada south to Florida and Mexico, but is absent from New England, the Appalacians, and the far West.

The eastern tiger salamander, *Ambystoma tigrnum tigrinum,* (Green), is found (Fig. 1) from Long Island to northern Florida, Ohio to Minnesota, and south to the Gulf of Mexico (Conant, 1975). Findings from the Pleistocene (Kansan) some 600,000 years ago, however, show that the tiger salamander also existed in the Appalachian region of Maryland (Holman, 1977).

Figure 1. General distribution of subspecies of the tiger salamander *Ambystoma tigrinum,* in North America. (After Conant, 1975).



The adult eastern tiger salamander spends nine to ten months of each year in a terrestrial habitat. During winter thaws, when rain, melting snow and fog often time accompany elevated temperatures, they leave their hibernacula to breed in ponds. The aquatic phase of the adult organism's life cycle lasts an average of 60 days, after which they leave the pond and return to land. The eggs of the tiger salamander hatch in 30 to 45 days with a typically low (3-10%) mortality rate in good breeding ponds. Larvae remain in the pond usually until the end of June, at which time the metamorphs leave the pond (Fig. 2). Any interruptions in the life cycle of the tiger salamander seriously impinges on the overall success of the population (Fig. 3).

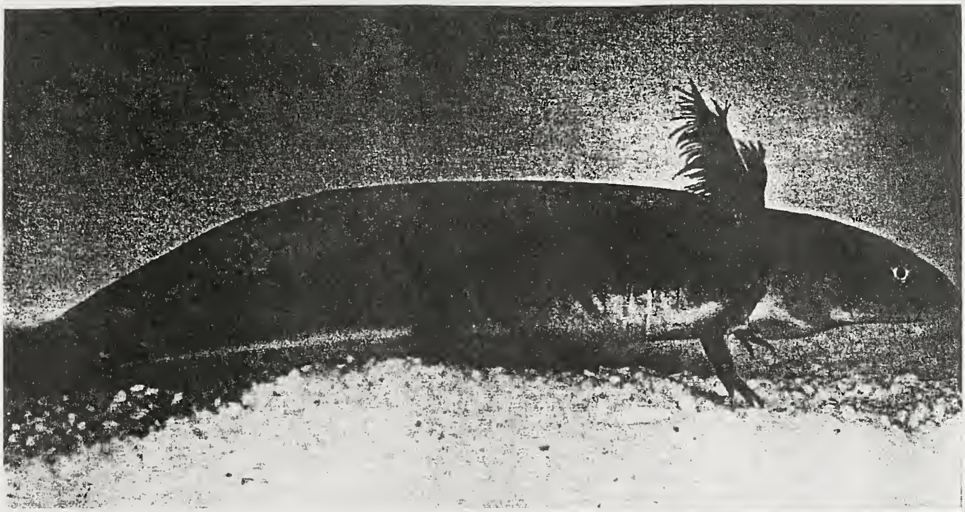


Figure 2. Larva (above) and eggs (right) of tiger salamander *(Ambystoma tigrinum).* Charles J. Stine, Jr.

The range of *A. t. tigrinum* in Maryland has been appreciably reduced in the last 30-40 years and, at present, breeding populations are found only on the Eastern Shore of Maryland. Cooper (1965) and Harris (1969) reported that *A. t. tigrinum* was found formerly in Anne Areundel, Caroline, Charles, Dorcester, Kent, Queen Anne and Worcester counties. In 1971, the Maryland General Assembly passed an Endangered Species Act. When the first list of endangered species was issued on March 1, 1992, it contained only seven mammals. It was not until October 12, 1972, that the Secretary of Natural Resources, upon recommendation from the Committee on Rare and Endangered Amphibians and Reptiles of Maryland, issued an order amending the Endangered Species List to include nine species of indigenous Maryland amphibians, including *A. t. tigrinum.* By this time, Cooper et al. (1993), in a special report stated that the only known breeding sites were at Golts and Massey ponds in Kent County. Stine (1984) indicated that only four ponds in Kent County and three in Caroline County had populations of eastern tiger salamander. Eng (19867) reported that *major* breeding populations were found only at one pond in Kent County and one in Caroline County (Fig. 4). Although egg masses and larvae had been observed in other ponds within the area, they showed very low egg deposition and high rates of mortality.

Figure 3. Life cycle of an amphibious salamander (Cogger, 1992).

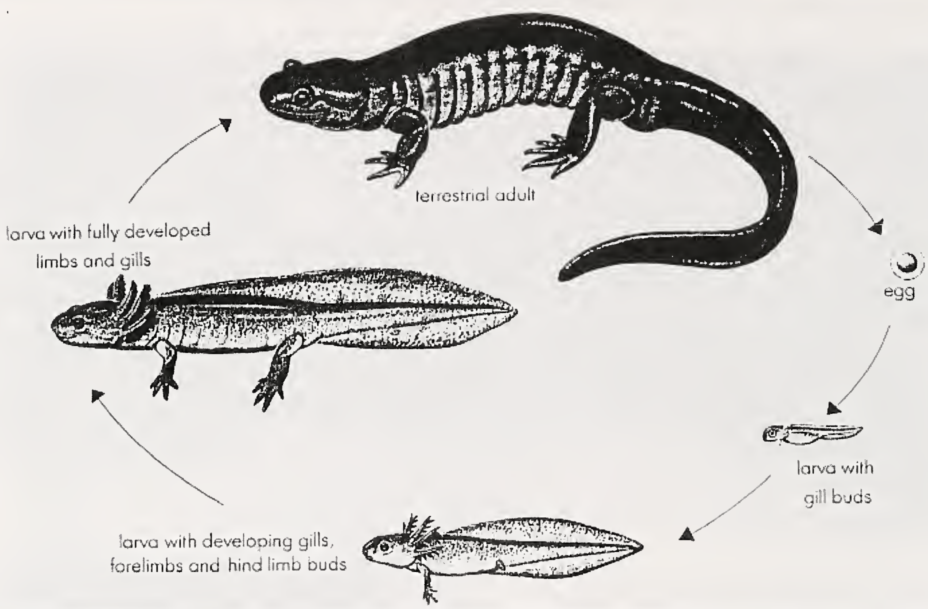
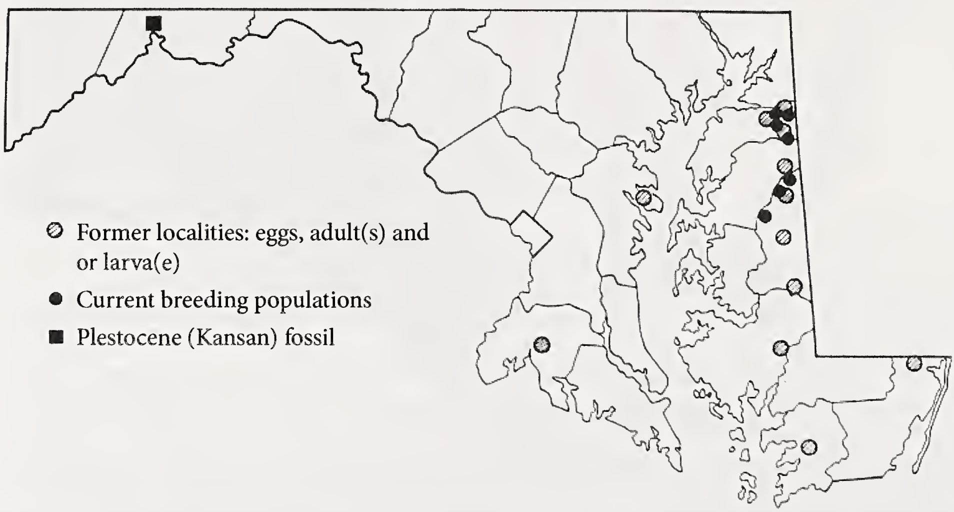


Figure 4. Distribution of eastern tiger salamander *Ambystoma tigrinum tigrinum* in Maryland. Shaded circles represent former localities for eggs, adult(s) and or larva(e). Closed circles represent current breeding colonies and closed square represents Pleistocene fossil record. (Stine, 1984).

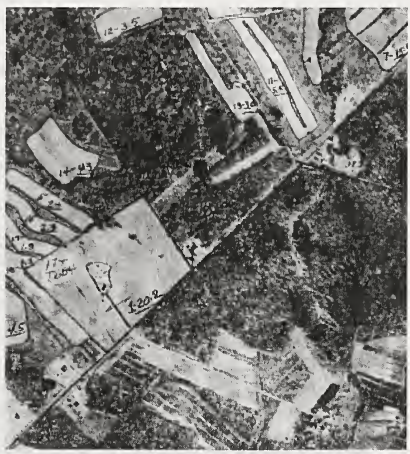


Stine (1984) proposed several hypotheses for the reduction of *A. t. tigrinum*'s range in Maryland. One of these was contamination of breeding ponds by toxic agrichemical pollutants. This study addresses that particular question. It involves three ponds in Kent County, Maryland: Massey, TP-1, and TP-3.

Massey pond has approximately a one-acre surface area in a year with average precipitation. Along its northern perimeter, Massey pond is bordered by a state road and on the east lies a large cultivated field. The west and south edges are heavily wooded. There is also a fringe of woody shrubs and small trees that surrounds the pond on the two sides that are bordered by road and cropfield. The TP ponds both lie within a right-of-way that is maintained by the Delmarva Power Company. This area appears to be mowed annually. The right-of-way is bound on both sides by woods. TP-3 pond lies totally within this right-of-way, and is two- to three-tenths acres. Although TP-1 also lies within the right-of-way. TP-1 is one- to two-tenths acres (Fig. 5).

It is this investigator's hypothesis that runoff from agricultural land has influenced in a deleterious way, the eggs and larvae of at least one of the ponds in this study, to wit, TP-1.

Figure 5. Aerial photos of Massey (above) and TP ponds (right).



Materials and Methods.

Volume/Number of Eggs Study

In any population study of salamanders, counting eggs is an essential but tedious and time consuming process. It was hypothesized that a faster, yet equally accurate method of determining egg numbers could be devised that would involve measuring the volume of the entire egg mass rather than counting the individual eggs (Fig. 6) in each mass.

Approximately 120 hours of field work were spent collecting data for this study. Numerous field visits were made to Massey pond during *A. t. tigrinum* breeding season – late December, 1992 through March, 2003. On each visit, the egg masses which had been deposited since the prior visit were located and marked with flagged four foot bamboo stakes. On the first visit, all the egg masses in the pond at that time were located and flagged. On each subsequent visit, all the recently deposited egg masses were flagged with a different colored ribbon, which allowed for categorization of the egg masses according to time of deposition (Fig. 7). On January 30-31, 1993, 10% of the total egg masses in each time/color group were selected by assigning each egg mass a number and then using a computer-generated random number sequence to select which masses would be measured. The individual numbering the masses had no prior knowledge of the numbers selected by the computer.

Figure 6. Eggs of *A. t. tigrinum* just prior to hatching.

Figure 7. Massey pond with flagged egg masses, January 30, 1993.

Each selected egg mass was carefully removed form the pond using a fine mesh aquarium net. The mass was then placed in a photographer's developing tray, the surface of which had been lined in a uniform grid pattern to facilitate counting. The eggs in each individual mass were counted independently, using a hand-held tally-meter, by this researcher and by an assistant until the tallies agreed; these figures were recorded. Each egg mass was then placed in a plastic beaker marked in milliliters. Any water in the beaker was suctioned off with a pipette until only the egg mass itself remained. The volume of the mass was noted.

Egg Mortality Study

After the eggs were counted, the masses were encapsulated. The encapsulating device was fabricated from one-liter clear plastic beverage containers and nylon stockings. Both ends of the bottles were removed, leaving a clear plastic sleeve which was then inserted into a nylon mesh sleeve (knee-high stockings). The plastic insured that the nylon would not collapse around the developing eggs and/or larvae, and the nylon mesh allowed free circulation of water and nutrients. Each capsule was then closed by knotting the open end of the stocking and tying it to its original marked stake with jute twine, in its original location and depth. Additionally the original flagged stake was also tagged with a striped green and white flagging ribbon so that it could be easily located and distinguished from all the other egg masses in the pond that were marked, but not part of this study. A total of twenty-two (22) egg masses were treated in this manner at Massey pond. In addition, six (6) more egg masses from the most recently deposited group were selected in similar fashion, measured volumetrically, counted, encapsulated and immediately transloctaed to ponds TP-1 and TP-3, three (3) egg masses per pond. The developing embryos were checked weekly until they hatched. At that time, the capsules were removed from the pond. The number of dead larvae and/or undeveloped eggs was counted. This number was subtracted from the total number of eggs that had been placed in the capsule to determine the number of surviving larvae. These larvae were then released into the pond in which they had developed and hatched.

The water quality at each pond – Massey, TP-1, and TP-3 – was also tested for dissolved oxygen (D.O.), pH, nitrates, phosphates, and tannic acide. Two borings were done at each pond at a distance of 25 feet from the pond edge. At Massey, these were done on the east side of the pond near the cropfield. At TP-1, the borings were dug at the northwest and southwest corners of the pond, near the cropfield. At TP-3, the boring locations were north and south of the pond along either edge of the right-of-way.

The following equipment was utilized for testing the appropriate water quality parameters:

Nitrates:	LaMotte Chemical Company Nitrate Nitrogen Water Test Kit Model 3110 NCR
Phosphates:	LaMotte Chemical Company Phosphate-Low Range Water Test Kit Model 3121/PAL
pH:	LaMotte Chemical Company Wide Range pH Test Kit Model P-3065
Dissolved 0_2:	YSI Dissolved Oxygen Meter
Water Temp:	Model 51B Probe Model P-3065

Tannic Acid: HACH Direct Reading Environmental Laboratory, Tyrosine Method (Ferros iron interference was eliminated by the addition of 0.2g of sodium pryophosphate to each 25ml water sample prior to the addition of the Tanni Ver3 reagent.)

Water temperature readings were also taken with a pocket thermometer (-10° to 110°C/1°/1, temperature/division). Ground water was accessed by using a five-foot soil auger with a three inch diameter open bucket. The borings were dug to a depth of five feet and allowed to fill with water for 3-4 hours before the water samples were taken. This provided a clean water sample.

A list of commonly used agrichemicals that are applied to soybeans (the crop planted in the fields near both Massey and TP-1) was compiled. These products are listed by their chemical formulae and also include the LD_{50} assigned to each (Table 1). The LD_{50}, except where noted, refers to the dose administered to laboratory rats orally (Merck, 1987). Leaching and runoff potentials are rated low, medium and high (Cooperative Extension Services, 1993).

Table 1. Pesticides commonly used on soybeans.

Chemical Formula	**Common Name**	**LD_{50}**	**Leaching Potential**	**Runoff Potential**
$C_4H_{10}NO_3PS$	Acephate	700	low	low
$C_5H_{20}NO_3PS_2$	Dimethoate	250	med	med
$C_7H_{17}O_2PS_3$	Phorate	1.1 (2.5*)	high	med
$C_8H_{10}NO_5PS$	Methyl Parathion		14 (67)*	low med
$C_8H_{19}O_2PS$	Ethoprop	n/a	high	med
$C_8H_{19}O_2PS_3$	Disulfoton	2.3 (6.0)*	low	med
$C_9H_{11}Cl_2NO_3PS$	Chlorpyrifos	145	low	high
$C_{10}H_{19}O_6PS_2$	Malathion	1000	low	low
$C_{12}H_{19}O_2PS_3$	Sulprofos	227	low	high
$C_{12}H_{21}N_2O_3PS$	Diazinon	250	n/a	n/a

*The doses in parenthesis were administered dermally.

The 1992 Crop Protection Chemicals Reference (CPCR) lists the Environmental Hazards for each of these pesticides. In addition the Extension Toxicology Network (EXTOXNET) presents short descriptions on issues such as environmental and ecological effects.

$C_4H_{10}NO_3PS$ (Acephate): This pesticide is toxic to birds. Applications may adversely affect birds in rangeland treatment areas. Do not apply directly to water, or areas where surface water is present (CPCR).

$C_5H_{20}NO_3PS_2$ (Dimethoate): This pesticide is toxic to wildlife and aquatic invertebrates. Do not apply directly to water or wetlands. Runoff from treated areas may be hazardous to aquatic organisms in neighboring areas (CPCR). Dimethoate is toxic to most aquatic life. A virtue is that it degrades in the environment and does not bioaccumulate. Also, since dimethoate is rapidly metabolized, biomagnification hazard is unlikely (EXTOXNET).

$C_7H_{17}O_2PS_3$ (Phorate): This pesticide is extremely toxic to fish and wildlife. Do not apply directly to water or wetlands. Drift and runoff may be hazardous to aquatic organisms in neighboring areas (CPCR). Phorate is very highly toxic to and extremely fast-acting on bird species, freshwater fish, and aquatic invertebrates. Phorate has low water solubility, is fat soluble, is slowly degraded and slowly eliminated in the body and thus has a moderate to high potential to accumulate within organisms. Phorate has some potential to leach through the soil and contaminate ground water, particularly where soils are sandy and aquifers are shallow (EXTOXNET).

$C_8H_{10}NO_5PS$ (Methyl Parathion): This pesticide is highly toxic to aquatic invertebrates and wildlife. Birds in treated areas may be killed. Shrimp and other aquatic organisms may be killed at recommended application rates. Do not apply directly to water or wetlands... Runoff and drift from target areas may be hazardous to aquatic organisms in adjacent aquatic sites (CPCR). Methyl Parathion is toxic to fish and to the animals eating the fish. Other studies indicate that fish kills may be caused by a series of events. The methyl parathion kills the insects and crustaceans... that feed on algae. Without them present, the algae "bloom." This overgrowth is rapid and rains the oxygen from the pond water. It is this lack of oxygen which kills the fish (EXTOXNET).

$C_8H_{19}O_2PS$ (Ethoprop): This product is toxic to aquatic organisms (fish and invertebrates), and extremely toxic to birds... Do not apply directly to water or wetlands... Drift and runoff from treated areas may be dangerous to aquatic organisms in neighboring areas. Do not apply within 140 feet of inland freshwater habitats. (CPCR).

$C_8H_{19}O_2PS_3$ (Disulfoton): This pesticide is toxic to fish and wildlife. Do not apply directly to water or wetlands. Drift and runoff from treated areas may be hazardous in neighboring areas (CPCR). Disulfoton-containing products are highly toxic to fish, crabs, shrimp, birds and other wildlife (EXTOXNET).

$C_9H_{11}Cl_2NO_3PS$ (Chlorpyrifos): This pesticide is toxic to birds and wildlife, and extremely toxic to fish and aquatic organisms. Do not apply directly to water. Drift and runoff from treated areas may be hazardous to aquatic organisms in adjacent aquatic sites (CPCR). Chlorpyrifos can be very toxic to aquatic organisms. The 96-hour LC_{50} for fathead minnows is 331.7 *u*g/1. When fathead minnows were exposed for a 200-day period during which they reproduced, the first generation of offspring had decreased survival and growth as well as a significant number of deformities; this occurred at approximately 2.68 micrograms per liter (*u*g/1) exposure for a thirty day period. Algal blooms are known to frequently follow applications to nearby fields (EXTOXNET).

$C_{10}H_{19}O_6PS_2$ (Malathion): This pesticide is toxic to fish and aquatic invertebrates. Do not apply directly to water except as specified on the label. Drift and runoff may be hazardous to aquatic organisms in areas near the application site (CPCR).

$C_{12}H_{19}O_2PS_3$ (Sulprofos): This pesticide is toxic to fish and wildlife... Keep out of lakes, streams and ponds. Do not apply when weather conditions favor runoff or drift from target areas (CPCR).

$C_{12}H_{21}N_2O_3PS$ (Diazinon): This product is toxic to fish, birds and other wildlife...

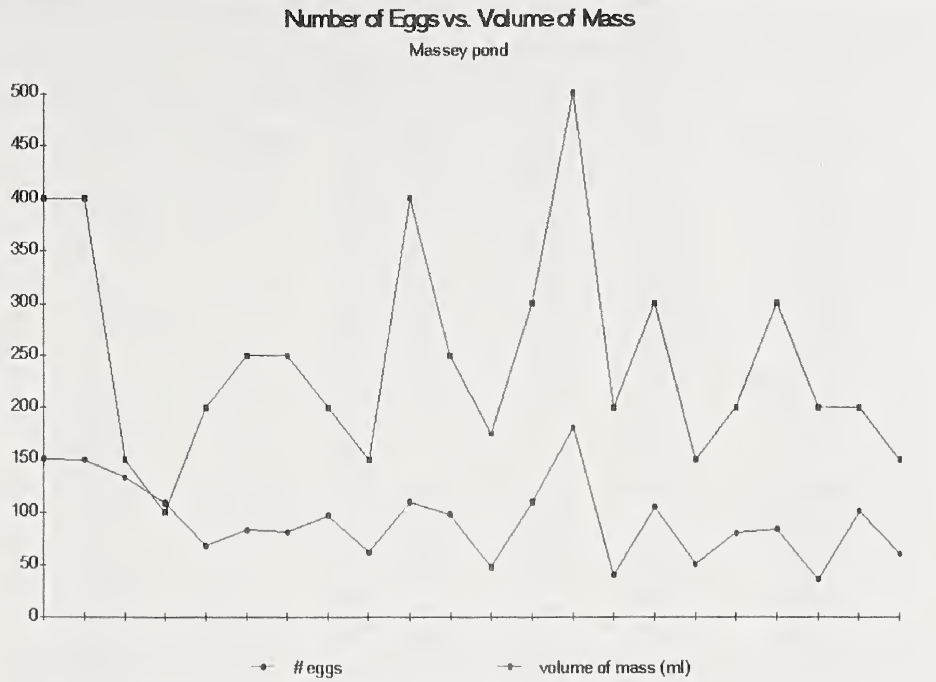
Keep out of lakes, stream or ponds. Do not apply where runoff is likely to occur (CPCR). Most fish are very sensitive to diazinon. Warm water fish such as fathead minnows and goldfish have LC_{50}s of 5100-15,000 *u*g/1 for 96-hour exposures. The bullfrog has a 96-hour LC_{50} > 2000 *u*g/1. Bioconcentration ratios ranged from 152 for gudgeon to 17.5 for guppies. The bioconcentration for crayfish is 4.9 (EXTOXNET).

Results.

Table 2. represents the data obtained counting the eggs in each mass and measuring the volume of each mass. Chart 1. is a plot of this data.

Color Tag	#Eggs	Volume Mass
B-1	151	400
B-2	150	400
C-1	133	150
C-2	109	100
P-1	68	200
P-2	81	250
P-3	81	250
P-4	97	200
P-5	62	150
P-6	110	400
O-1	98	250
O-2	48	175
O-3	110	300
O-4	181	500
O-5	40	200
Y-1	105	300
Y-2	51	150
Y-3	80	200
W-1	84	300
W-2	36	200
W-3	101	200
W-4	61	150

Chart 1. Number of eggs per mass vs. volume of mass.



In Table 3 are listed the number of eggs counted and the number of live larvae that hatched from each egg mass. The mean hatch rates were: Massey, 94.2%; TP-1, 40.7% and TP-3, 85.9%. TP-1 was overrun with filamentatious algae in March; this progressively worsened in following months.

Table 3. Live Larvae

Color Tag	# Eggs	# Live Larvae
Massey Pond		
B-1	151	146
B-2	150	143
C-1	133	127
C-2	109	106
P-1	68	65
P-2	83	78
P-3	81	78
P-4	97	93
P-5	62	61
P-6	110	89
O-1	98	93
O-2	48	42

Table 3. *Continued*

Color Tag	**# Eggs**	**# Live Larvae**
O-3	110	98
O-4	110	172
O-5	40	38
Y-1	105	91
Y-2	51	50
Y-3	80	74
W-1	84	78
W-2	36	31
W-3	101	100
W-4	61	58
TP-1 Pond		
TP-1-A	65	0
TP-1-B	20	8
TP-1-C	72	59
TP-3 Pond		
TP-3-A	52	49
TP-3-B	70	58
TP-3-C	67	54

The following are the results of tests for nitrates, phosphates and tannic acid.

Table 4. Water Quality: Nitrate, Phosphates and Tannic Acid.

4/11/93	**Nitrates**	**Phosphates**	**Tannic Acid**
Massey	0	0	1.4 mg/l
TP-1	0.2 mg/.	1.0 mg/l	6+ mg/l
TP-3	0	0	1.5 mg/l
6/11/93			
Massey	0	0	1.6 mg/l
TP-1	0.9 mg/l	0.6 mg/l	6+ mg/l
TP-3	0	0	1.6 mg/l

Ground water samples tested for the presence of phosphates produced the following results:

Table 5. Groundwater Phosphate Levels at each Pond.

6/11/93	**Sample #1**	**Sample #2**
Massey	0	0
TP-1	1.0 mg/l	0.8 mg/l
TP-3	0	0

Water temperature, dissolved oxygen and pH measurements were also taken at several locations in each pond. The time of day is indicated since the ambient temperature obviously affected both water temperature and dissolved oxygen readings.

Table 6. Water Quality: Dissolved Oxygen, pH and Temperatures.

4/11/93	**Air Temp °C**	**H_2O °C**	**D. O. ppm**	**pH**	**H_2O Depth cm**
Massey	18	17	9.8	6.0	10-15
2:00 pm		16	7.5	6.0	30
		15	10.5	6.0	76
TP-1	14	17	9.6	5.0	5-10
11:00 am		16	10.8	5.0	15-20
		15	10.1	5.0	30
		13	12.0	5.0	61
TP-3	12	13	11.6	5.0	10-15
9:00 am					
6/11/93	**Air Temp °C**	**H_2O °C**	**D. O. ppm**	**pH**	**H_2O Depth cm**
Massey	30	32	7.8	6.0	10-15
2:00 pm		27	7.0	6.0	61
TP-1	23	26	1.7	5.0	10-15
9:00 am		25	0.2	5.0	61
TP-3	27	26	6.6	5.0	10-15
11:00 am		25	2.2	5.0	61

Discussion.

Volume/number of eggs study

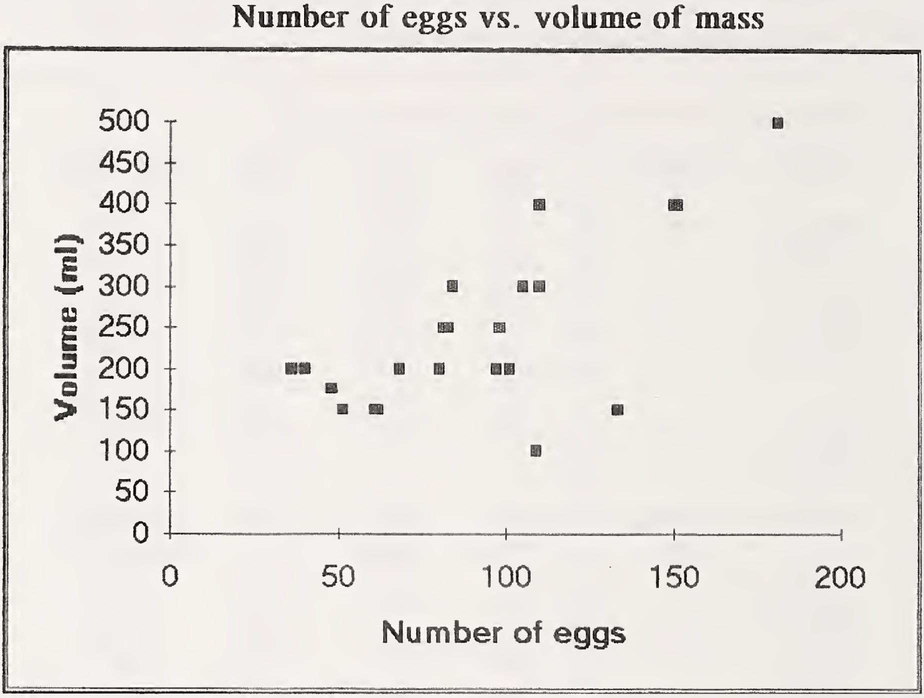
The data obtained from the egg count/mass volume portion of this study was analyzed statistically using the linear regression method (Fig. 8). This represents a correlation coefficient (r) = 0.7018, and a p value – 0.0003, which is interpreted as statistically significant. However, this study did not take into account the exact age of each egg mass and, therefore, does not adjust for this variable. Given that salamander eggs are hygroscopic (Stine, 1984), differences in the exact age of the eggs may influence the results.

It appears that, if very rough estimates of egg numbers are needed, this would be an acceptable method for obtaining the estimate. It would also seem that a larger sample representing the same developmental stage egg masses would produce even more significant results. This warrants further study.

Egg Mortality

It is apparent from the data present in Tables 3, 4 and 6, that Massey pond provides a much more favorable environment for breeding of *A. t. tigrinum* than does TP-3. Larval population estimate for the 1993 breeding season, at Massey, are 26,000 individuals. In contrast, TP-1's environment seems veritably hostile to *A. t. tigrinum* eggs and larvae. Of the 41% of the eggs translocated to TP-1 hatched successfully, only one individual larva was found when the pond was

Figure 8. Linear plot of the number of eggs vs. volume of masses of *A. t. tigrinum* at Massey pond.



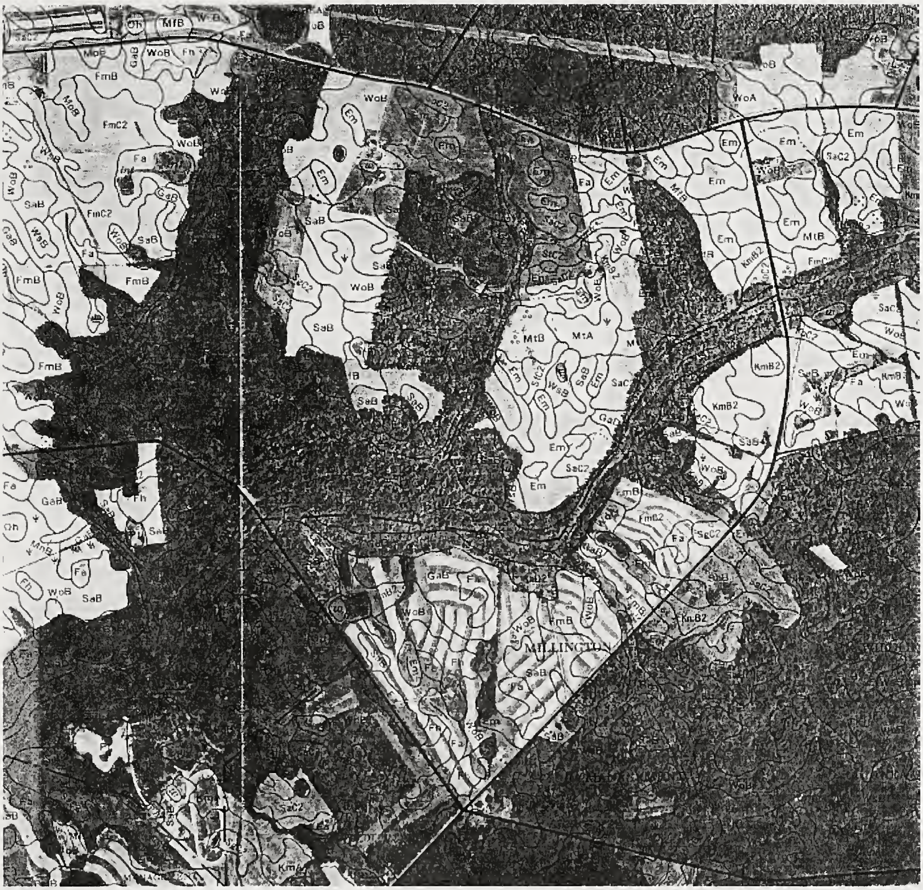
seined one month after the larvae were released, and none were found two months later (Maex, pers. comm., July, 1993). Representative samples seined from Massey, however, yielded 62 larvae one month after release; 15 larvae two months later (Maex, pers. comm., July, 1993). No similar data is available for TP-3. However, invertebrate life at TP-3 is visibly quantatively and qualitatively inferior to that of Massey pond (Stine, pers. comm., July, 1993) and therefore it seems unlikely that larvae at TP-3 would fare much better than those at TP-1 since food would be a limiting factor. The TP ponds are both located on the same Fallsington soils and within the same drainage area of the watershed (Fig. 9). Also, the land around TP-3 is unsuitable for the non-aquatic phase of the tiger salamander: these is swamp to the northeast, high ground to the southwest that is too dry, and a small pine forest along a county road to the west-southwest.

Algae seems to be a major problem at TP-1. Even in January, when the encapsulated egg masses were translocated to TP-1, there were algae present. As temperatures rose, the algae continued to grow in a manner that is commonly referred to as an algal bloom. The water quality data provides some insight into what is, perhaps, the driving force behind this particular algae problem.

TP-1 was the only pond with detectable levels of nitrates and phosphates. It was also the only site where phosphates were also detectable in the ground water within 25 feet of the pond's edge. Ground water data obtained from *USGS Water Resources Basic Data Report, No. 10*, data site BG-15, located in Massey, Maryland, (Fig. 10) indicates a natural phosphate level of <.05 mg/l (USGS 1978). Ground water samples taken at TP-1 range from 16 to 20 times that amount, while phosphate levels in the pond itself were 12 to 20 times what is normally expected.

Additionally, TP-1 had a much higher level of tannic acid than did Massey and TP-3, an indication that there is much more decaying matter in TP-1 (Table 4). Dissolved oxygen levels at

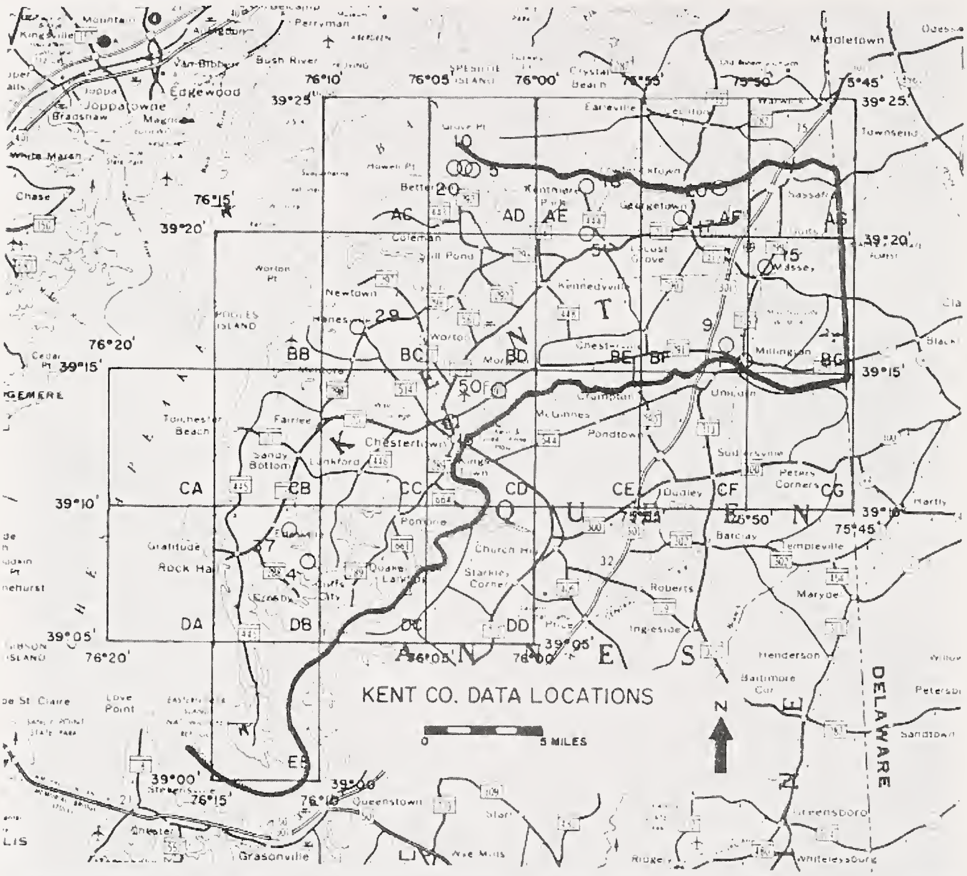
Figure 9. Soils map of Massey and TP ponds. Massey ois located on Elkton (Em) soiles, while the TP ponds are on Fallsington (Fa).



TP-1 declined 51-98% over a two month period, while levels at TP-3 dropped 43-81% and those at Massey 6-33% (Table 6). Dissolved oxygen levels at TP-1 were actually higher than those at Massey and TP-3 in April, probably because: 1) the algae present had not yet overrun the pond; 2) the water temperatures were 12-13° C lower than in June and; 3) at 11:00 a.m. on a sunny day, the algae were at peak oxygen output from photosynthesis. Massey and TP-3, though each had acceptable dissolved oxygen levels, did not have the additional input of oxygen from algae photosynthesis. Conversely, by June, as the algae bloomed in TP-1, and then began dying and decomposing and the dissolved oxygen levels dropped at TP-1, the dissolved oxygen levels at Massey remained stable, although TP-3 levels were also in decline.

The feature which most distinguishes Massey from the TP ponds, however, is the topographic situation (Fig. 11). Though all the ponds are at approximately the same elevation (60-65 feet above mean sea level), Massey pond receives negligible amounts of surface runoff, none of which comes from the adjacent cropland and very little of which comes from the roadway. TP-1, however, receives direct runoff from the cropfield alongside it, and even though TP-3 does not

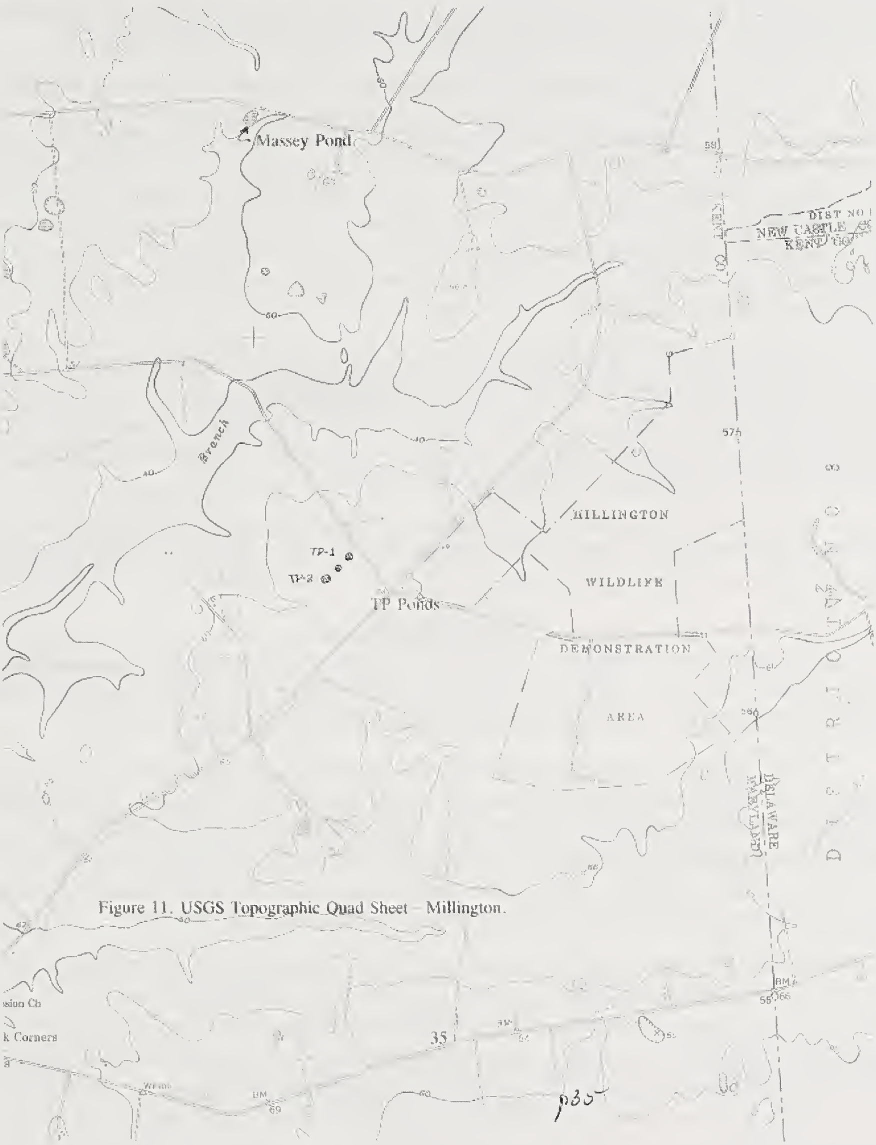
Figure 10. USGA Water Resources Basic Data Report No. 10., Maryland Ground-Water Information: Chemical Quality Data.



have any cropfields immediately adjacent, it is downstream from TP-1. The TP ponds, therefore, are subject to severe environmental perturbations as a result of agricultural practices applied to the nearby land, specifically agrichemicals. TP-1 could probably be classified as eutrophic. Seasonally, it supports prolific aquatic plant growth and this tremendous biomass generates abundant oxygen during photosynthesis. However, when environmental factors inhibit or retard photosynthesis, dissolved oxygen drops drastically. The oxygen level also drops considerably with depth because the algae restricts sunlight penetration.

Phosphates appear to be the key to the overabundant algae found in TP-1. It is introduced to the pond primarily through surface runoff from the cropfield. Phosphorus is applied as a component of some fertilizers, but more damaging are the organophosphates that are a part of the various pesticides used on corn and soybeans – the major crops grown on the Delmarva peninsula, where annually, nearly three million pounds of agricultural herbicides, insecticides and fungicides are used to enhance production (USDA, 1992). The field next to TP-1 was planted in soybeans in 1992 and again in 1993.

Figure 11. USGS Topographic Quad Sheet.



Phosphorus has a high adsorption partition coefficient, a measure of the absorption phenomenon. Therefore, it adsorbs quite readily to soil particles and is delivered to the pond with sediment during storms. Phosphorus-fueled algae blooms are not the only adverse effect that these pesticides have on *A. t. tigrinum.* All of the pesticides researched are toxic to either wildlife, birds, fish, aquatic invertebrates, aquatic organisms or a combination of these. Though not mentioned specifically, surely *A. t. tigrinum* is affected by these agrichemicals as well. These pesticides would be toxic not only to the adult organism, but even more so to the eggs and larvae since the limits of tolerance for developing organisms are narrower.

At 15° C, larval *A. t. tigrinum* take in about 59% of their total oxygen from the water (Whiteford and Sherman, 1968). It is likely that an organism utilizing cutaneous respiration in addition to pulmonary respiration would be at even greater risk from toxic chemicals.

All of the pesticides studied were insecticides. Only one, phorate, is fat soluble and, therefore, has a high potential to accumulate within organisms. If bioaccumulation is a hazard, then one can extrapolate that biomagnification is also a consequence of pesticide-contaminated ponds.

Dimethoate, on the other hand, claims to not be an environmental hazard since it readily degrades and does not bioaccumulate. If, however, this chemical is toxic to insects – its intended target – then it is still an environmental hazard since it will also kill aquatic invertebrates and alter the food supply for *A. t. tigrinum*; and it will contribute phosphates to surface and ground water that, in turn, create algal blooms.

All the pesticides researched were selected because they are commonly used in the state of Maryland on soybeans and they contain phosphates. It is not known which, if any, were actually applied to the crops grown in the field next to TP-1, but it is reasonable to infer that the elevated phosphate levels are not a natural occurrence, and that applications of agricultural fertilizers and pesticides are the most likely avenues for their introduction.

The mechanisms for delivery of contaminants to the pond are rainfall events which detach soil particles that are transported with surface runoff, percolation of phosphates into ground water, and direct spray or drift onto surface waters.

On the Delvmarva peninsula, where ground water is the major source of surface water streamflow, ground water can significantly affect the quality of surface water. Ground water movement is very slow, typically moving one-quarter to two feet per day in the water-table aquifer; consequently, water can remain in the water-table aquifer for several decades (USDA, 1992).

Ground water is particularly fragile. Since it lacks biological activity, it has no assimilative capacity (USDA, 1988). Dissolved constituents in natural ground water are derived from the mineral components of the aquifer materials. The Delmarva peninsula aquifers are comprised primarily of quartz sand that does not dissolve readily, and, therefore, the concentration of dissolved constituents is very dilute. Additional inputs such as agrichemicals with moderate to high leaching potentials could significantly change the chemical properties of the water (USDA,1992).

There is no historic data on pesticide use on the Delmarva peninsula. Ground water testing that has been done for pesticides reveals their presence, but at reportedly low levels, less than the U.S. Environmental Protection Agency maximum contaminant and health advisory levels for drinking water. However, very little is known about the effects of long-term exposure of aquatic animals and plants to ground water that contains low concentrations of pesticides (USGS, 1992). Furthermore, there is sparse data on the breakdown products of pesticides and the environmental effects of combinations of pesticides are as yet unknown.

The universal concern among the herpetological community regarding the disappearance of amphibians is more than justified. Land development demands from an expanding human population are putting ever increasing stresses on an already abused ecosystem. The tiger salamander ranges over most of the North American continent, and indication of a rather resilient and adaptive creature, yet in the state of Maryland their numbers are dwindling to the point that they are now considered an endangered species. That in itself should be cause for alarm.

Summary.

Although a volumetric approach to quantifying the number of eggs in *A. t. tigrinum* egg masses seems to be possible, further study involving greater numbers of egg masses seems warranted before it can be used definitively as an accurate field method.

Topography seems the single most important factor influencing the overall success of *A. t. tigrinum* in the ponds studied. Massey pond, by virtue of its topographic position in the watershed, seems to have escaped the long term effects of pollutants delivered to ponds adjacent to agricultural fields by surface runoff and ground water contamination. The algae blooms that occur in TP-1, and the lack of a suitable invertebrate food supply at TP-3, seem to be directly related to agricultural runoff and in turn, the depletion of dissolved oxygen necessary for survival of *A. t. tigrinum* developing embryos and larvae. In addition, a study of the many pesticides that are used in the production of corn and soybeans on the Delmarva peninsula reveals that they are toxic to *A. t. tigrinum* in all of its life stages and a host of other organisms as well. There is a dearth of information on the long term environmental effects of not only the pesticides themselves, but also of their breakdown products singly and in combination with each other. The exact quantities of each of a multitude of agrichemicals that have been applied to the land over the years and how much of these have reached the aquifers underlying the Delmarva, and the resultant effects are also unknown. The information contained in this study should not be extrapolated to explain species decline in other areas or ponds, rather, further study in other ponds, with greater attention to the effect of topography, seems indicated.

Literature Cited.

Anderson, J.D., D.D. Hassinger, and G.H. Dalrymple.
1971. Natural morality of eggs and larvae of *Ambystoma t. tigrinum*. *Ecology*, 52 (6): 1107-1112.

Arndt, R.G.
1989. Note on the natural history and status of the tiger salamander. *Ambystoma tigrinum*, in Delaware. *Bulletin of the Maryland Herpetological Society*, 25 (1):1-21.

Bishop, S.C.
1943. *Handbook of Salamanders*. Comstock Publishing Company, Garden City, New York.

Budavari, S.
(ed). 1987. *The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals*. Merck and Company, Inc. Rahway, New Jersey.

Buskirk, J. W. and D.C. Smith.
1991. Density-dependent population regulation in a salamander. *Ecology*, 72 (5):1747-1756.

Cochran. D.M.
1961. *Amphibians of the world*. Doubleday and Company, Garden, New York.

Cogger, H.G., and R.G. Sweifel.
1992. *Reptile and Amphibians*. Smithmark Publishers Inc. New York.

Conant, R.
1975. *Field Guide to Reptiles and Amphibians of Eastern and Central North America*. Houghton Mifflin Co., Boston.

Cooper, J.E.
1965. Distributional survey: Maryland and the District of Columbia. Reprinted from *Bulletin of the Philadelphia Herpetological Society*, 8 (3): 18-24, and revised by H.S. Harris, Jr., (November, 1965) *Bulletin of the Maryland Herpetological Society*, 1 (1):3-14

Cooper, J.E., L.R. Franz, et al.
1973. Endangered amphibians and reptiles of Maryland: a special report. *Bulletin of the Maryland Herpetological Society*,9 (3):42-100.

Crop Protection Chemicals Reference.
1992. John Wiley and Sons, New York.

Druad, M.J.
1993. North American tiger salamander. Reptile and Amphibian Magazine, January-February, pp 62-68.

Duellman, W.E., and L. Trueb.
1986. *Biology of Amphibians*. McGraw-Hill, New York.

Hamilton, P. A., and R.J. Shedlock.
1992. *Are Fertilizers and Pesticides in the Ground Water?* U.S. Geological Survey Circular 1080. U.S. Government Printing Office, Washington, DC.

Hassinger, D.D., J.D. Anderson and G.H. Dalrymple.
1993. The early life history and ecology of *Ambystoma tigrinum* and *Ambystoma opacum* in New Jersey. *Midatlantic Naturalist*. 84:474-495.

Hitchman, M.L.
1978. *Measurement of Dissolved Oxygen*. John Wiley and Sons, Inc., and Orbisphere Laboratories, Geneva, Switzerland.

Holman, J.A.
1977. The Pleistocene (Kansan) herpetofauna of Cumberland Cave, Maryland. *Anals of Carnegie Museum*, 46:157-172.

Hotchkiss, B.E., J.W. Gillett, et al.
1992. *EXTOXNET*. Extension Toxicology Network, Cornell University, Ithaca, New York

Lee, D.S. and R. Franz.
1974 Comments on the feeding behavior of larval tiger salamanders, *Ambystoma tigrinum*. *Bulletin of the Maryland Herpetological Society*, 25 (1):1-21.

Lynn, A.M.
1987 *Maryland Fishpond Management Guide*. U.S. Department of Agriculture, Soil Conservation Service, College Park, Maryland.

Maex, C.
1993. Unpublished data.

Maryland Department of Natural Resources.
1984. *Wildlife Management for the '80's*. Maryland Department of Natural Resources, Forest, Park and Wildlife Management Program.

________________.
1991. *Rare, Threatened, and Endangered Animals of Maryland*. Maryland Natural Heritage Program.

Moore, D.S. and G.P. McCabe.
1989. *Introduction to the Practice of Statistics*. W.H. Freeman and Co., New York.

Odum, E.P.
1971. *Fundamentals of Ecology*. W.B. Saunders Co., Philadelphia.

Petranka, J.W.
1989. Density-dependent growth and survival of larval *Ambystoma:* evidence from whole pond manipulations. *Ecology*, 70 (6):1752-1767.

Sever, D.M., and C.F. Dineen.
1978. Reproductive ecology of the tiger salamander, *Ambystoma tigrinum tigrinum* in Northern Indiana. *Proc. Ind. Adac. of Sci.* 87:189-203.

Stine, C.J., J.A. Fowler and R.S. Simmons.
1954. Occurrence of the eastern tiger salamander, *Ambystoma tigrinum tigrinum* (Green) in Maryland, with notes on its life history. *Annals of the Carnegie Museum*, 33:145-148.

Stine, C.J.
1984. The life history and status of the eastern tiger salamander, *Ambystoma tigrinum tigrinum* (Green) in Maryland. *Bulletin of the Maryland Herpetological Society*, 20 (3):65-108.

____
1993. Personal communication.

University of Maryland Cooperative Extension Service.
1992. Unpublished information.

U.S. Department of Agriculture.
1988. Water Quality Field Guide. U.S. Government Printing Office, Washington, DC.

Weier, T.E., C.R. Stocking, and M.G. Barbour.
1974. *Botany, An Introduction to Plant Biology*. John Wiley and Sons, New York.

White, E.A.
1983. *Soil Survey of Kent County, Maryland*. United States Department of Agriculture, Soil Conservation Service, in cooperation with the Maryland agricultural Extension Service and Kent Soil Conservation District.

Whiteford, W.G., and R.E. Sherman.
1968. Aerial and aquatic respiration in axolotl and transformed *Ambystoma tigrinum*. *Herpetologia*, 24:233-237.

Woll, R.S.

1978. Water Resources Basic Data Report No. 10 Maryland Ground Water Information: Chemical Quality Data. United State Geological Survey, Department of the Interior, U.S. Government Printing Office, Washington, DC.

Received: 20 January 2014
Accepted: 12 June 2014

Leucistic Wood Frog tadpole *(Lithobates sylvaticus)* from central Ohio

Abnormally pigmented anurans, both adults and tadpoles, have been observed for several species, with abnormalities including both albinism and leucism (no skin pigment but eyes pigmented) (reviews in Hensley, 1959; Dyrkacz, 1981). Here I report leucism in a tadpole Wood Frog (*Lithobates sylvaticus*) from central Ohio. Previously, Mitchell and White (2005) reported a leucistic Wood Frog tadpole from northern Virginia. Toledo et al. (2011) suggested that we need more reports of such abnormal pigmentation to better understand their appearance and distribution in nature.

As part of a mesocosm experiment, I introduced a total of 2550 Wood Frog tadpoles into 48 mesocosms (1135 L cattletanks filled with 800 L of well water) on 6 April 2014. Tadpoles were Gosner Stage 25 (Gosner, 1960) when introduced into the experiment. Wood Frog tadpoles were derived from 8 partial clutches collected from a local pond on the Denison University Biological Reserve, Granville, Licking Co, Ohio, USA (40°05'07.32"N, 82°30'33.92"W; datum: WGS84, elev. = 341 m). During the course of the experiment, the abnormally pigmented tadpole was noticed in one of the mesocosms (Fig. 1), and its status was noted throughout the experiment. The tadpole had pigmented eyes, and there appeared to be a cream color to its body, but it was lacking any darker pigments observed in other Wood Frog tadpoles. Thus, it appears this tadpole exhibited leucism, and is similar to the description by Mitchell and White (2005). Thompson and Rea (2013) reported a leucistic adult *L. sylvaticus* from a population in British Columbia, Canada. To my knowledge, the only other previous observation of leucism in tadpoles of *L. sylvaticus* was that reported by Mitchell and White (2005) (see reviews in Hensley, 195.; Dyrkacz 1981).

Fig. 1. Leucistic Wood Frog (*Lithobates sylvaticus*) tadpole observed in a mesocosm in Granville, Licking Co., Ohio, USA. Note the normally pigmented Wood Frog tadpole to the right of the leucistic tadpole.

The abnormally pigmented tadpole did not metamorphose by the end of the experiment on 27 June 2014. It had reached Gosner Stage 39-40. I attempted to continue raising the tadpole to metamorphosis in the laboratory but the tadpole died 2 days later. Mitchell and White (2005) observed that the leucistic tadpole they found developed slower relative to other Wood Frog tadpoles in their ponds.

The observed abnormal pigmentation is quite rare since only one such tadpole was observed (= 0.039% of those introduced to the experiment; = 0.054% [1 of 1851] recovered at the end of the experiment). These estimates of frequencies are likely overestimates since I did not observe any abnormal pigmentation in the tadpoles not used in the experiment. In addition, I have observed no similar tadpoles or metamorphs in local ponds over the past 13 years. Taken together with the observations of Mitchell and White (2005), it is clear that leucism is very rare in Wood Frog tadpoles.

Acknowledgments

I thank M. Smyk, L. Smith, G. Eng-Surowiec, J. Hollis, M. Jones, D. Mirzashvili, J. Rettig, and W. Smith for help throughout the experiment. I thank S. Graham for pointing out the Mitchell and White (2005) observation. The experiment was conducted with approval of the Denison University Institutional Animal Care and Use committee (14-002), and eggs collected and used under Ohio Division of Wildlife permit (17-51).

Literature Cited

Dyrkacz, S.
1981. Recent instances of albinism in North American amphibians and reptiles. Herpetological Circular 11: 1-31.

Gosner, K.L.
1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16: 183-190.

Hensley, M.
1959. Albinism in North American amphibians and reptiles. Publications of the Museum, Michigan State University, Biological Series 1: 133-159.

Mitchell, J.C., and J. White.
2005. Leucistic Wood Frog (*Rana sylvatica*) tadpoles from northern Virginia. Banisteria 25: 52-53.

Thompson, M., and R.V. Rea.
2013. *Rana sylvatica*. Leucism. Herpetological Review 44: 128-129.

Toledo, L.F., N. Rodirgues da Silva, and O.G. dos Santos Araújo.
2011. Albinism in two Amazonian frogs; *Elachistocles carvalhoi* (Microhylidae) and *Lithobates palmipes* (Ranidae). Herpetology Notes 4: 145-146.

Geoffrey R. Smith, Department of Biology, Denison University, Granville, Ohio, USA (e-mail: smithg@denison.edu)

Received: 13 October 2014
Accepted: 1 November 2014

Predation of *Leptodactylus melanonotus* (ANURA: LEPTODACTYLIDAE) by *Cupiennius salei* (ARANEAE: CTENIDAE)

Predation is the greatest cause of mortality in natural populations and can occur in any life history stage, (Vitt and Caldwell, 2009). Amphibians are preyed by invertebrates including spiders at all life stages. Small-bodied and juvenile amphibians are prey for numerous arthropods including insects, amblypygids and spiders, however no invertebrate species is recognized as specialist predator of this group. Most species are generalist predators that feed opportunistically on available food items (Wells, 2007; Vitt and Caldwell, 2009). Anurophagy by spiders is well documented in other reviews on the subject (Toledo, 2005; Menin et al., 2005; Maffei et al., 2010).

On 4 February 2014 I observed the first record of one individual of *Leptodactylus melanonotus* Hallowell, 1861 being preyed upon by *Cupiennius salei* Keyserling, 1877 in La Mancha, Municipality of Actopan, State of Veracruz, in eastern México (19.618180 N, 96.444569 W 6 m elevation). The animals were on the border of a water body, They were observed for a few minutes and photographed. The back of the frog was damaged and being eaten by the spider (Figure 1). Anurans have a key role in food webs, acting as either important predators or significant prey, and link terrestrial and aquatic ecosystems (Wilbur, 1997; Whiles et al., 2006). This record contributes to the knowledge of the predator-prey relationship between amphibians and arthropods especially the spiders.

I thank Miguel Angel Avila Lazcano for the spider identification

Fig. 1. Leptodactylus melanonotus being preyed by Cupiennius salei..JPG (6.1MB)

Literature Cited

Maffei F., Ubaid, F.K., Jim J.
2010. *Predation of herps by spiders (Araneae) in the Brazilian Cerrado* Herpetology Notes, volume 3: 167-170.

Menin, M., Rodrigues, D.J., Azevedo, C.S.
2005. *Predation on amphibians by spiders (Arachnida, Araneae) in the Neotropical region*. Phyllomedusa. 4(1): 39-47.

Toledo, L.F.
2005. *Predation of juvenile and adult anurans by invertebrates: current knowledge and perspectives*. Herpetol. Rev. 36 (4): 395-400.

Vitt, L.J. and J. Caldwell.
2009. *Herpetology: an introductory biology of amphibians and reptiles. San Diego:* Academic Press. 697p. upiennius salei Keyserling, 1877.

Wells, K. D.
2007. *Ecology and behavior of amphibians*. The University of Chicago Press. 1148 pp.

Whiles, M.R., Lips, K.R., Pringle, C.M., Kilham, S.S., Bixby, R.J., Brenes, R., Connelly, S., Colon-Gaud, J.C., Huntebrown, M., Huryn, A.D., Montgomer, Y.C., Peterson, S.
2006. *The effects of amphibian population declines on the structure and function of Neotropical stream ecosystems*. Frontiers in Ecology and the Environment 4(1): 27-34.

Wilbur, H.M.
1997. *Experimental ecology of food webs: Complex systems in temporary ponds*. Ecology 78(8): 2279-2302.

Rafael Alejandro Calzada-Arciniega, Avenida de los Barrios Número 1, Colonia Los Reyes Iztacala Tlalnepantla, Estado de México, C.P. 54090 (raejandrocalzada89@gmail.com)

Received: 17 October 2014
Accepted: 20 October 2014

News and Notes:

News and Notes:

Society Publication

Back issues of the Bulletin of the Maryland Herpetological Society, where available, may be obtained by writing the Executive Editor. A list of available issues will be sent upon request. Individual numbers in stock are $5.00 each, unless otherwise noted.

The Society also publishes a Newsletter on a somewhat irregular basis. These are distributed to the membership free of charge. Also published are Maryland Herpetofauna Leaflets and these are available at $.25/page.

Information for Authors

All correspondence should be addressed to the Executive Editor. Manuscripts being submitted for publication should be typewritten (double spaced) on good quality 8 1/2 by 11 inch paper with adequate margins. Submit original and first carbon, retaining the second carbon. If entered on a word processor, also submit diskette and note word processor and operating system used. Indicate where illustrations or photographs are to appear in text. Cite all literature used at end in alphabetical order by author.

Major papers are those over five pages (double spaced, elite type) and must include an abstract. The authors name should be centered under the title, and the address is to follow the Literature Cited. Minor papers are those papers with fewer than five pages. Author's name is to be placed at end of paper (see recent issue). For additional information see *Style Manual for Biological Journals* (1964), American Institute of Biological Sciences, 3900 Wisconsin Avenue, N.W., Washington, D.C. 20016.

Reprints are available at $.07 a page and should be ordered when manuscripts are submitted or when proofs are returned. Minimum order is 100 reprints. Either edited manuscript or proof will be returned to author for approval or correction. The author will be responsible for all corrections to proof, and must return proof preferably within seven days.

The Maryland Herpetological Society
The Natural History Society of Maryland
P.O. Box 18750
6908 Belair Road
Baltimore, MD 21206

Natural History Society of Maryland

Maryland
Herpetological
Society