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Density, length frequency distribution, growth rate, and condition of the freshwater mussel, Anodonta imbecillis Say 1829, in six ponds at the Clifton F. McClintic Public Hunting and Fishing Area, Mason County, West Virginia

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Density, Length Frequency Distribution, Growth Rate, and Condition of the Freshwater Mussel, *Anodonta imbecillis* Say 1829, in Six Ponds at the Clifton F. McClintic Public Hunting and Fishing Area, Mason County, West Virginia

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ABSTRACT

Populations of the freshwater mussel *Anodonta imbecillis* Say 1829, were sampled from six ponds at the Clifton F. McClintic Public Hunting and Fishing Area in Mason County West Virginia from April 1986 to May 1987. One of the six ponds, pond 12, is contaminated with byproducts from the manufacture of TNT which occurred at the site during World War II. Population density, length frequency distribution, growth rates and condition indices were examined for each population to establish baseline data for the species and to determine what effects the contaminants might be having on these organisms.

Population densities ranged from 4.8 mussels per square meter in pond 6 to 26.0 individuals per square meter in pond 27. All ponds except pond 12 were dominated by the 70.0 - 80.0 mm size class. The 60.0 - 70.0 mm size class was dominant in pond 12. Combined growth data revealed rapid growth for this species resulting in attainment of 90% of their estimated maximum size by the fifth year. Growth rates for pond 12 mussels were lower than for other ponds although these mussels were not significantly less healthy. Pond 6 mussels were found to be healthier by

all measurements used.

While there is no strong evidence for decreased health in pond 12 mussels, decreased growth rates and some degree of stunting seem to exist. Further investigation such as soft tissue analysis to test for possible accumulation of the contaminants is necessary in order to fully understand the effects of the presence of these chemical compounds on this species.

Table of Contents

Table of Contents (cont.)

List of Plates

List of Figures

List of Figures (cont.)

List of Figures (cont.)

List of Tables (cont.)

List of Tables

Chapter I

INTRODUCTION

> "Not war, but a plethora of manmade things ... is threatening to strangle us, suffocate us, bury us, in the debris and by-products of our technologically inventive and irresponsible age ". "Along with the possibility of the extinction of mankind by nuclear war, the central problem of our age has therefore become the contamination of man's total environment with such substances of incredible potential for harm - substances that accumulate in the tissues of plants and animals and even penetrate the germ cells to shatter or alter the very material of heredity upon which the shape of the future depends ".

These statements by Margaret Mead and Rachel Carson (1962), respectively, express their grave concern over a manmade nightmare that perhaps will haunt man for many generations. War is a threat, but chemical contamination of our environment, as potentially deadly in the long run, is a fact that we must deal with daily. Our insistence on "improving" life through technological advances, otherwise known as progress, has lead to the contamination of our entire world with thousands of synthetic chemical compounds with which the earth and its inhabitants must find ways to cope.

Pesticides and industrial byproducts are two major groups of synthetic chemicals which pollute our environment so thoroughly that virtually no living organism has escaped contamination. Even those that are the most susceptible to such substances, the unborn, are not free from such contamination. The scope of damage caused by such agents is not likely to be known before such damage has had time to occur.

Our ignorance of the power possessed by the chemicals we create is one of the major contributors to the problem. In the case of pesticides, much more is commonly used than actually needed as the result of the "if some is good, a lot is great" mentality of its users. As a result, levels of the well known pesticide DDT often exceed 12 parts per million (ppm) in human fat, and in the early 1970s, most mother's milk in the United States would have been considered illegal for interstate commerce because it contained such high levels of this substance (Ehrlich, et al. 1977). The view of "the solution to pollution is dilution" has been and still is wide spread, resulting in the contamination of not only inland waters but the oceans themselves.

Beginning with the discovery of dynamite in 1867 by the Swedish chemist Alfred Bernhard Nobel, many explosive chemical compounds were synthesized that could be used in war. In many cases, however, contamination of the environment resulted from production of such compounds. Commercially, the period from 1865 to 1955 could be called the dynamite era. During this era TNT (trinitrotoluene), first synthesized in Europe around 1907, was second only to nitroglycerine in importance as an explosive. It was the major component of high explosive shells, bombs, torpedoes, and mines during World Wars I and II.

The process employed in the production of TNT, known as the batch process, involves the nitration of toluene by the addition of nitric acid and sulfuric acid. Major by-products resulting from this process include 2,4-dinitrotoluene (2,4-DNT) and 2,6-dinitrotoluene (2,6-DNT). Toluene and phenol also occur as by-products. These byproducts are often found as contaminants of both soil and water systems in and around the areas of production. One such former TNT production site is presently occupied by the Clifton F. McClintic Public Hunting and Fishing Area (formerly McClintic Wildlife Station) in West Virginia (Figure 1).

The Clifton F. McClintic Public Hunting and Fishing Area occupies 2,788 acres along W. Va. Route 62 approximately six miles northeast of Point Pleasant in Mason County, West Virginia (USGS Topographic Map, Cheshire Quadrangle, 1968, photorevised 1975). This state owned property is managed as a public hunting and fishing facility by the West Virginia Department of Natural Resources. Within the station's boundaries are 35 manmade ponds, most of which were constructed between 1953 and 1955.

Chapter II

HISTORY OF THE STUDY SITE

The boundaries of the McClintic Hunting and Fishing Area lie within the former boundaries of the 8,323 acre West Virginia Ordnance Works (WVOW) that from 1942 to 1945 produced trinitrotoluene for use in World War II (Figure 2). Soil contamination occurred in several areas as a result of this production (Figure 3). One of these, the former "TNT area" where the actual production process occurred, lies in close proximity to several of the station's ponds. Another source was the burning grounds where below specification TNT was destroyed. This area is also close to several McClintic ponds. The former waste water disposal reservoirs which received acid wastes from the TNT production area and were known as "red water" and "yellow water" reservoirs, are now actually sites of three of the station's ponds. Sewer lines that carried liquid wastes from the process sites to these reservoirs, as well as underground process lines, have deteriorated and are also involved in the contamination of soil and water in the area.

In 1945, when WVOW ended operations at this site, decontamination of the facilities was carried out. No records are known to exist revealing the methods used or the extent to which this work was done, but it now appears that decontamination efforts concentrated on above ground structures. A second decontamination effort took place in the late 1950s in the area of process lines 8, 9 and 10 (Figure 4). Process lines 11 and 12 were never activated, thus no decontamination was required.

No further decontamination efforts were undertaken until a red water seepage was noticed in May 1981 near pond 13 (Figure 4). This pond is located near a sewer line pumping station that carried wastes from the TNT production site to the red water reservoirs. Investigations by the W. Va. DNR and U. S. EPA led to ranking the site 84th on the National Priorities List under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) (PL 96-510 amended by PL 97-272). Contamination migration has recently been assessed by Environmental Science Engineering, Inc. (ESE 1984 and 1986) through contract no. DAAK 11-83-D-007 awarded by the U. S. Army Toxic and Hazardous Materials Agency.

Chapter III

THE PROBLEM

The NUS Corp. (1983) tested the water seeping into pond 13 from the sewer line in 1981 and found it to be contaminated by 2,4-DNT at levels up to 710 ppm (parts per million), 2,6-DNT up to 130 ppm, TNT up to 16.6 ppm and phenol at 3.1 ppm. Sampling by ESE has revealed the presence of TNT, 2,4-DNT and 2,6-DNT in the water of pond 13. Levels as high as 3.2 ppm were found for TNT. In addition, pond 12, which receives water from pond 13, was also shown to be contaminated, though to a lesser extent (0.08 ppm for 2,6-DNT) than found in pond 13.

Fish tissue samples from ponds 12 and 13 revealed the presence of 2,4-DNT but the levels were below the quantifiable limit (ESE, 1986). As a result of this finding, fishing in ponds 12 and 13 has been banned.

While the ESE Corp. chose to monitor water and sediments for the presence and migration of these contaminants, no direct research into the effects of these chemicals on the local biota was planned. Concern over the environmental effects had taken a back seat to mere monitoring the location and movement of the problem causing agents. To remedy this situation, I chose to use a freshwater mussel, *Anodonta imbecillis* Say 1829, the Paper Pond Shell, as a biological monitor (Plate 1).

Anodonta imbecillis is the only Unionid bivalve found in the McClintic ponds. Fingernail clams (Sphaerids) are occasionally found in the spring in some of the ponds but their numbers are very few. The presence of these "pure" cultures made it especially easy to perform a study such as this. The presence of other bivalve populations may have altered the results through competition for space and/or food. *Anodonta imbecillis*, as the sole representative of the Unionid bivalves in these aquatic communities, comprise a major component of the benthic biomass.

A combination of factors makes *A. imbecillis* unique among the freshwater bivalves. First, its ability to pass through the larval (glochidial) stage without parasitism on the gills of fish enables populations of this species to become established and remain viable in the absence of a fish host(s). Second, as an hermaphroditic species, it is well suited for survival in standing water habitats such as occur in ponds and lakes. In addition, this allows for easy evaluation of health since allowances for differences in male-female reproductive states are unnecessary. Third, A. imbecillis has been shown to respire normally at dissolved oxygen levels as low as 0.73 ml/L (Hiestand, 1938). These, and other factors, enable this species to survive in locations and situations otherwise inhospitable to many species of freshwater mussels.

For this research, I chose to examine three parameters of the existing mussel populations: 1) population densities and length frequency distributions, 2) growth rates, and 3) condition indices (CI). Population density and length frequency distribution measurements reveal the status of the population within a given pond at a given time and provide baseline information that is useful in growth and CI evaluation. Growth rate of mussels, in terms of both length and weight, were investigated in an attempt to establish baseline growth patterns (not available in the literature) for this species in non-contaminated ponds and compare this with growth in contaminated ponds. Condition indices, a measure of health, were examined in order to further understand the effects of contamination and seasonal variations on the mussel populations.

Several advantages accompany the use of freshwater mussels in this type of work. Their sedentary life style assures their continuing presence and accessibility in the area of study. Being filter feeders, they come in contact with the contaminants through both water and food. In addition, most mussel species are considered to be long lived, allowing for extended observation.

Chapter IV

DESCRIPTION OF STUDY SITES AND SAMPLE LOCATIONS

Early investigations of the ponds in the McClintic Wildlife Station by Dr. James Joy and myself revealed populations of Anodonta imbecillis existing in only six (Ponds 6, 12, 14, 15, 27 and 30) of the approximately 35 ponds.

All of the study ponds cover less than 12,000 square meters (1.2 hectares) with the smallest covering approximately 6,000 square meters (0.6 hectares). All ponds except 6 and 12 receive treatment with the herbicide 2,4-D on an "as needed" basis to control aquatic and bank vegetation (Doerfer, pers. comm., 1987).

Pond 6 is a long, narrow pond located along what was once a WVOW patrol road (Figure 1). This pond served as the control pond since it is uncontaminated by TNT or its byproducts (ESE, 1986) and is also left untreated by the DNR with regards to herbicides. This pond is open to fishing from Memorial Day to October 1 and is set aside as one of the station's bird sanctuaries.

All sampling in this pond was restricted to the south and east banks. Mussels being studied for growth were placed at MG (Military Grid) 07530748 (Plate 2) (all MG locations included in this study are from USGS Topographic Map, Cheshire Quadrangle, 1968, photorevised 1975).

Water levels in this pond fluctuated more than in others (0.5 - 1.0 Meter) due to the activity of very persistent beavers clogging the overflow pipe and our subsequent efforts to unclog it. The substrate is composed of organic material such as decaying leaves etc. overlaying a soft silty base layer. Without herbicide treatment, the growth of aquatic plants is often substantial. Few trees are located in the area of the sample stations, but trees line the opposite bank. Water depth increases fairly rapidly from shore to a maximum depth of 2.0 - 3.0 meters.

Pond 12, shown to be contaminated with TNT and its byproducts, is a nearly square pond located at the end of the main entrance road to the station (Figure 1). This pond is off limits to fishermen. Sampling in this pond was restricted to the southeast side, with the growth study site established at MG 07200725 (Plate 3).

Water fluctuation of approximately 0.5 meter was noted to occur during the course of this study. This pond appears to have the greatest depth of the six study ponds. Depth increases rapidly as distance from shore increases, and probably exceeds 4.0 meters. For this reason, sample stations were confined to very near the shore. In the sample area, the substrate is mostly soft clay and silt. Much of the bank line of this pond is lined with trees, including the sample area.

Pond 13, the main site of contamination, lies to the northwest of pond 12 (Figure 1). This is a very shallow, swampy pond clogged with vegetation and in advancing stages of succession (Plate 4). An overflow from this pond feeds into the northwest side of pond 12.

Pond 14 is located to the northwest of and drains into pond 13 (Figure 1). This large, square pond is open for fishing throughout the year. The southwest and southeast banks served as sample areas with the growth study population being placed at MG 06930743 (Plate 5).

Water fluctuation in this pond was less than 0.5 meter. Maximum depth is estimated at 2.0 - 3.0 meters with a gradual slope from the shore. Substrate in the pond is more variable than in the others in that it ranges from areas of clay to those of high organic content. Many trees line the shore of this pond, but few are located in the vicinity of the sample area.

Pond 15 is to the northwest of pond 14 along a side road that leads to the DNR offices (Figure 1). This is an irregular, more or less C shaped pond in which fishing is allowed year round. The experimental algae control dye Aquashade has been used in this pond on one occasion in the recent past. Sampling was done along the southern end of the pond. The growth study population was placed at MG 06780759 (Plate 6).

A water fluctuation between 0.5 to 0.75 meter was noted over the study period. Maximum depth is approximately 2.0 to 3.0 meters. The substrate in this pond is more variable than in any of the others. Areas of clay, sand, silt and organic matter were all noted. Trees are nearly absent in the sampling area.

Pond 27 is located to the southwest of the pond 12-15 complex along a side road (Figure 1). This relatively small (6000 square meters) pond of irregular shape is open to all year fishing. Samples were taken primarily from the southeast and southwest banks. The growth study population was placed at MG 05900642 (Plate 7).

Water fluctuation in this pond was minimal during the study period (less than 0.5 meter) and the maximum depth is less than 2.0 meters. A fairly uniform, highly organic substrate exists here and is much thicker than in the other ponds, making collecting of samples by wading difficult. Few trees are located near the water's edge in the sampling area.

Pond 30 is located to the southwest of pond 27 (Figure 1). The topography is such that water flows from pond 27 to pond 30. This pond is fished throughout the year. Samples were taken from the east side of the pond and the growth population was located at MG 05800617 (Plate 8).

Little water fluctuation was noted here and there is a maximum depth of less than 2.0 meters. The substrate is less organic than found in pond 27. Clay and sand are the dominant substrates with interspersed areas of organic matter. Trees are lacking from the banks along the sample area.

Chapter V

REVIEW OF THE LITERATURE

The monitoring of freshwater environments by examination of the uptake and accumulation of certain organic compounds and heavy metals is not a new idea. Examination of river pollution by Hynes (1957) is one of the early works in this area.

Biological Monitors

Bivalves tend to accumulate several heavy metals including, mercury, lead, silver, nickel, cadmium, copper and zinc in their soft tissues, or viscera. Schuster and Pringle (1969) demonstrated that *Crassostrea virginica*, the oyster, can accumulate zinc to over 3000 ppm after 18 to 20 weeks exposure to 0.2 ppm zinc in sea water. While this may not be an example of a freshwater situation, the same type of accumulation is known to occur in freshwater species. For example, *Corbicula fluminea*, the Asiatic Clam, was shown by Joy, et al., (1983) to accumulate manganese and chromium, but not zinc, suggesting that some species may be well suited for monitoring only certain metals. Other bivalve species have been shown to accumulate one or more of the above mentioned metals (Roosenberg, 1969; Rogers, et al., 1977; Graney, et al., 1978).

Most of the literature concerning accumulation of organics in bivalve tissues pertains to marine species. Crassostrea virginica was shown to be an excellent indicator of the presence of PCBs in the marine environment (Duke, et al., 1970). The freshwater import, Corbicula fluminea, concentrates chlorinated organics according to a study in the Columbia River by Claeys, et al., (1975). Joy (1983) supported this finding in the Kanawha River, West Virginia population of C. fluminea. In his study, four caged populations were maintained at different locations in the river and were shown to accumulate PCBs.

Bedford, et al., (1968) and Bedford and Zabik (1973) examined the accumulation of DDT and its metabolites in freshwater mussels. They determined that these materials concentrated to considerably higher levels in the soft tissues than found in the water. Leard, et al., (1980) studied seven species of freshwater mussels and concluded that freshwater bivalves are " ... effective monitors of pesticide content."

While no literature was found dealing specifically with the uptake and accumulation of TNT and its byproducts by mussels, it may be assumed that such could likely occur.

TNT and its byproducts are suspected carcinogens. Mori, et al., (1984) examined the metabolic conversion in mice of 2,4 - DNT to 2,4 - diaminotoluene, which is highly suspected of being carcinogenic. Guest (1982) points to intestinal microflora as being a site of 2,4 - DNT metabolism in man. Here he found production of two toxic and possibly carcinogenic metabolites of this TNT byproduct. Whong, et al., (1984) and Sundvall, et al., (1984) have studied the toxic and mutagenic effects of waste water components from TNT manufacturing sites on the bacterium *Salmonella typhimurium*. They found that these nitroaromatic compounds are mutagenic in several strains of this species of bacteria. They also discovered that metabolites of TNT and DNT have a greater mutagenicity than the parent compounds.

Growth

Published attempts to analyze growth rates in freshwater mussels appeared first in the early 1900s. At that time, the pearl button industry, with shells of mussels as its raw material, was in its zenith. This served as the initiator of interest in the life histories of the mussels. Investigators such as Lefevre and Curtis (1912), Isely (1913), Coker, et al., (1920), Grier (1922), Howard (1922) and Chamberlain (1931) began to establish some basic principles concerning the growth of mussels. In general, it was established that, like most animals, the most rapid growth occurs among the smaller individuals, with a slowing of the growth process as size increases (Lefevre and Curtis 1912). In addition, the rates of growth were found to range from very rapid in the thin shelled mussels to very slow in the thick shelled species (Isely, 1913; Grier, 1922). Howard (1922) found that seasonal growth occurs in the Mississippi River bivalve fauna, but ceases when water temperature falls below 13°C. Isely (1913) also noted high variability in growth rates among the same species in the same river.

While this information provided a good base for further investigation, little in-depth work into growth rates of individual mussel species has followed. This, in part, is due to the loss of much of the commercial value of mussels. The pearl button industry collapsed with the introduction of plastic buttons in the 1940's. In the 1960's, a renewed interest in mussels was sparked as the result of the demand for shells as a source of raw material in the Japanese cultured pearl industry.

Little and Gentner (1970) examined growth of *Amblema perplicata*. Their work established good baseline data for this species. Few other species of freshwater mussels have been studied as well.

The only published growth data found concerning *Anodonta imbecillis*, the target species in this study, was that of *one* individual examined by Lefevre and Curtis (1912). They reported an increase in length from 30 mm to 61 mm in two years with a corresponding weight gain of from 8.0 gms to 13.3 gms for a single caged specimen in the Mississippi River.

Much more information exists on the growth rates of the commercially important edible bivalves such as the clam *Mya arenaria* and the Hard Clam *Mercenaria mercenaria*. The effects of temperature, substrate and salinity on growth rates of *Mya arenaria* were first examined by Belding (1916). Results corresponding to the growth principles found in freshwater mussels have also been discovered for these salt water species (Newcombe, 1936; Newcombe and Kessler, 1936; and Loesch and Haven 1973).

Extensive investigation into the growth rates of Corbicula fluminea has taken place due to its rapid invasion and "pest" status in the United States (O'Kane, 1976; Poole and Tilly, 1977; Britton, et al;, 1979; Mattice, 1979; Dreier and Tranquilli, 1981; Welch and Joy, 1984; Joy, 1985; Mattice and Wright, 1985). This species also follows the general principles of growth found for other bivalves.

Health

The use of mathematical formulas to express the condition or health of clams was first advocated by Hopkins (1937). He used a formula based on the inner volume to dry weight ratio to define the condition of oysters. Medcof and Needler (1941) slightly modified this formula. Information that suggests a correlation between dry weight-inner volume ratios and glycogen content in oysters was presented by Ingle (1949).

Haukioja and Hakala (1978) examined the ratio of the weight of soft body parts to the cube of length as a measure of condition indices (CI). They noted that annual variation in CI can be high depending on availability of food resources. Gunter (1938), working with oysters, and Joy (1983), working with Corbicula fluminea, both pointed out that CI values vary with location of sample populations, even within the same water system. Jørgensen (1976) points out variability in soft tissue to length ratios with food availability, reproductive state, and size of the bivalve. In other words, season and habitat will play a role in the CI values of mussels.

Chapter VI

MATERIALS AND METHODS

Density and Length Frequency Distribution

Between 26 April 1986 and 1 June 1986, each of the six sample ponds was examined by quadrat sampling in order to determine the density and length frequency distribution of their respective mussel populations. In addition, this sampling provided a test population for growth studies.

This quadrat sampling procedure involved the use of a square wooden frame, constructed of 1" by 1" stakes, with an interior area of 1.0 square meter. The frame was randomly placed into each pond a total of five times at or near the bank. Water depth in these sample areas varied from a few centimeters to a maximum of one meter. After each placement of the frame, all mussels within the square were removed by hand and placed in a 10 liter plastic bucket containing pond water. Special care was taken in order to reduce biasing of the length frequency distribution caused by overlooking the smaller individuals within the sample area. After all five samples were completed, the mussels from each bucket were wiped clean of epiflora and epifauna and dried on

Approximately 120 days after the initial measurements were recorded on the numbered specimens, attempts were made to recover as many of these numbered individuals as possible from each pond for analyses of summer growth rates. Those individuals recovered were cleaned, dried, measured to the nearest 0.1 mm, and weighed to the nearest 0.1 gm as before. They were then returned to the area from which they were taken. This process was repeated the following spring, approximately one year after the initial marking date. Thus growth data was obtained for periods of four summer months and a full calender year.

Data derived from summer and annual growth studies in each pond were summarized using Walford transformation plots (Walford, 1946). Regression lines were fitted to the data using the least squares method. The resulting line could be expressed by the general linear equation: Y = a + bX, but a more informative equation is:

$$L_{(t+1)} = L_{(t)}(1-k) + kL_{(t)}$$

where $L_{(t)}$ = X (length at time t), $L_{(t+1)}$ = Y (length at end of some time interval), $L_{(t)}(1-k)$ = a (the Y-intercept), and k = b (the slope of the line) (Loesch and Haven 1973). L_{∞} is an expression of the theoretical maximum mean length and is reached when the derived regression line meets a line 45° above the X-axis (when $L_{(t)} = L_{(t+1)}$).

In addition to growth in length, weight gain can also be analysed using this same formula by simply substituting W for L.

The Walford transformation is used to estimate growth independently of age. In order to relate length to age, the average size of at least one age group must be known. Hudson and Isom (1984) used juveniles of Anodonta imbecillis with an initial length of 0.28 mm at 2-3 days old in their experiments on laboratory rearing of the species. Using this value as $L_{(t)}$ where t = 0 and substituting it into the derived growth equations, an estimated length at age one ($L_{(t+1)}$) results. The age one length can then be used to derive an estimated age two size, and so on. From this information, growth curves can be constructed. While a known length at some age other than zero would most likely give better results, obtaining such information in much more difficult.

Health

The Condition Index (CI) of mussels in pond 27 was determined from samples taken on 2 May and 30 August 1986. Mussels representing the size range of the population available by hand-picking were collected and returned to the lab in a plastic bucket of pond water. After 24 hours, the mussels were dried and cleaned of debris. They were then measured to the nearest 0.1 mm (length = L) with vernier calipers and weighed to the nearest 0.01 gm (total weight = TW) on an Ohaus Galaxy 400 toploading digital balance. The mussels were then opened by inserting a scalpel to sever the anterior and posterior adductor mussels. The soft tissues (viscera) were then removed and blotted dry on Teri-towels and placed in pre-weighed aluminum pans for weighing to the nearest 0.01 gm. This weight represented the wet viscera weight (WVW) of the individual. The pans containing the viscera were then placed in a drying oven at 45°C for 24 hours to reach a dry viscera weight (DVW) which was also recorded to the nearest 0.01 gm. Shells were allowed to dry at room temperature for 24 hours. Individual dry shell weights (DSW) were then recorded to the nearest 0.01 gm.

A third sample for Condition Index measurement was conducted on 17 April 1987 and included mussels from all six study ponds. Mussels in the 60.0 - 69.9 mm length class range were examined in order to reduce variation in CI values due to length. In cases where an insufficient number of mussels within this size range were available, slightly smaller or larger individuals were included. These mussels were processed in the same manner as the previous samples.

Many approaches have been employed in the analysis of condition, or health, of bivalves. While no one formula is standard among the researchers investigating the health of bivalves, most use some measure of weight, but may or may not incorporate length into their calculations. The ideal formula would be expected to give somewhat consistent values among all size groups within a population at a given time. Variations from time to time and from population to population could then be detected. I chose to examine this problem by analysing two formulas found in the literature which have been used to study bivalve health. In addition, length-weight curves were examined for their usefulness in determining health. One goal was, therefore, to determine which method, if any, could best be used to evaluate health of mussels. The second goal was to determine if the mussel population in the contaminated pond showed signs of decreased health in comparison to those in the non-contaminated ponds.

Joy's (1985) formula using total weight (TW) and wet viscera weight (WVW) to arrive at a CI expressed as a percentage, was the first method employed in this study. A high value derived from the equation:

$$WVW/TW \times 100 = \_\_\_\% = CI$$

should theoretically represent a greater degree of health than a low value. Values obtained from this formula for the three pond 27 samples were then regressed with shell length for analysis of variations in health over time.

The Newman-Keuls method of multiple comparisons (Glass and Hopkins, 1984) was employed to analyze the values derived by this method from the six samples collected on 17 April 1987.

Regression analyses for total weight, water weight, wet viscera weight, dry shell weight and dry viscera weight vs. length were performed on the data obtained from the first two samples from pond 27. The resulting regression lines were then used to compare the samples as well as to examine the use of this tool in determining mussel health.

While Joy's formula omits length as a parameter for determining CI, Haukioja and Hakala (1978) used the viscera weight to length relationship of:

$$CI = weight/\ length^3$$

to express condition. Dry viscera weight was used in their calculation. Total weight, water weight, wet viscera weight, dry shell weight and dry viscera weight were all used in this analysis. The resulting values were then plotted against shell length.

Using this formula, a higher degree of health is indicated by a larger value, regardless of whether dry viscera weight or total weight is used. Changes in values derived from shell weight or water weight with length do not necessarily indicate variations in health but may be used to reveal their effects on the condition index derived from total weight, of which they are components. In other words, a change with length in the values derived from total weight may be due to changes in one or more of the other components of total weight. It is important to be able to determine which of the components of total weight is the varying factor from one sample to another in order to make judgements concerning health of the populations in question.

Analysis of the CI values derived from this formula for the 17 April 1987 sample data also employed the Newman-Keuls method of multiple comparisons.

Water Quality

Water samples were taken at regular intervals from each pond. Analysis of the parameters of dissolved oxygen, total hardness, carbon dioxide and pH was accomplished using Hach Kits. Water temperature was also recorded.

Chapter VII

RESULTS

Density and Frequency Distribution

Populations of Anodonta imbecillis showed considerable variation among study ponds with respect to density and length frequency distribution during the initial sampling in the spring of 1986. Density ranged from a low of 4.8 individuals per square meter in pond 6 to a high of 26.0 per square meter in pond 27. Intermediate density values of 5.6 in pond 15, 8.6 in pond 14, 12.8 in pond 30 and 20.2 in pond 12 were obtained.

Length frequency distributions also varied somewhat among populations (Figure 5). Populations in four ponds, 14, 15, 27 and 30, were dominated by individuals in the 70.0 to 79.9 mm size class range with the 60.0 - 69.9 mm size class being second in dominance in ponds 14, 15 and 30. Pond 6 was found to be dominated by the 80.0 - 89.9 mm length class, followed by the 70.0 - 79.9 mm class. The pond 12 population was unusual due to the virtual lack of 70.0 + mm mussels and the presence of a dominant 60.0 - 69.9 mm size class. The 50.0 - 59.9 mm class was found to be next in dominance. In all ponds, larger mussels (50.0 - 89.9 mm) greatly outnumbered smaller individuals (0 - 49.9 mm). This may be due in part to the sampling method which would undoubtedly be biased against the smaller individuals.

Growth

Summer growth data were obtained for <u>Anodonta imbecillis</u> in four of the six study ponds. Searches of the sites where mussels had been placed in ponds 27 and 30 yielded mostly dead individuals, both numbered and unnumbered. Subsequent attempts to collect mussels in these ponds for condition analysis and other, non-related studies revealed drastically altered population compositions and densities as compared to those found during the initial work in the spring of 1986.

Of the four ponds in which summer growth data is available, mussels in pond 12, the contaminated pond, showed by far the least growth (Figure 6). While the size range studied in this pond was limited, it was not restricted to the larger, normally slow growing mussels, but was represented by a size range that, in other ponds, showed considerable more growth. The largest increase in length of the 15 recovered mussels was 2.0 mm for an individual with an initial length of 65.0 mm.

The weight measurements for the late summer sample showed some slight increases and several decreases over the initial spring measurements (Figure 7).

Pond 6 mussels showed considerable advances in both length and weight gains over the four month study period (Figures 8 and 9). The Y-intercept value of 25.7 can be used as a prediction of the approximate length (in mm) a mussel would reach after only four months growth. An increase of 2.0 to 3.0 mm in the larger mussels (70.0 + mm) of pond 6 was not unusual. No losses in weight were noted in pond 6 individuals, and several of the smaller individuals had substantial weight gains.

Fourteen mussels were recovered from pond 15 and fifteen were recovered from pond 14. While these were not extremely low sample numbers in comparison to the other samples, they were more restricted in their representation of size ranges, and therefore of limited value when considered alone. These ponds were represented mostly by individuals with initial measurements above 70.0 mm, and in both ponds this group showed slight increases in length as would be expected for this size group (Figures 10 and 12). A number of mussels in each of the two ponds were found to have lost weight over the summer (Figures 11 and 13). This appears to have been limited to larger individuals and involved only slight declines in weight.

Annual growth data were available in three of the six study ponds. As previously mentioned, study populations in ponds 27 and 30 had been nearly eliminated prior to the fall recapture date. In addition, no numbered mussels were found at the pond 12 location at the end of one year.

The pond 6 sample seems to give a realistic growth pattern for all sizes (Figure 14). The resulting annual growth equation for this population predicts a growth to 35.0 mm in the first year after the glochidium settles to the substrate. No weight loss was noted for any of the pond 6 mussels (Figure 15). Weight gain was much greater in the initially small individuals than in the larger ones.

Small sample sizes and restricted size ranges in ponds 14 and 15 again made the growth equations for these ponds of limited value. Even so, both samples indicate that mussels in the 70.0 mm size class range and above continue to increase in length at a slow rate (Figures 16 and 18). However, the growth equations derived from such limited data tend to overestimate the growth rates of smaller individuals, and therefore should be examined with caution. Larger individuals appeared to loose weight to a small extent in pond 15 while those of the same size and weight in pond 14 continued to gain weight (Figures 17 and 19).

Growth curves derived from the equations for annual growth representing these three ponds reveal similar overall patterns (Figure 20). In ponds 6 and 14, 90% of the estimated maximum length (83.0 - 84.0 mm) is reached during the fourth year of growth. After six to seven years, annual increments of less than 1.0 mm are added to the length of the mussel. Pond 15 mussels reach 90% of their estimated length (75.0 mm) after only three years and slow to less than 1.0 mm annual growth after only five years. These growth curves are, again, based on a limited number and size range of individuals, especially in ponds 14 and 15.

By combining the annual growth data obtained in this study to that acquired by Dr. James Joy (unpublished data) for the same populations from 1984 to 1986, composite Walford transformation plots for length and weight made available growth equations based on large numbers of individuals (Figures 21 and 22). The composite growth equation for length, therefore, is based on 81 individuals with initial lengths from 36.1 mm to 83.7 mm, and for weight, 80 individuals from 2.3 gms to 40.3 gms.

From the derived equation for length, it can be seen that a growth of approximately 31.0 mm is predicted by the Y-intercept for the first year of life in Anodonta imbecillis (Figure 21). A cumulative growth curve based on this equation estimates a maximum length of 81.0 mm for this species, 90% of which is reached in the fifth year of growth (Figure 23). After 8 years, annual growth increments are less than 1.0 mm.

The equation derived from the composite Walford transformation for annual weight gain estimates a weight of 8.9 grams for a one year old mussel (Figure 22).

Health

The Anodonta imbecillis population in pond 27 was sampled three times for determination of condition indices. The first sample was conducted on 2 May 1986 and involved 49 mussels with a size range of 35.4 mm to 88.2 mm. This represents the largest and most diverse of the three samples. CI values determined from Joy's formula ranged from a low of 14.4 for an individual 79.6 mm in length to a high of 51.9 for a mussel of 45.0 mm.

The mean CI value derived from this group was 28.6. When regressed with length, a distinct decline in CI values were noted with increasing length (Figure 24).

Twenty-four mussels ranging from 48.4 mm to 84.9 mm were collected in the second attempt to analyze health, this time in the late summer of 1986. CI values from this sample, again using Joy's formula, ranged from 12.6 to 21.9 with a mean of 17.1 (Figure 24). As with the spring sample, this group also shows a decline in condition values with increasing shell length. Eventhough the sample means for the spring and summer samples are significantly different at the 99% confidence level, the slopes of their regression lines are nearly identical.

The third sample from pond 27 was taken on 17 April 1987 and was limited to a size range of 60.9 mm to 70.8 mm. Joy's condition index values ranged from 19.5 to 25.6 for this sample with a mean of 21.4 (Figure 24). The regression line for this group falls between those of the previous two and shows some increase in CI with increasing length although this may be misleading due to the small sample size.

Joy's condition index values for samples taken on 17 April 1987 from the other five study ponds were also determined (Table 1). Analysis of this data from each of the six ponds using the Newman-Keuls method of multiple comparisons indicates significant differences at the 95% confidence level between pond 6 and two of the remaining five ponds, ponds 14 and 15. All other comparisons between ponds yielded no significant difference at this confidence level.

Examination of the data collected from the 2 May 1986 and 30 August 1986 samples also involved plotting length vs. various weight measurements (Figure 25). Examination of these plots reveal differences in the data from spring to late summer. While total weight vs. length appear uniform from one sample to the other (Figure 25A), declines in dry shell weight and dry viscera weight are evident (Figure 25D and E), especially among larger individuals. Wet viscera weight declines, apparently in response to the drop in dry viscera weight (Figure 25C). A slight increase in water weight among larger individuals is also apparent (Figure 25B).

Analysis of the 30 August 1986 sample data from pond 27 using Haukioja and Hakala's formula indicates a slight loss in total weight in all sizes as compared to the spring sample (Figure 26A). This was not evident from Joy's CI method or the length-weight curves. A slight gain in water weight among larger mussels, as indicated by the length-weight curves, is also shown for the August sample by this method (Figure 26B). Unquestionably, declines in dry shell weight and dry viscera weight do occur from the May to August samples (Figure 26D and E).

The samples taken from all six study ponds on 17 April 1987 were analysed for health using Haukioja and Hakala's formula of DVW/L^3 as the CI parameter (Table 1). Analysis of this data using the Newman-Keuls method of multiple comparisons indicates that pond 6 mussels had significantly greater values of health than those in the other five ponds at the 95% confidence level. Pond 30 mussels were also found to be significantly healthier than those from pond 15.

Water Quality

Water quality results throughout the study period reveal variations within as well as among ponds (Tables 2-7). One noteworthy finding was the rise in pH during late summer in all ponds except 15. Temperatures peaked in mid July in all ponds with a high reading of 33 °C in pond 30 on 19 July 1986. Dissolved oxygen levels ranged from 4 to 15 mg/L but were generally in the 7 - 10 mg/L range during most of the year.

Chapter VIII

DISCUSSION

Density and Length Frequency Distribution

Individuals of Anodonta imbecillis were noted lying on the substrate surface in pond 27 as early as 14 March in 1986. Many of these were lying on their sides instead of being burrowed into the substrate as was most often the case at later observations. It is assumed that they winter below the substrate surface and then assume this position temporarily upon reaching the surface.

The length frequency distributions may, to some, suggest populations in a state of decline (Figure 5), however, this is not believed to be the case. Admittedly, the sampling method in itself is somewhat biased against finding young individuals. In addition, the growth results show that growth slows in this species at around 70.0 to 75.0 mm. This, then, would lead to an accumulation of age classes, and therefore a disproportionate number of individuals in the 70.0 + mm size range. From the cumulative growth curve (Figure 23), it can be seen that the 70.0 to 80.0 mm size class would be made up of individuals from approximately 4 to 9 years old while the smaller size classes represent only a single year class. The relative lack of 80.0 + mm mussels in five of the ponds suggests that these are the few "old" individuals left and are approximately ten years of age or older. A dominant 80.0 to 90.0 mm size class in pond 6 is supporting evidence that the mussels in this non-contaminated, non herbicide treated pond may be in a better environment than those in the other ponds. In addition, mussels up to 99.0 mm have been found in this pond, by far the largest from any pond.

From their length frequency distribution (Figure 5), pond 12 mussels appear stunted. Very few individuals over 70.0 mm have been found here. One possible explanation for this might be that the population is of recent origin and has not yet had enough time for individuals to accumulate in the larger size classes. Two points refute this possibility. First, the summer growth data from this study show very little growth during the time of year when most growth should occur (Figure 6). Second, growth studies initiated by Dr. Joy in the spring of 1985 indicated a dominance by individuals in the 50.0 to 70.0 mm range at that time also. Many of these should have had ample time to reach the 70.0 + mm range by the spring or fall of 1986, yet few did. It appears as though the size range at which growth tends to cease in this pond is at least 10.0 mm less than in other ponds.

Mussel populations in ponds 27 and 30 suffered a dramatic decline during the summer of 1986. Great numbers of empty shells were found in both ponds, including many from the growth study populations. Some of the empty shells had unquestionably been preyed upon by muskrats as evidenced by claw marks on the shells. However, the claw marks were found on only a small number of the empty shells, indicating death by some other unexplained means.

Examination of the water quality data for these two ponds reveal surface water temperatures in excess of 30 °C on two occasions in ponds 27 and 30 from late June to late July 1986 (Tables 6 and 7). The surface temperature in pond 6 also exceeded 30 °C on 29 July (Table 2). However, ponds 6, 12, 14, and 15 are all deeper and considerably more shaded than ponds 27 and 30. This suggests that the temperatures encountered by mussels in ponds 27 and 30 may have been greater than in the other ponds and may have contributed to the deaths. Salbenblat and Edgar (1964) reported that the freshwater mussel Anodontoides ferrusacianus could not withstand temperatures above 29 °C although Anodonta grandis and Lampsilis radiata luteola could. Hudson and Isom (1984) found no ill effect on juvenile A. imbecillis grown at 30°C.

Another water quality parameter that varied in the ponds was pH. Ponds 27 and 30 were not alone in this fluctuation, however. Values up to 9.5 were measured in pond 12 with values of 9.0 reached in ponds 6 and 27 (Tables 2, 3 and 6). Harman (1969) reported that "soft, poorly buffered waters may experience rapid changes in pH, which can, in turn, do harm to mussels". The rate of pH change could, therefore, affect mussels more than the actual pH values. The water monitoring system used here gives no indication of rate of pH change and therefore no judgement can be made about the contribution of pH to the deaths of these mussels. The sudden decline in A. imbecillis populations in ponds 27 and 30, then, remains an enigma.

Growth

Knight (1968) argued convincingly against the use of samples with limited size ranges for analysis of growth data, especially when making predictions about size ranges which are not represented in the sample. For this reason, strong emphasis is not placed on the individual sample population growth equations obtained in this study. Variations from 35.0 to 50.0 mm for the predicted amount of first year growth in ponds 6, 14 and 15 emphasize this point (Figure 20). These individual samples are useful where groups of a narrow size range are available for comparison or where two dissimilar size groups have similar growth rates, such as occurred in summer growth between ponds 6 and 12 (Figures 6 and 8).

By combining all of the annual growth data available from this study with that obtained in Dr. Joy's work, it was felt that all variations in initial size, yearly environmental conditions, and habitat as well as individual variations would be represented.

While no data were obtained for the growth of an individual with an initial size less than 36.1 mm, a prediction of 31.0 mm growth during the first year seems reasonable. Hudson and Isom's (1984) study on rearing juvenile A. imbecillis in the lab supports this prediction. In their study, individuals of this species were shown to reach 5.1 mm after approximately 10 weeks growth, an average of 0.51 mm per week. They reported, however, that growth was initially slow (0.16 mm/wk) but had increased substantially to 0.95 mm/wk. If a seven month (28 weeks) natural growth period (April to October) is assumed, the maximum length obtainable over this time period at the rate of 0.95 mm/wk is approximately 26.0 mm. While this is 5.0 mm short of the prediction from this study, it can be assumed that a somewhat slower growth may be occurring in the lab than in natural settings and therefore the two predictions are not believed to be significantly different.

The cumulative growth curve derived from the combined growth data indicates a rapid attainment of full size in *A. imbecillis* (Figure 23). From this curve, it appears as though only five years is necessary in order for this mussel species to reach 90% of its predicted maximum length of 81.0 mm. This compares to 11 years for the Hard Clam, *Mercenaria mercenaria*, (Loesch and Haven, 1973), 8 + years for the Lake Pepin Mucket, *Lampsilis siliquoidea pepinensis* and the Yellow Sand Shell, *Lampsilis anodontoides* (Chamberlain, 1931) and 10 + years for *Elliptio complanata* (Paterson, 1985). This rapid growth for a thin shelled species corresponds to Isely's (1913) findings in which he indicated that the shells may be built up at a greater expense of food and energy than the soft parts of the animal.

The equation derived from the Walford transformation for combined annual weight gain predicts a growth to 8.9 gms at the end of the first year for

A. imbecillis (Figure 22). While this may not appear unreasonable at first glance, further examination reveals a problem. If 31.0 mm is an acceptable length for a one year old mussel, it would have to weigh around 8.9 gms according to the two equations. The length-weight curve in Figure 27 shows that this cannot be the case. According to this curve, a 31.0 mm individual should weigh approximately 1.0 gm. (The weight of 8.0 gms for a 30.0 mm individual reported by Lefevre and Curtis (1912) in their growth study on a single *A. imbecillis* is doubtful. A weight of 0.8 gms is more reasonable.) A weight of 8.9 gms would not be reached until a mussel is approximately 52.0 mm in length, or approximately two years old according to the cumulative growth curve (Figure 23).

If the value of 8.9 gms is substituted into the Walford equation for combined weight gain (Figure 22) as that of a two year old mussel, a value of 15.9 gms is predicted for a three year old individual. From the cumulative growth curve (Figure 23), a three year old mussel would measure approximately 62.0 mm. A 62.0 mm, three year old mussel should weigh approximately 15.4 gms according to the length-weight curve (Figure 27) and this value is very near that of 15.9 predicted from the Walford transformation equation.

Figure 28 fits the cumulative growth curve to the length-weight curve to show the predicted length and weight at the end of each years growth. After two years a nearly linear relationship occurs between length and weight. The growth pattern for the first two years does not follow this pattern.

From this it can be seen that the 8.9 gms predicted for an average one year old individual is much too high and is instead more likely the weight of an average two year old mussel. Even though no data points are available, it can be seen from the length-weight curve (Figure 27) that as the young individuals increase in length up to around 30.0 mm, relatively little weight gain occurs. Up to this point, it could be argued, there is little space within the valves of the shell for accumulation of the water that becomes a major contributor to mussel weight, especially in larger individuals. An example of this was found for the 2 May 1986, pond 27 CI sample. The percent of total weight made up by water was found to increase from 75% at 30.0 mm to 85% at 85.0 mm (Figure 26B).

Health

The health of any population is thought to reflect the surrounding environment. While mussels may appear isolated from the environment by their shell, they are not. Water must be nearly constantly pumped over their gills and mantle for oxygen and carbon dioxide exchange and to bring in food and remove waste. This process brings the animal in direct contact with its environment.

Haukioja and Hakala (1978) have shown by transfer experiments with the freshwater mussel Anodonta piscinalis that the weight of the soft parts of the animal in relation to shell length is determined by the environment to which they were transplanted. Newcombe (1936) recognized that food availability may be a more important factor in growth of mussels than temperature. Jørgensen (1976) noted that the reproductive state, in addition to the nutritional state, may affect the relation of the weight of soft parts to shell length of bivalves.

All three methods used in an attempt to determine health of Anodonta imbecillis indicated a decline in condition from 2 May to 30 August 1986 in pond 27 (Figures 24, 25 and 26). Fourty-eight of the fourty-nine mussels taken in May were partially or fully gravid. Even the smallest (35.0-40.0 mm) were noted to contain some glochidia, suggesting sexual maturity may be reached during the second year, as also suggested by Allen (1924). While many of the individuals taken in August were gravid, it was to a lesser extent than in May. Therefore the reduced reproductive state may well have led to the decline in CI values, eventhough the animal itself may not have been less healthy. It must also be kept in mind that the August sample was taken after the mass die-off of mussels in this pond. Reduced CI values may have occurred because of reduced weight brought on by stress in addition to a natural (or unnatural) decrease in reproductive state.

A partial recovery is evident in the 17 April 1987 sample from pond 27 by the fact that the condition index values fall between those from the two previous samples (Figure 24).

The Newman-Keuls method of multiple comparison test on the data derived from Haukioja and Hakala's CI method revealed that mussels in pond 6 were healthier than those from the other ponds in the 17 April 1987 sample. This, in addition to the findings from the length frequency distributions and the growth studies, indicates a superior environment in this pond.

The Newman-Keuls test on the CI values derived from Joy's formula for these same samples only revealed significant differences between pond six and two of the others, ponds 14 and 15. This suggests that this may be a more conservative formula for determining health and can only be used to indicate the existence of major differences between populations.

None of the methods used for determination of health was found to be the "ideal formula". The values derived from Joy's formula show a considerable amount of length dependency. A 25 percent decrease in CI values is noted from the regression line between 35.0 and 85.0 mm for the 2 May 1986 sample in pond 27 (Figure 24). Joy (1985) found that CI values increased with length of Corbicula fluminea using this formula. From Figure 24, it should be obvious that a random sample of individuals should not be used to derive a mean from which comparisons are to be made. This formula appears best suited for the type of samples taken on 17 April 1987, those limited to a defined size range which can be compared among populations. If one does take a random sample of all sizes, length must be incorporated in the form of regression analysis as shown in this figure. This method does allow for easy health determination in that no drying of viscera or shells is necessary.

The use of regression analysis for various weight parameters vs. length appears very useful in CI evaluation (Figure 25). The results of health determination by Joy's formula can be predicted by examining Figures 25A and C. A decline in late summer wet viscera weight is not accompanied by a decline in total weight in this sample. This results in the decreased CI values found for the August sample using Joy's formula.

Joy's formula does not, however, give any indication of what is occurring with shell weight as Figure 25D does. Why shell weight would decline from spring to late summer in pond 27 mussels is unclear. Ellis, et al., (1931) have shown that some mussels combat rapid pH changes by drawing on shell materials for buffers. deWaele (1930), in studying the Eurasian freshwater mussel, Anodonta cygnea, found that they can take calcium from their valves when this element is at low levels in their diet. A. imbecillis may also be able to reabsorb some of its shell material for use under adverse conditions, and this in itself may be an indicator of their state of health or of environmental stresses, such as that which caused the mass deaths in this pond.

Results from Haukioja and Hakala's formula reflects those from the length-weight regression but carries the analysis one step further. Weight to length cubed ratios, plotted as CI vs. length, give indications of changes in one parameter with respect to the other not revealed by the length-weight curves. If length and weight increase at the same rate, a horizontal line will result. If the weight parameter is increasing at a faster rate than length, an upward curve will result. On the other hand, if length is increasing faster, a downward curve will occur. From the solid line representing the 2 May 1986 sample (Figure 26A), total weight is shown to increase at a faster rate than length from 35.0 mm up to approximately 75.0 mm when the trend reverses slightly. The same situation exists for water weight (Figure 26B). Wet viscera weight increases faster than length up to around 60.0 mm and begins to be outpaced by increasing length at 70.0 mm (Figure 26C). Dry shell weight is the only parameter shown to be increasing at a slower rate than shell length for all size classes (Figure 26D). This suggests a form change in the shell, probably resulting from increases in length with little increase in height. The line for dry viscera weight closely parallels that for wet viscera weight (Figure 26E).

The slowing of growth in the 70.0 to 75.0 mm length range determined by the growth studies seems to be accompanied by a notable cessation of weight gain of the visceral mass as Figure 26E clearly shows. A similar phenomenon was shown in Joy and McCoy's (1975) work with Corbicula. This may actually be the result of reduced reproductive capacity and therefore less weight due to glochidia in the gill pouches.

Galtsoff (1931) and Little and Gentner (1970) indicate that many bivalve species continue to gain weight with little or no corresponding increase in shell length among larger individuals. They contend that an increase in shell thickness is the cause of this phenomenon. However, in A. imbecillis this growth pattern is not observed. In fact, from Figure 26A, it can be seen that total weight is not increasing faster than shell length for larger individuals but the reverse may actually be occurring. In addition, shell weight is not increasing faster than length in older mussels (Figure 26D), further supporting the belief that shells of this species do not increase in thickness after growth in length slows.

No definite conclusion can be made from this study concerning the effects of the nitroaromatic contaminants on these mussel populations. I was unable to do the tissue analysis necessary to ultimately link observed differences in growth rate in pond 12 with the contaminants. Even if such analysis had been available, and accumulation of these materials had been found, other environmental factors may have been involved in the observed differences. Fuller (1974) summarized this problem with this statement: " It is very rare that we can quantitatively and/or qualitatively correlate the composition and size of mussel fauna with a specific disruption, be it chemical or physical."

Chapter IX

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Plate 1: Anodonta imbecillis.


Manostat
41
yes we can!

Plate 2: McClintic Public Hunting and Fishing Area; Pond 6 growth study site.

Plate 3: McClintic Public Hunting and Fishing Area; Pond 12 growth study site.

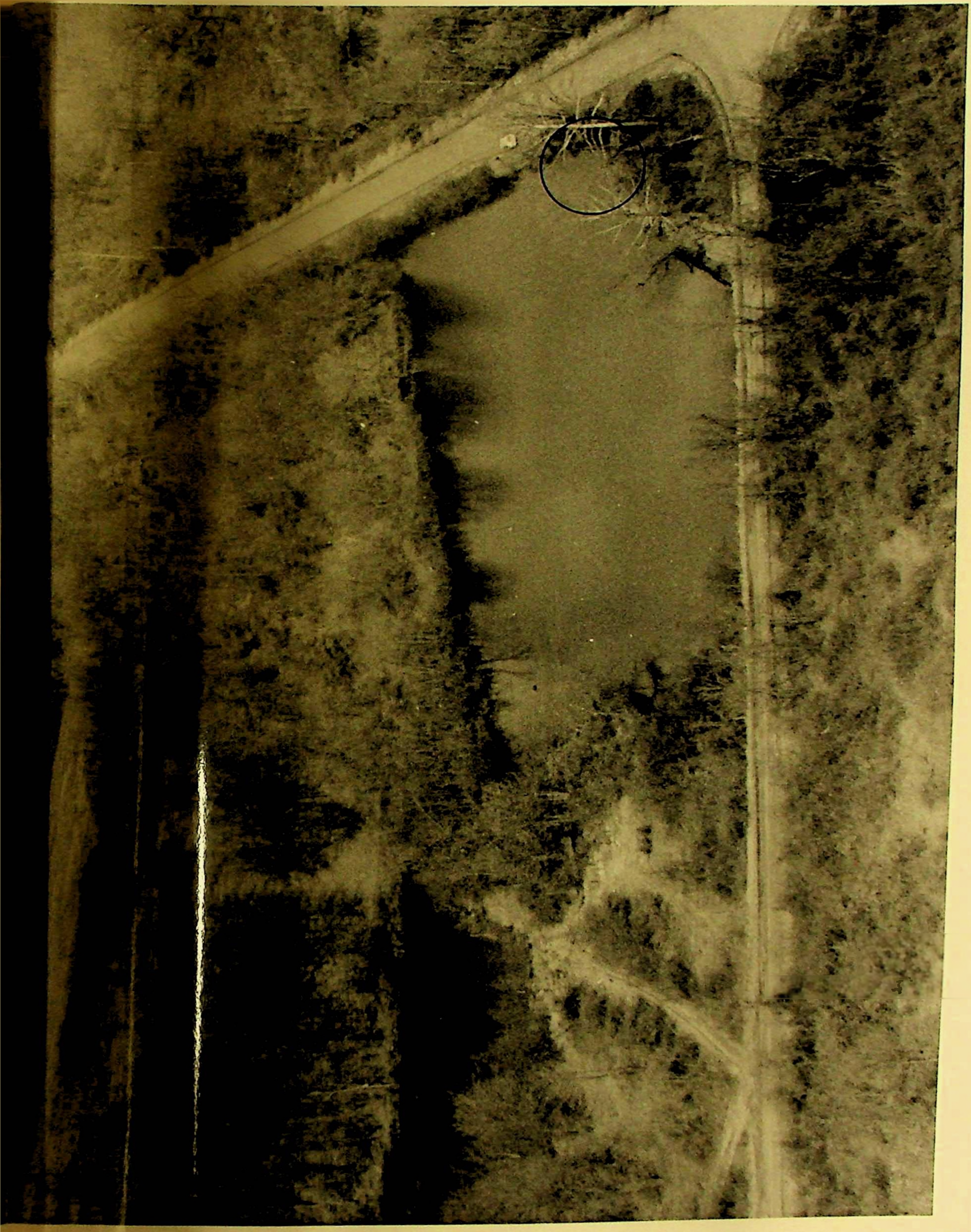


Plate 4: McClintic Public Hunting and Fishing Area; Pond 13.

Plate 5: McClintic Public Hunting and Fishing Area; Pond 14 growth study site.

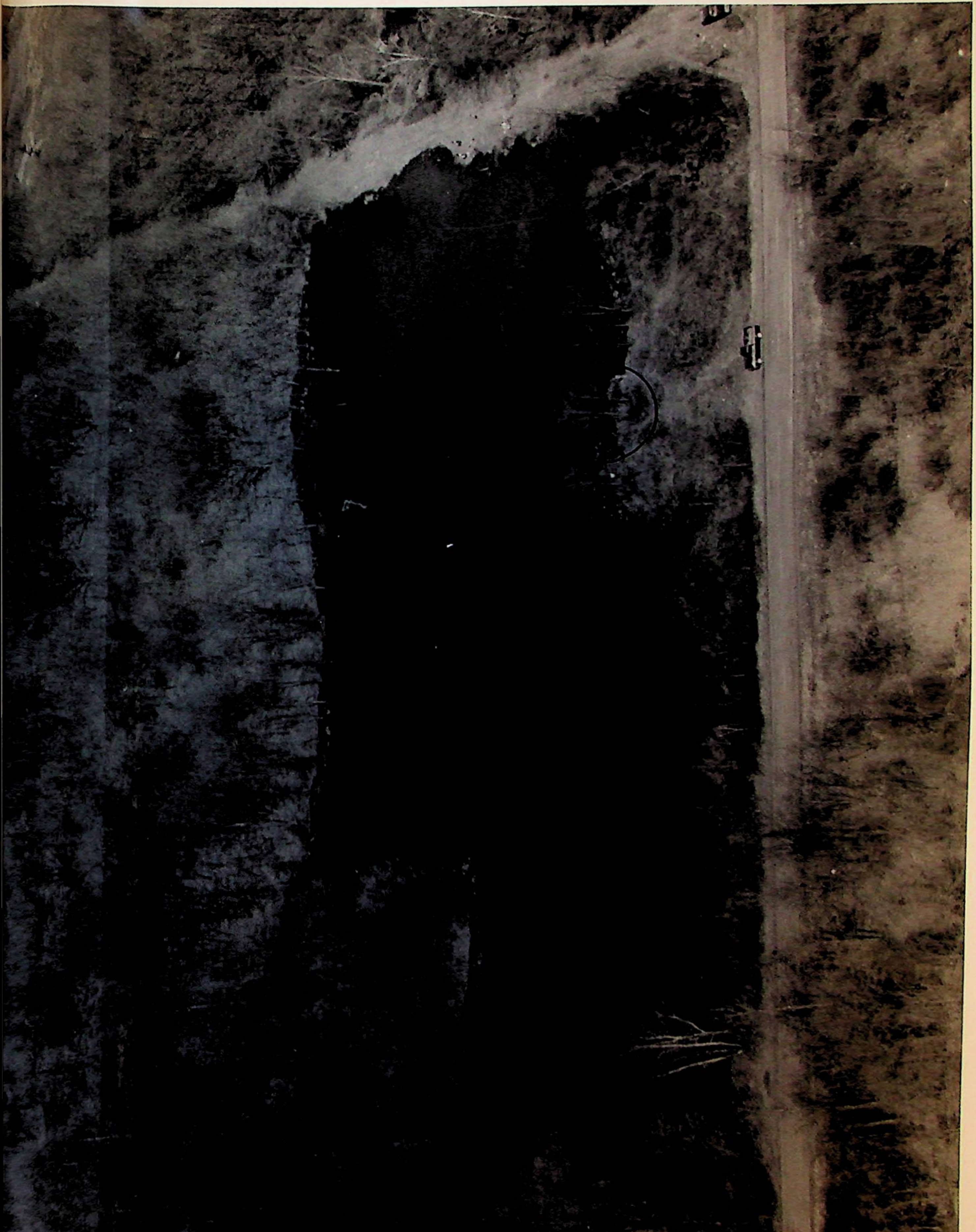


Plate 6: McClintic Public Hunting and Fishing Area; Pond 15 growth study site.

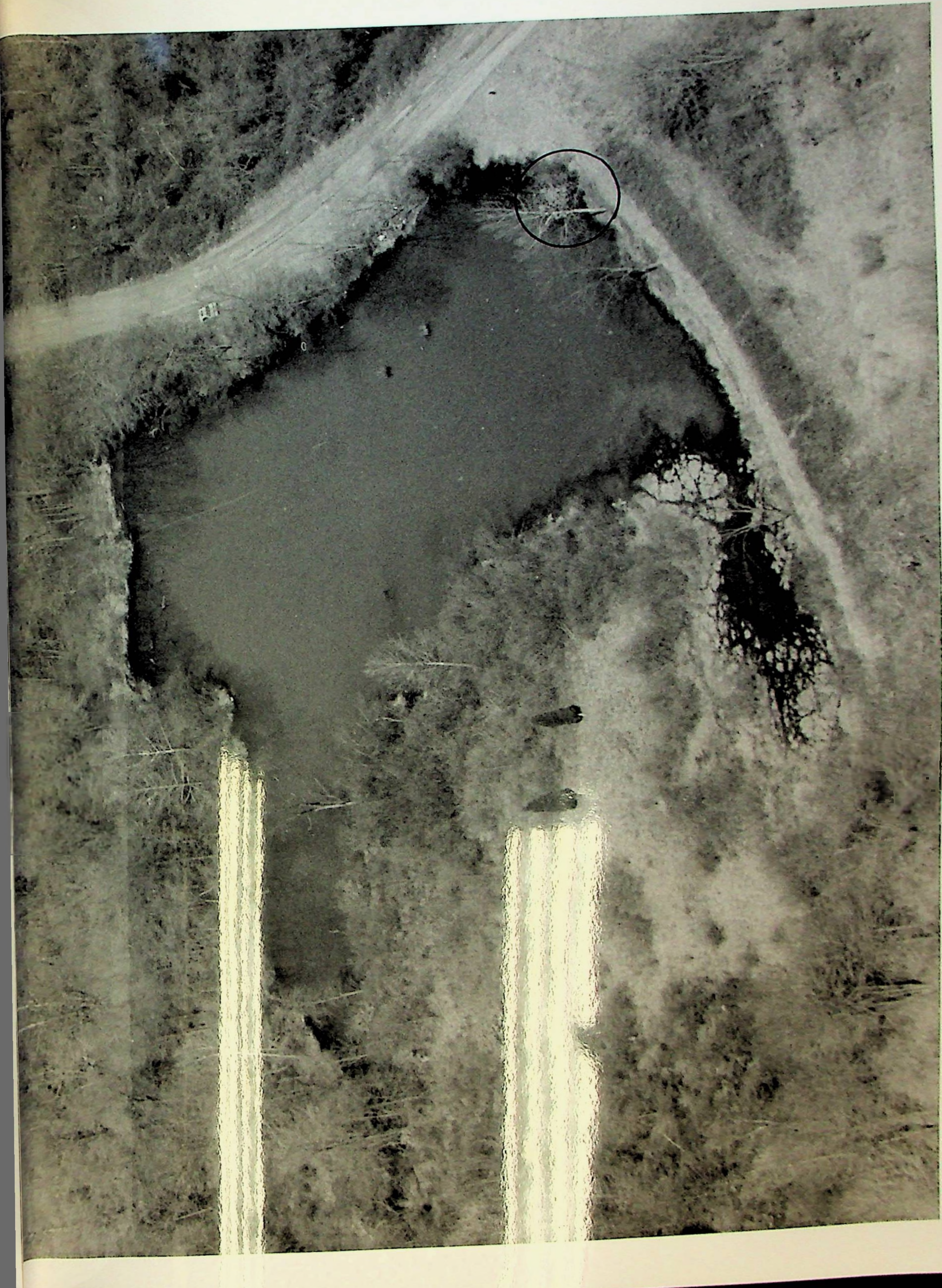


Plate 7: McClintic Public Hunting and Fishing Area: Pond 27 growth study site.

Plate 8: McClintic Public Hunting and Fishing Area; Pond 30 growth study site.

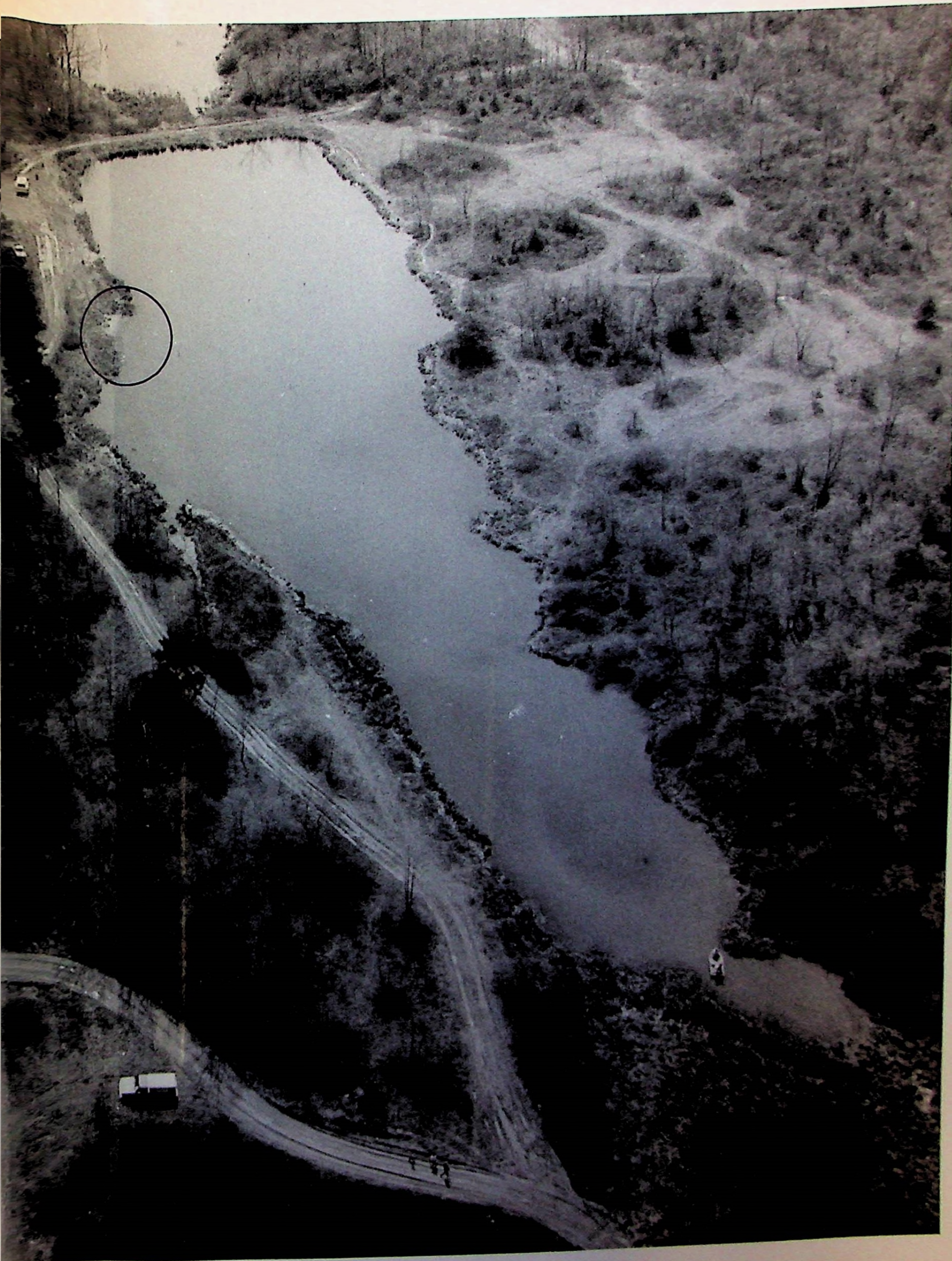
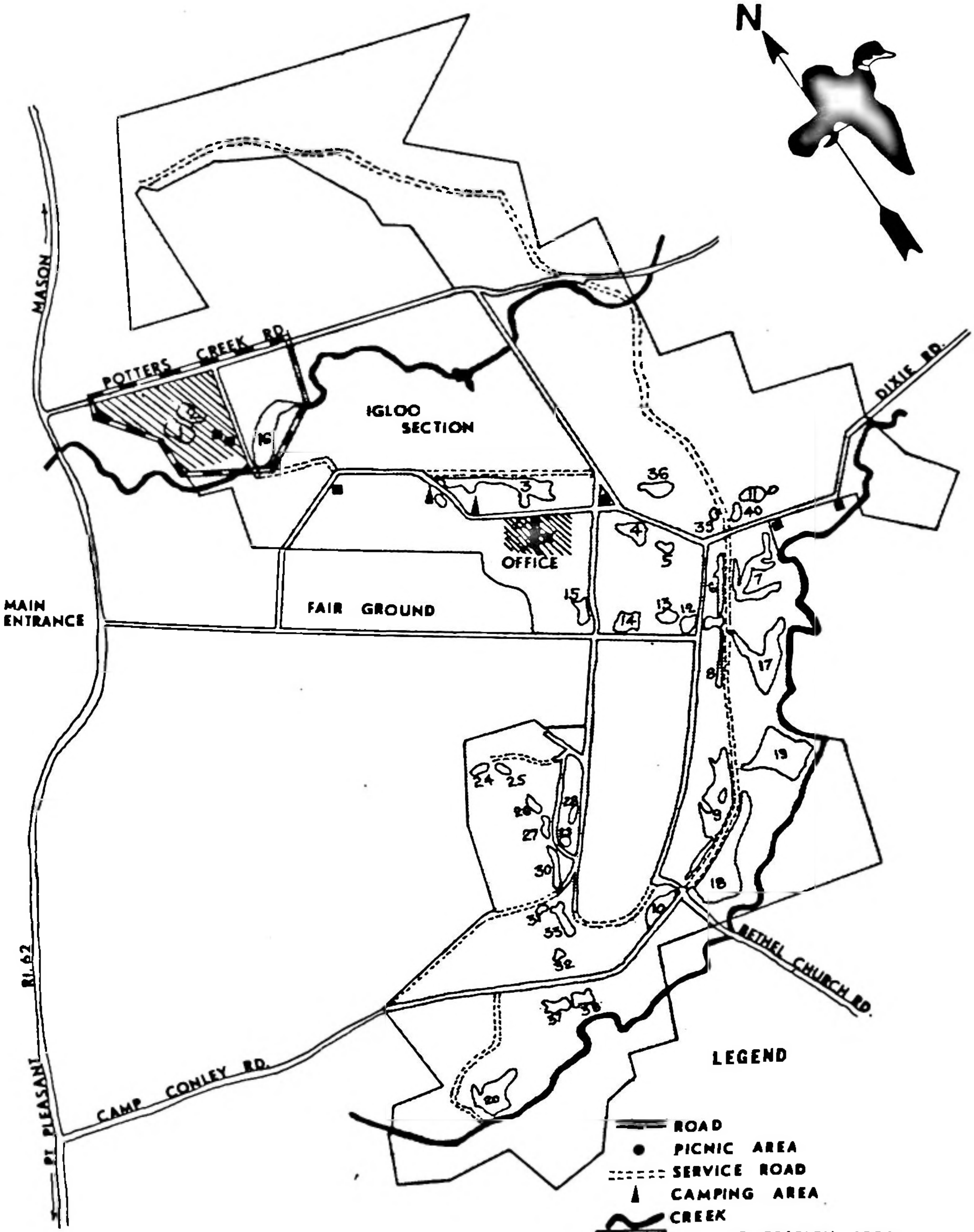


Figure 1: Clifton F. McClintic Public Hunting and Fishing Area with pond locations.



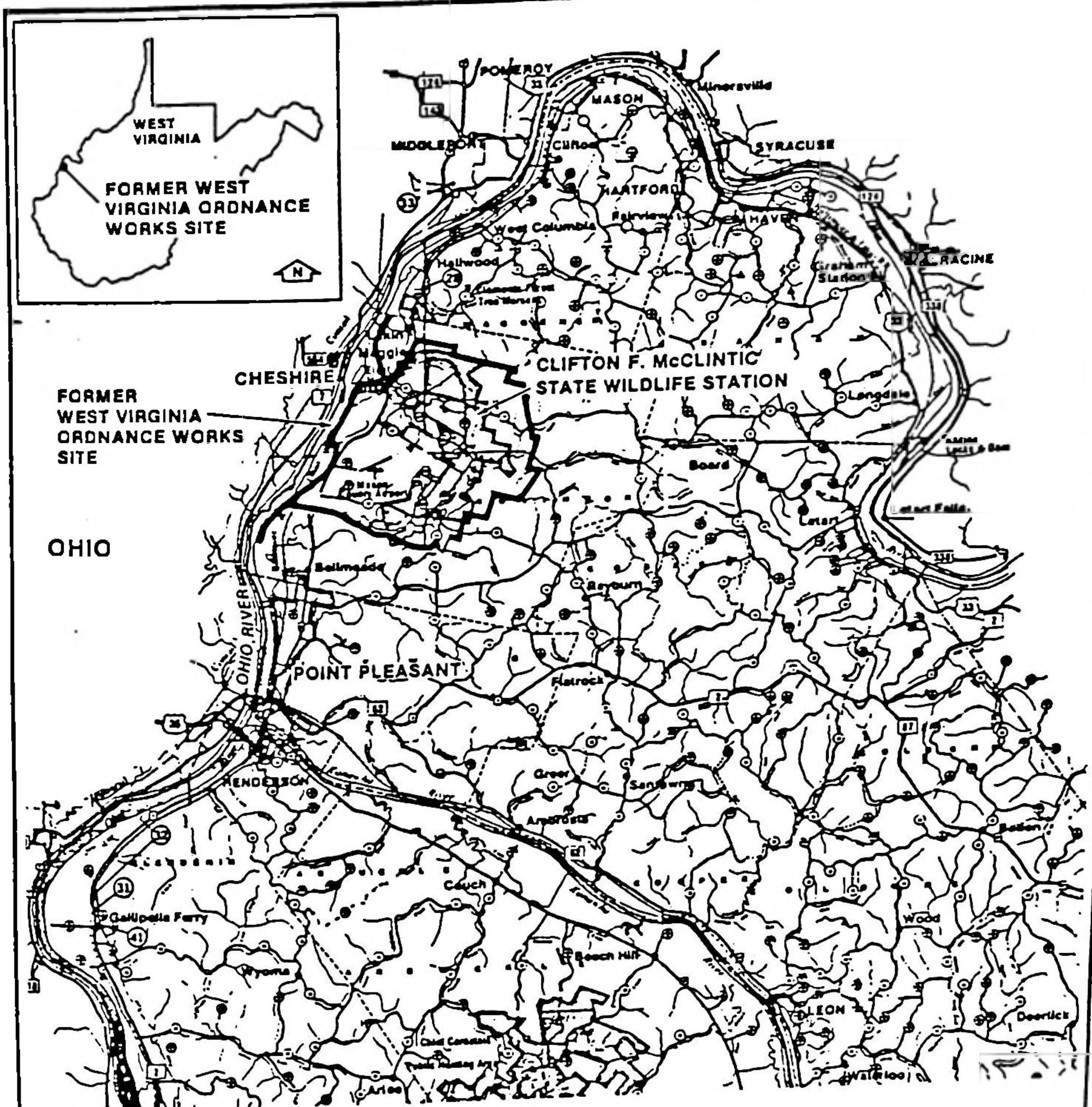
CLIFTON F. McCLINTIC WILDLIFE STATION

Rt. 1 Box 125

Pt. Pleasant, WV 25550

Ph. 675-4380

Figure 2: Location of the former West Virginia Ordnance Works (WVOW) Site.



N

SCALE

1 0 1

1 1 2 KILOMETERS

SOURCES: City of Point Pleasant-County of Mason, Chamber of Commerce, n.d.
ESE, 1984.

Figure 3: Location of WVOW facilities and sites of contamination resulting from TNT production (shaded). Source: ESE, 1984.

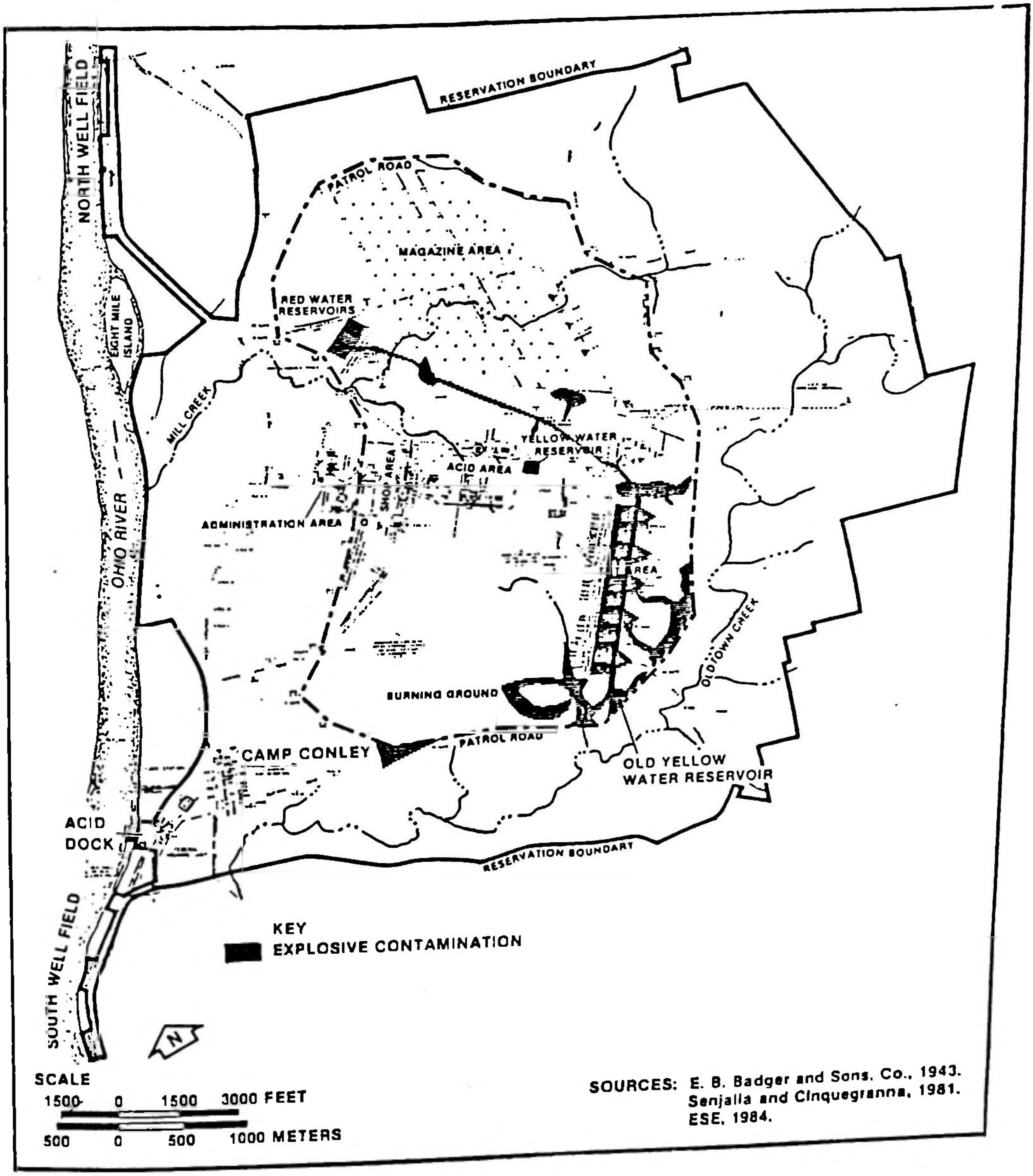
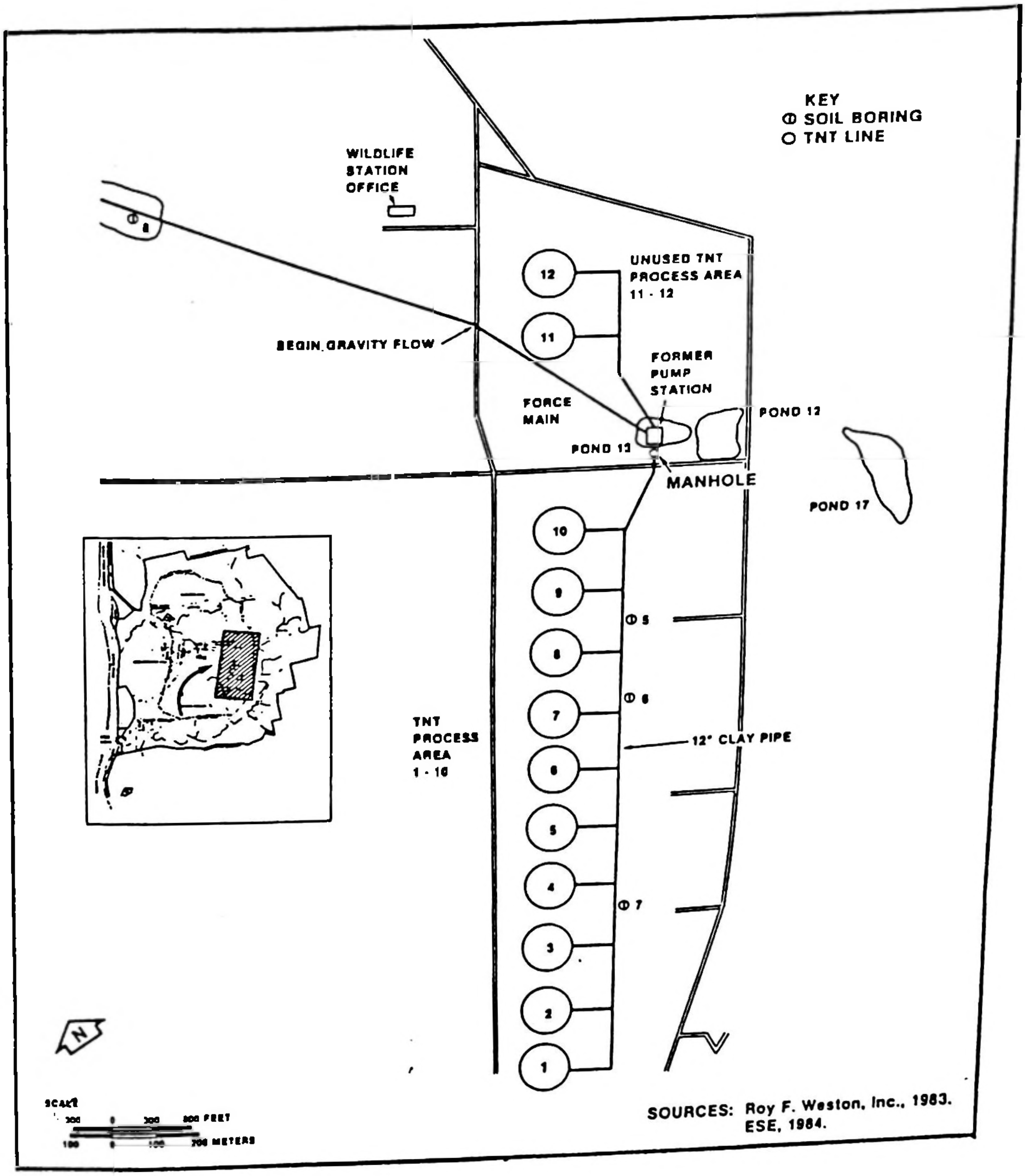
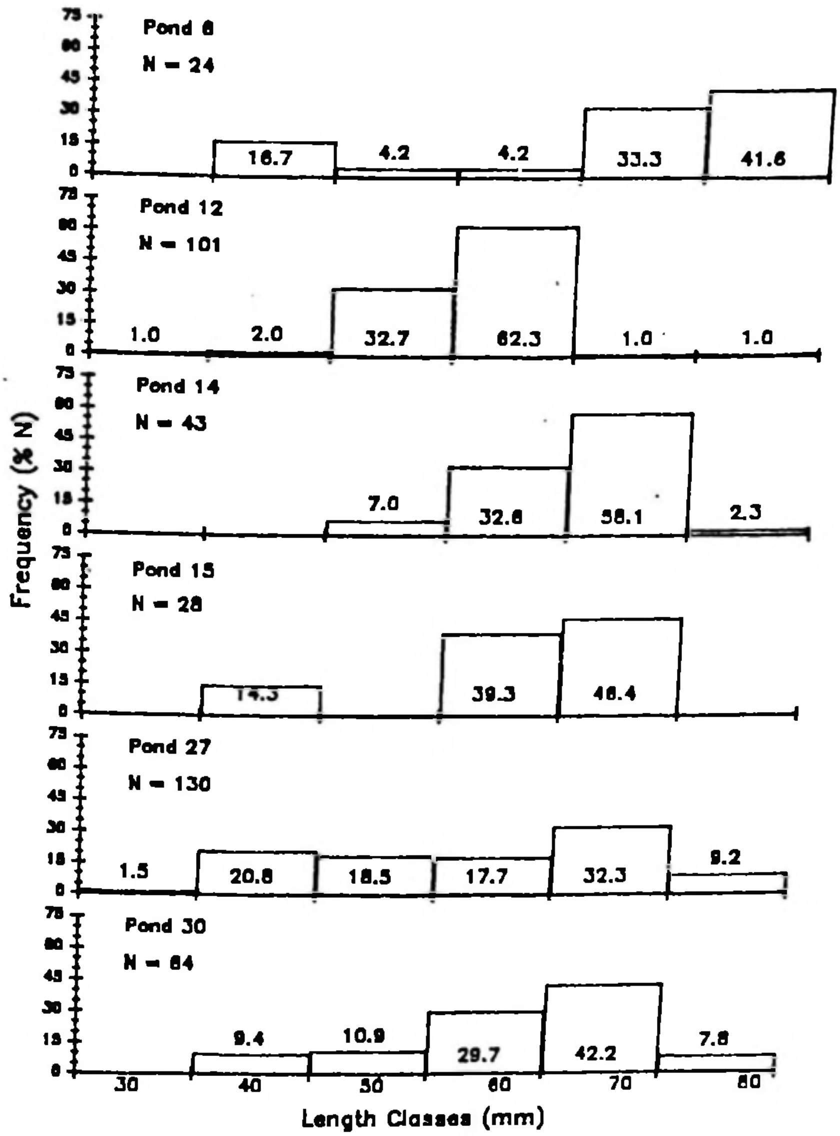

NORTH WELL FIELD
RESERVATION BOUNDARY
PATROL ROAD
MAGAZINE AREA
RED WATER RESERVOIRS
EIGHT MILE ISLAND
MILL CREEK
YELLOW WATER RESERVOIR
ACID AREA
ADMINISTRATION AREA
OHIO RIVER
AREA
OLDTOWN CREEK
BURNING GROUND
CAMP CONLEY
PATROL ROAD
OLD YELLOW WATER RESERVOIR
ACID DOCK
RESERVATION BOUNDARY
SOUTH WELL FIELD
KEY
EXPLOSIVE CONTAMINATION
N
SCALE
1500 0 1500 3000 FEET
500 0 500 1000 METERS
SOURCES: E. B. Badger and Sons. Co., 1943.
Senjalla and Cinquegranna, 1981.
ESE, 1984.

Figure 4: Location of WVOW process lines and pumping station (now pond 13) involved in pumping acid wastes from the TNT lines to red water reservoirs (now ponds 1 and 2).



SOURCES: Roy F. Weston, Inc., 1983.
ESE, 1984.

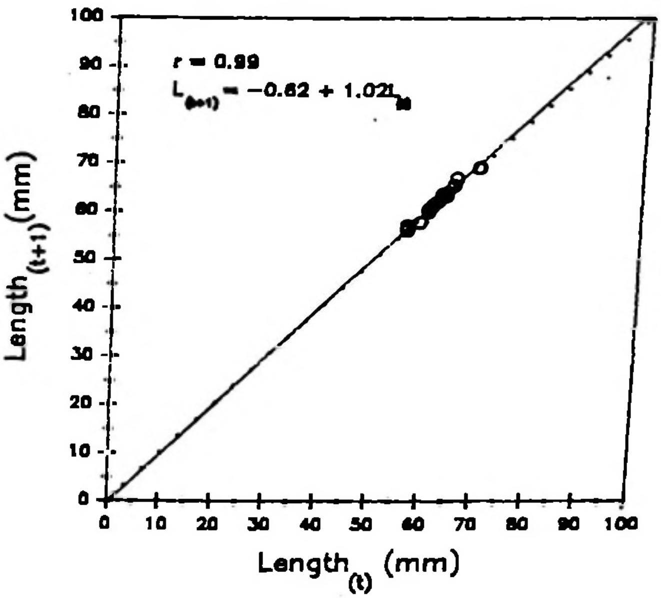
Figure 5: Shell length frequency distribution of *A. imbecillis* in the six study ponds during spring 1986 samples.



Pond 8
N = 24
16.7
4.2
4.2
33.3
41.6
Pond 12
N = 101
1.0
2.0
32.7
62.3
1.0
1.0
Pond 14
N = 43
7.0
32.6
58.1
2.3
Pond 15
N = 28
14.3
39.3
46.4
Pond 27
N = 130
1.5
20.8
18.5
17.7
32.3
9.2
Pond 30
N = 64
9.4
10.9
29.7
42.2
7.8
30
40
50
60
70
80
Frequency (% N)
Length Classes (mm)

Figure 6: Walford transformation for summer growth in length of *A. imbecillis* in pond 12 (1 Jun 1986 - 28 Sep 1986).

Figure 7: Walford transformation for summer weight gain of *A. imbecillis* in pond 12 (1 Jun 1986 - 28 Sep 1986).


r = 0.99
$L_{(t+1)} = -0.62 + 1.02L_{(t)}$
Length(t+1) (mm)
Length(t) (mm)
0
10
20
30
40
50
60
70
80
90
100

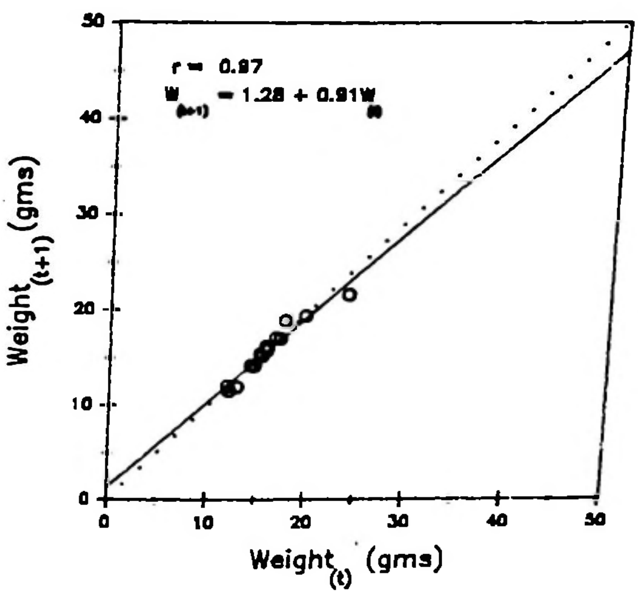
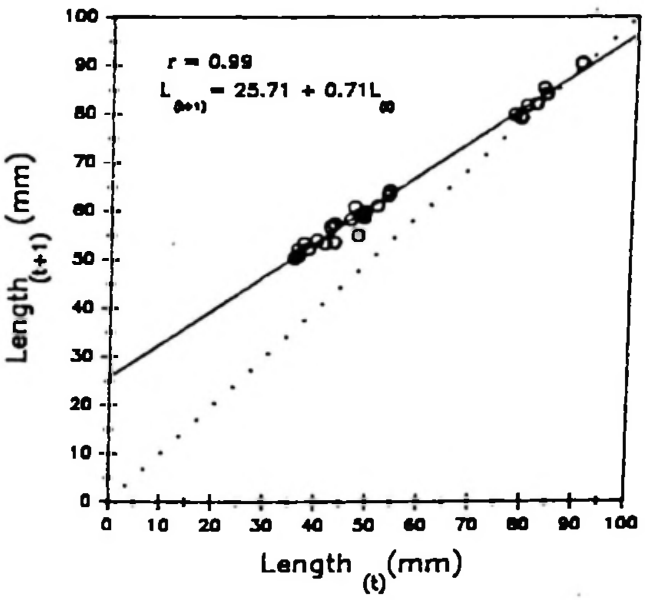
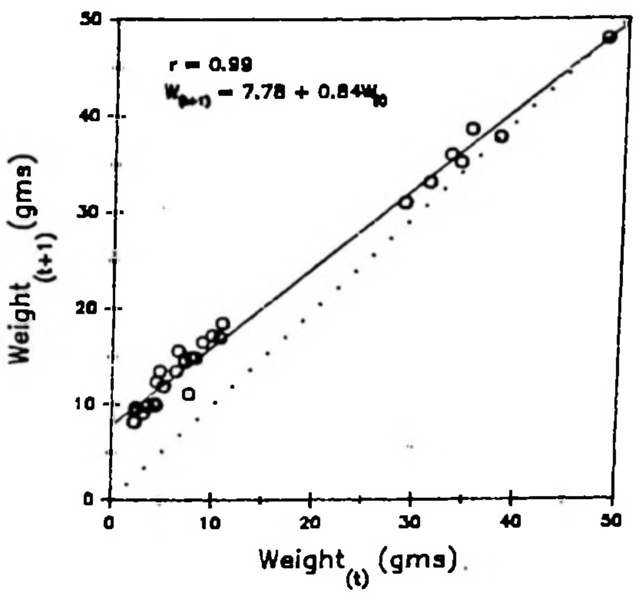

r = 0.97
$W_{(t+1)} = 1.28 + 0.91W_{(t)}$
Weight(t+1) (gms)
Weight(t) (gms)
0
10
20
30
40
50

Figure 8: Walford transformation for summer growth in length of *A. imbecillis* in pond 6 (18 May 1986 - 15 Sep 1986).

Figure 9: Walford transformation for summer weight gain of *A. imbecillis* in pond 6 (18 May 1986 - 15 Sep 1986).



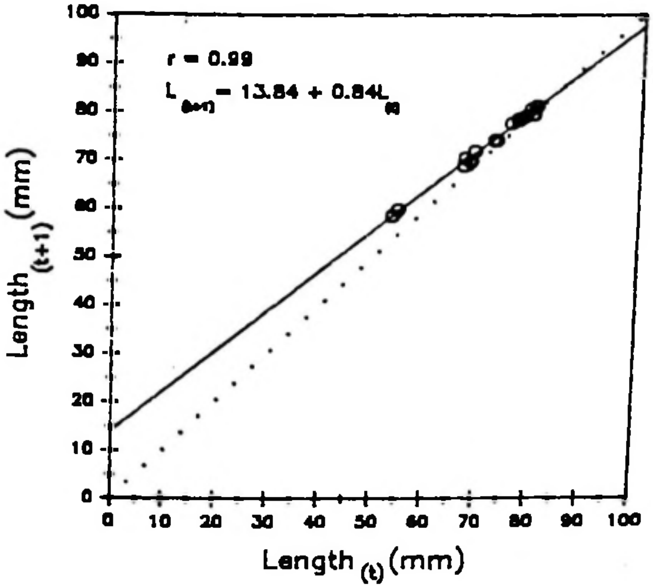
r = 0.99
L(t+1) = 25.71 + 0.71L(t)
Length(t+1) (mm)
Length(t) (mm)



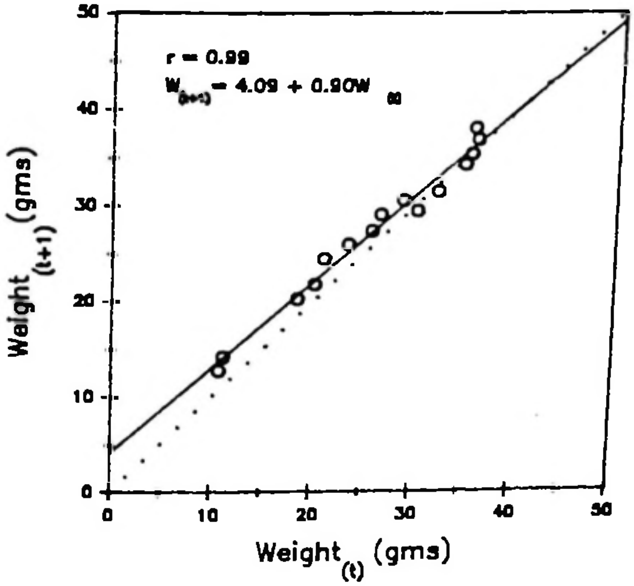
r = 0.99
W(t+1) = 7.78 + 0.84W(t)
Weight(t+1) (gms)
Weight(t) (gms)

Figure 10: Walford transformation for summer growth in length of *A. imbecillis* in pond 14 (6 May 1986 - 6 Sep 1986).

Figure 11: Walford transformation for summer weight gain of *A. imbecillis* in pond 14 (6 May 1986 - 6 Sep 1986).



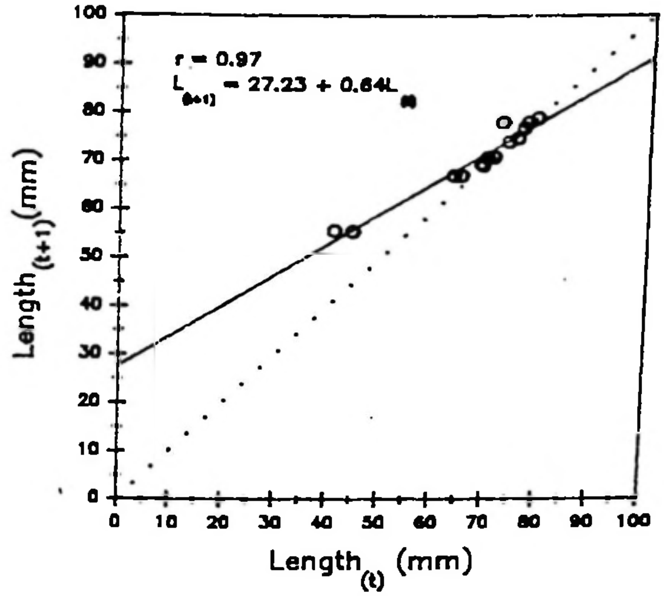
r = 0.99
L(t+1) = 13.84 + 0.84L(t)
Length(t+1) (mm)
Length(t) (mm)



r = 0.99
W(t+1) = 4.09 + 0.90W(t)
Weight(t+1) (gms)
Weight(t) (gms)

Figure 12: Walford transformation for summer growth in length of _A_. _imbecillis_ in pond 15 (6 May 1986 - 5 Sep 1986).

Figure 13: Walford transformation for summer weight gain of _A_. _imbecillis_ in pond 15 (6 May 1986 - 5 Sep 1986).


r = 0.97
$L_{(t+1)} = 27.23 + 0.64L_{(t)}$
Length(t+1) (mm)
Length(t) (mm)
0
10
20
30
40
50
60
70
80
90
100

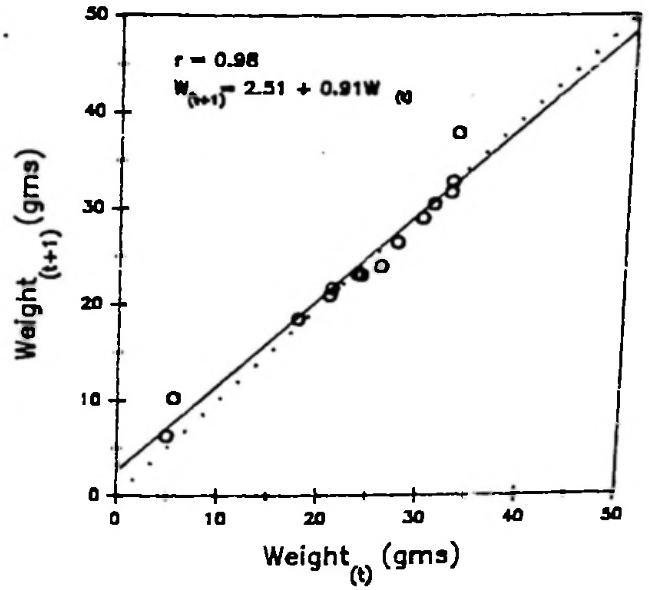
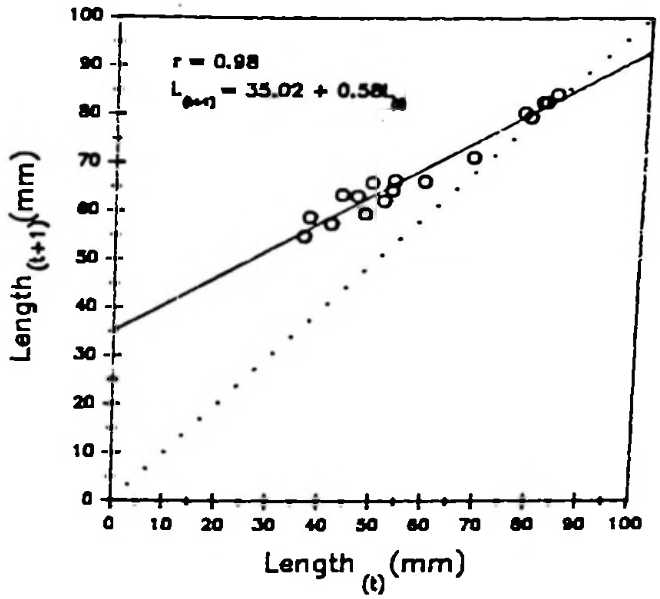

r = 0.98
$W_{(t+1)} = 2.51 + 0.91W_{(t)}$
Weight(t+1) (gms)
Weight(t) (gms)
0
10
20
30
40
50

Figure 14: Walford transformation for annual growth in length of *A. imbecillis* in pond 6 (18 May 1986 - 18 May 1987).

Figure 15: Walford transformation for annual weight gain of *A. imbecillis* in pond 6 (18 May 1986 - 18 May 1987).


r = 0.98
L(t+1) = 35.02 + 0.58L(t)
Length(t+1) (mm)
Length(t) (mm)

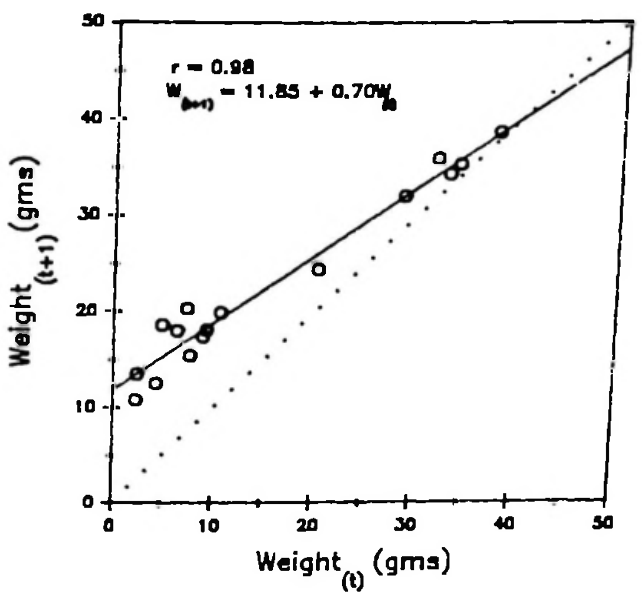
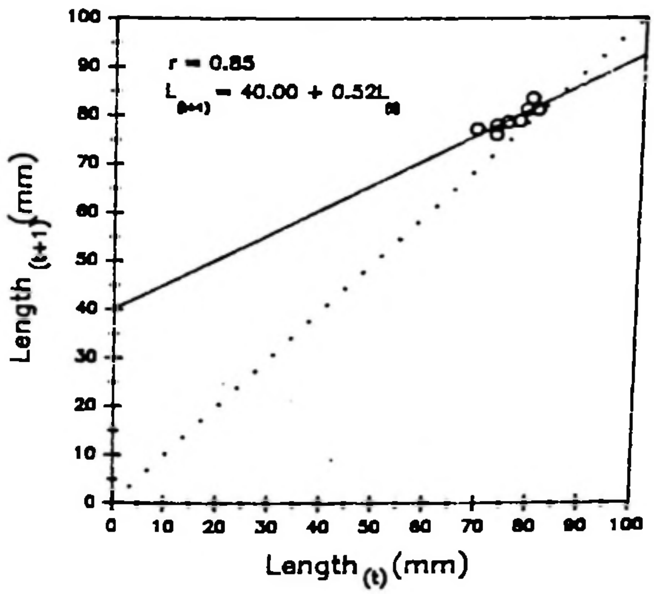

r = 0.98
W(t+1) = 11.85 + 0.70W(t)
Weight(t+1) (gms)
Weight(t) (gms)

Figure 16: Walford transformation for annual growth in length of *A. imbecillis* in pond 14 (6 May 1986 - 26 May 1987).

Figure 17: Walford transformation for annual weight gain of *A. imbecillis* in pond 14 (6 May 1986 - 26 May 1987).


r = 0.85
L(t+1) = 40.00 + 0.52L(t)
Length(t+1)(mm)
Length(t)(mm)
0
10
20
30
40
50
60
70
80
90
100

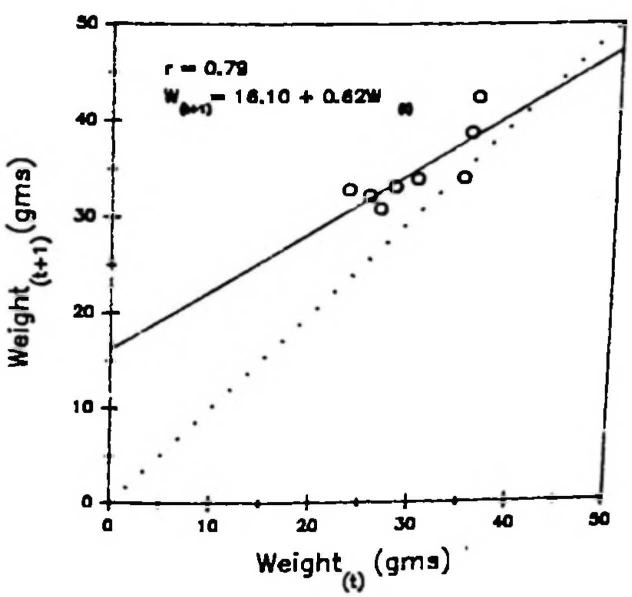
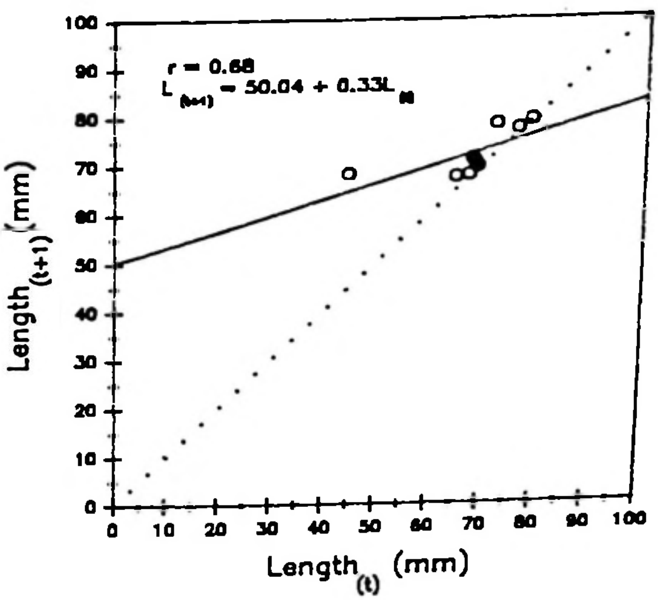

r = 0.79
W(t+1) = 16.10 + 0.62W(t)
Weight(t+1)(gms)
Weight(t) (gms)
0
10
20
30
40
50

Figure 18: Walford transformation for annual growth in length of *A. imbecillis* in pond 15 (6 May 1986 - 26 May 1987).

Figure 19: Walford transformation for annual weight gain of *A. imbecillis* in pond 15 (6 May 1986 - 26 May 1987).


r = 0.68
L(t+1) = 50.04 + 0.33L(t)
Length(t+1) (mm)
Length(t) (mm)
0
10
20
30
40
50
60
70
80
90
100

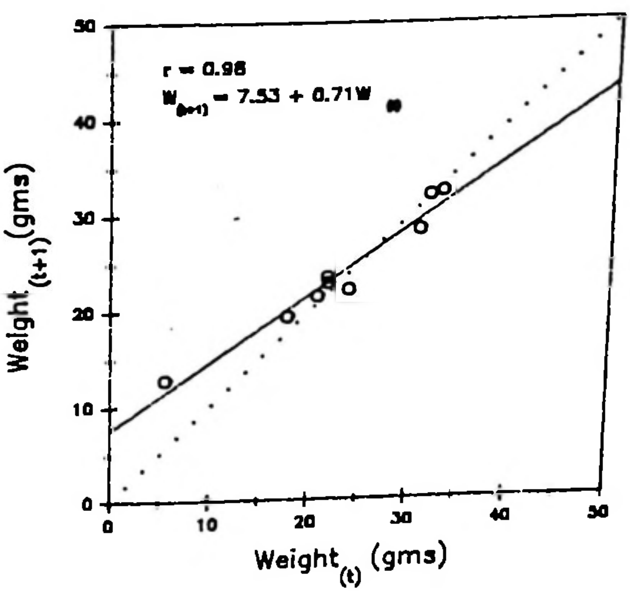
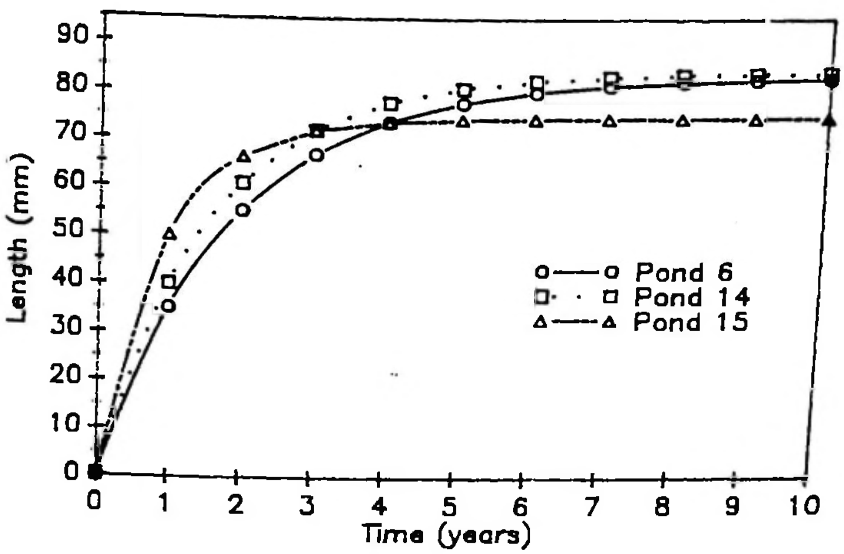

r = 0.98
W(t+1) = 7.53 + 0.71W(t)
Weight(t+1) (gms)
Weight(t) (gms)
0
10
20
30
40
50

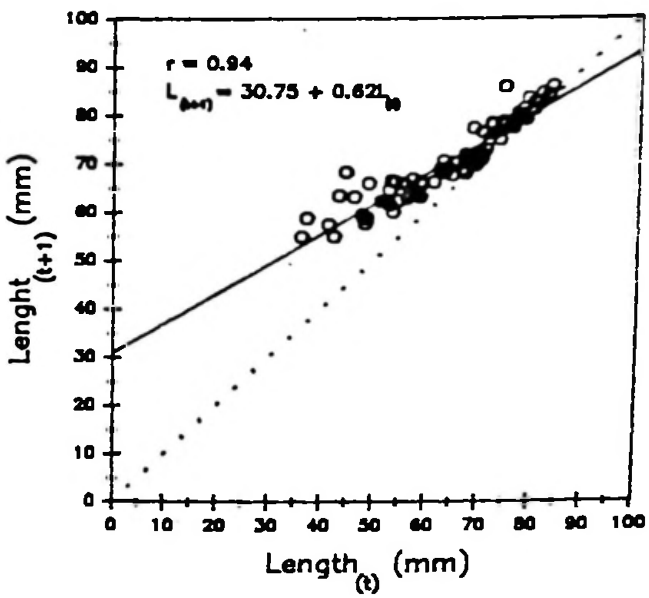
Figure 20: Estimated growth curves of
A. imbecillis in ponds 6, 14 and 15
as derived from Walford
transformation equations.



Length (mm)
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10
Time (years)
Pond 6
Pond 14
Pond 15

Figure 21: Walford transformation for annual growth in length of 81 individuals of *A. imbecillis* as combined from studies in the six McClintic study ponds by Harmon and Joy from May 1984 to May 1987.

Figure 22: Walford transformation for annual weight gain of 80 individuals of *A. imbecillis* as combined from studies in the six McClintic study ponds by Harmon and Joy from May 1984 to May 1987.


r = 0.94
L(t+1) = 30.75 + 0.621(t)
Lenght(t+1) (mm)
Length(t) (mm)
0
10
20
30
40
50
60
70
80
90
100

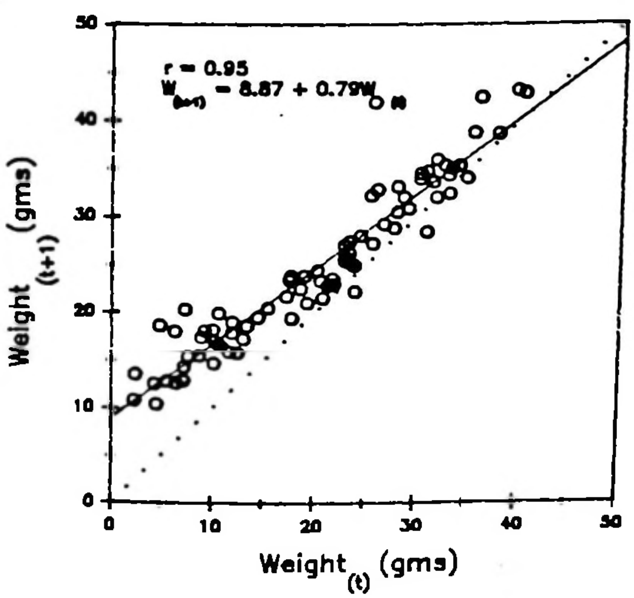
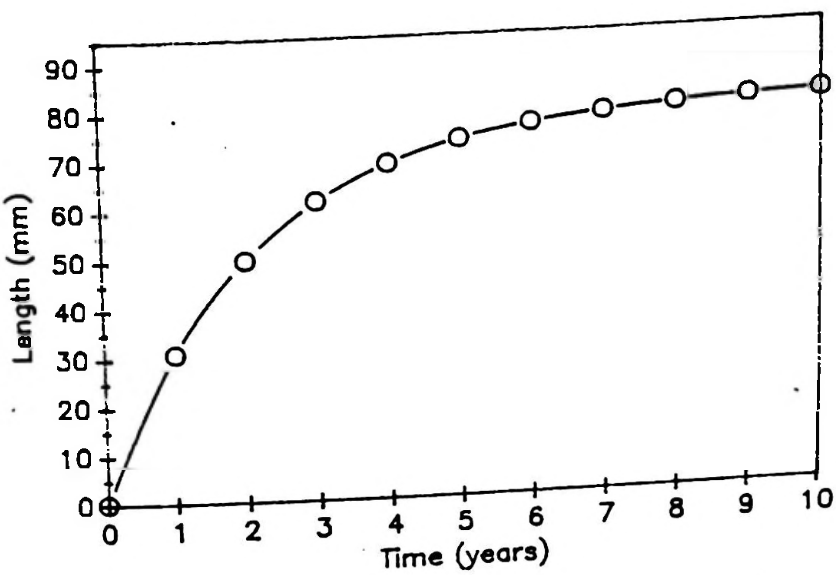
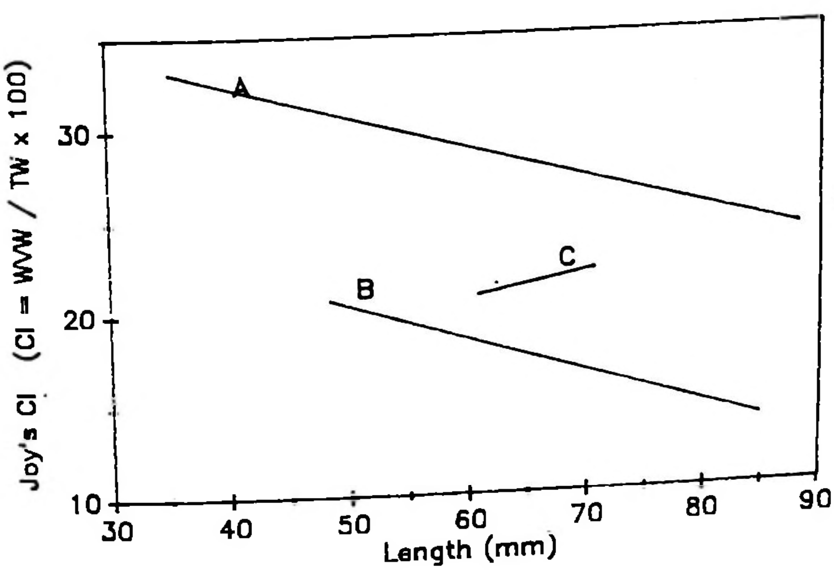

r = 0.95
W(t+1) = 8.87 + 0.79W(t)
Weight(t+1) (gms)
Weight(t) (gms)
0
10
20
30
40
50

Figure 23: Cumulative growth curve of *A. imbecillis* dervied from Walford transformation plot of combined annual growth in length.



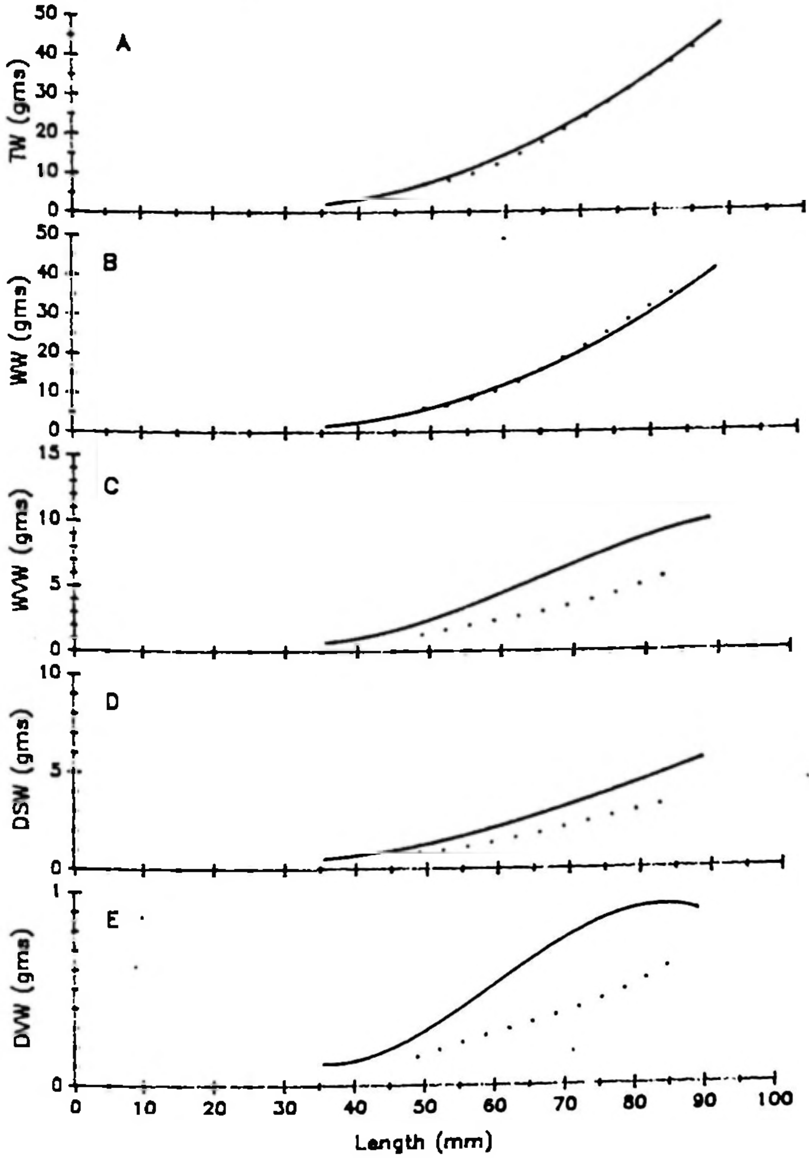
90
80
70
60
50
40
30
20
10
0
Length (mm)
0
1
2
3
4
5
6
7
8
9
10
Time (years)

Figure 24: Linear regression analysis of Joy's condition index vs. length for three samples taken from pond 27 on: A) 2 May 1986, B) 30 Aug 1986, C) 17 Apr 1987.



Joy's CI (CI = WW / TW x 100)
30
20
10
A
B
C
30
40
50
60
70
80
90
Length (mm)

Figure 25: Regression analysis of five weight parameters vs. length of *A. imbecillis* in pond 27 on 2 May 1986 (solid line) and on 30 Aug 1986 (dotted line): A) Total Weight; B) Water Weight; C) Wet Viscrea Weight; D) Dry Shell Weight; E) Dry Viscrea Weight.



A
TW (gms)
B
WW (gms)
C
WVW (gms)
D
DSW (gms)
E
DVW (gms)
0 10 20 30 40 50 60 70 80 90 100
Length (mm)

Figure 26: Regression analysis of five weight parameters (expressed in terms of weight/length) vs. length of *A. imbecillis* in pond 27 on 2 May 1986 (solid line) and on 30 Aug 1986 (dotted line): A) Total Weight; B) Water Weight; C) Wet Viscera Weight; D) Dry Shell Weight; E) Dry Viscera Weight. All values on the Y-axis are in units of 10^{-6}.

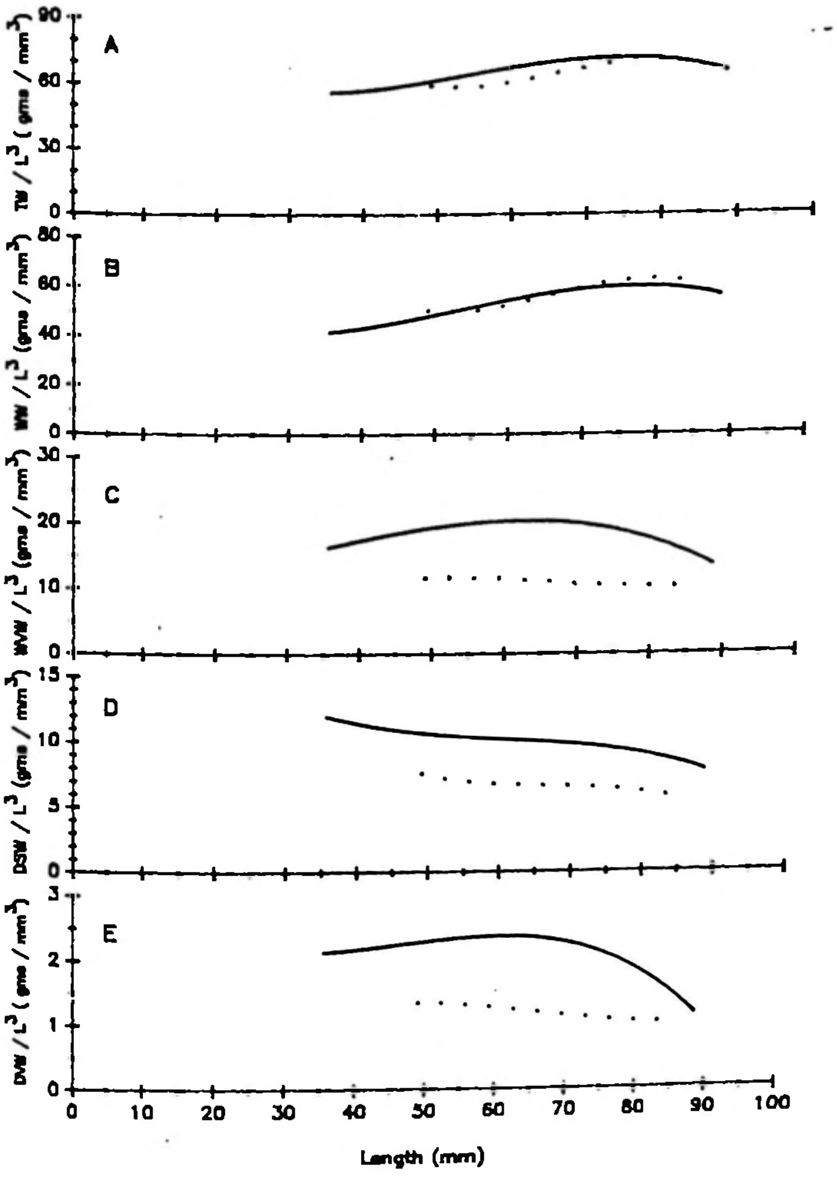
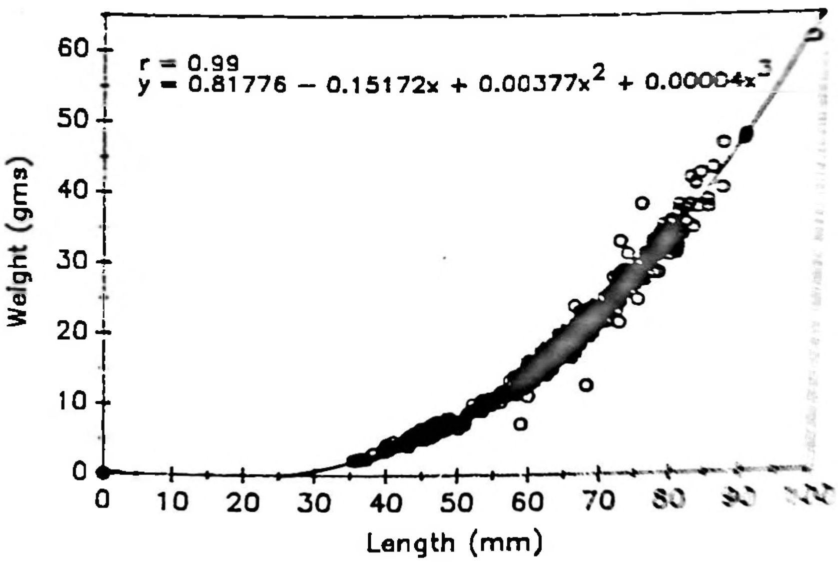
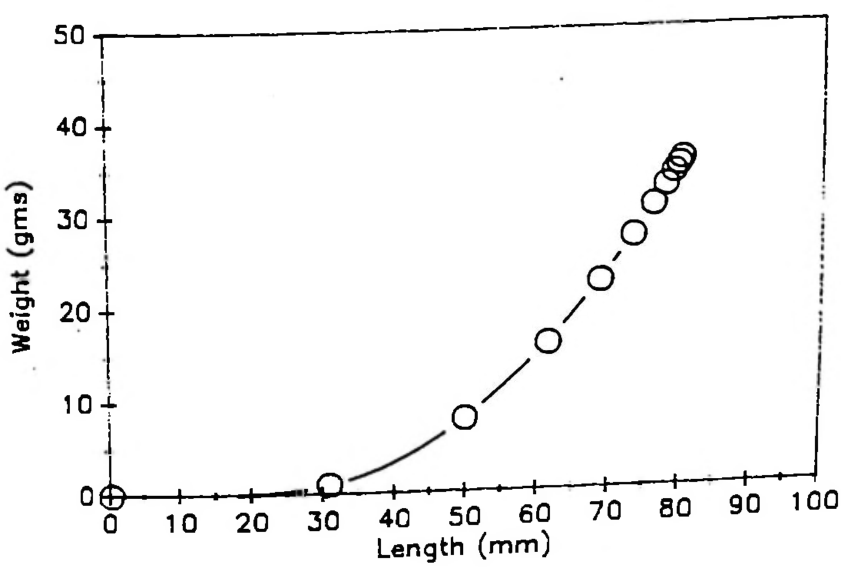

A
B
C
D
E
TW / L^3 (gms / mm^3)
WW / L^3 (gms / mm^3)
WVW / L^3 (gms / mm^3)
DSW / L^3 (gms / mm^3)
DVW / L^3 (gms / mm^3)
Length (mm)

Figure 27: Length-weight regression of 319 *A. imbecillis* individuals collected from the six study ponds between 26 Apr 1986 and 1 Jun 1986 for mark and recapture growth studies.



r = 0.99
y = 0.81776 − 0.15172x + 0.00377x2 + 0.00004x3
Weight (gms)
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
Length (mm)

Figure 28: Predicted length-weight curve through 10 year growth period for *A. imbecillis*. From combined data of Joy and Harmon (unpubl.).



50
40
30
20
10
0
Weight (gms)
0
10
20
30
40
50
60
70
80
90
100
Length (mm)

Table 1: Means and ranges derived from Joy's and Haukioja and Hakala's methods of condition index determination for samples of *A. imbecillis* taken from the six study ponds on 17 April 1987. (H and H's data is in terms of 10^{-6})

		Joy's CI		H & H's CI	
Pond	N	Mean	Range	Mean	Range
6	9	26.5	18.5-34.4	2.18	1.07-2.91
12	10	23.3	19.3-31.9	1.60	1.26-2.38
14	4	19.6	15.6-23.5	1.57	1.02-1.83
15	10	20.1	14.0-26.3	1.24	0.71-1.67
27	10	21.4	19.5-25.6	1.40	1.22-1.70
30	5	24.0	22.2-27.7	1.71	1.54-1.87

Tables 2-7: Water quality for McClintic Public Hunting and Fishing Area ponds 6, 12, 14, 15, 27 and 30 in addition to sampling dates for:

A) Population density and length frequency distribution
B) Growth: initial marking
C) Growth: summer growth recovery
D) Growth: one year growth recovery
E) Condition indices evaluation

Table 2: Pond 6 Water Quality.

Date '86-'87	Sample Type	Temp. °C	DO mg/L	CO2 mg/L	CaCO3 mg/L	pH
24 Mar		14	12	5	64	7.0
18 May	A,B	-	-	-	-	-
7 Jun		26	9	5	49	7.0
30 Jun		28	9	10	48	8.0
29 Jul		32	10	15	45	8.0
10 Aug		26	12	5	38	8.5
24 Aug		26	6	10	41	7.5
15 Sep	C	-	-	-	-	-
17 Apr	E	15	10	5	-	7.0
18 May	D	28	9	10	-	7.0

Table 3: Pond 12 Water Quality.

Date '86-'87	Sample Type	Temp. °C	DO mg/L	CO2 mg/L	CaCO3 mg/L	pH
26 Apr		26	9	10	60	7.5
10 May		27	7	5	51	7.5
1 Jun	A,B	-	-	-	-	-
4 Jun		27	11	5	49	8.0
30 Jun		28	10	10	62	8.0
17 Jul		29	8	10	53	7.5
10 Aug		27	12	0	58	9.5
24 Aug		26	8	5	54	9.0
6 Sep		25	11	0	51	9.5
28 Sep	C	-	-	-	-	-
17 Oct		14	10	20	38	8.0
14 Nov		6	10	5	46	7.0
17 Apr	E	16	10	5	-	7.0
18 May		28	8	10	-	7.5

Table 4: Pond 14 Water Quality.

Date '86-'87	Sample Type	Temp. °C	DO mg/L	CO_2 mg/L	$CaCO_3$ mg/L	pH
26 Apr		27	9	10	58	8.0
6 May	A,B	-	-	-	-	-
13 May		21	4	10	58	7.0
30 Jun		30	11	10	60	8.0
17 Jul		29	8	5	55	7.5
8 Aug		26	6	10	61	7.5
24 Aug		28	9	10	50	8.0
6 Sep	C	25	11	5	51	8.0
17 Oct		14	9	-	65	7.5
14 Nov		6	-	-	-	-
12 Dec		2	-	-	-	-
10 Jan		5	-	-	-	7.0
17 Apr	E	15	10	5	-	7.0
18 May		27	5	10	-	7.0
26 May	D	-	-	-	-	-

Table 5: Pond 15 Water Quality.

Date '86-'87	Sample Type	Temp. °C	DO mg/L	CO2 mg/L	CaCO3 mg/L	pH
27 Mar		14	10	5	39	7.0
6 Apr		22	6	10	38	6.5
16 Apr		13	8	5	43	7.0
20 Apr		17	-	5	38	7.0
4 May		18	9	5	45	7.0
6 May	A,B	-	-	-	-	-
28 May		24	8	10	41	7.0
30 Jun		28	8	10	43	7.0
17 Jul		27	7	10	41	7.0
10 Aug		26	7	10	49	7.0
24 Aug		28	8	10	40	7.0
6 Sep	C	26	11	5	33	7.0
17 Apr	E	15	9	5	-	6.5
18 May		27	9	5	-	7.0
26 May	D	-	-	-	-	-

Table 6: Pond 27 Water Quality.

Date '86-'87	Sample Type	Temp. °C	DO mg/L	CO2 mg/L	CaCO3 mg/L	pH
14 Mar		12	10	5	-	7.0
24 Mar		13	13	5	51	6.5
6 Apr		21	8	10	58	7.0
16 Apr		13	9	5	70	7.0
20 Apr		18	-	5	59	7.0
2 May	A,B	22	11	5	52	8.0
13 May		21	5	10	71	7.0
4 Jun		26	8	10	84	7.5
30 Jun		28	8	15	65	7.5
17 Jul		30	8	15	72	7.5
29 Jul		32	15	5	50	9.0
8 Aug		26	10	-	79	8.5
24 Aug		26	8	10	58	8.0
30 Aug	C	-	-	-	-	-
17 Oct		14	9	-	58	7.0
14 Nov		7	11	5	42	7.0
12 Dec		4	-	-	-	-
10 Jan		5	-	-	-	6.5
17 Apr	E	14	9	20	-	6.5
18 May		27	9	5	-	7.0

Table 7: Pond 30 Water Quality.

Date '86-'87	Sample Type	Temp. °C	DO mg/L	CO2 mg/L	CaCO3 mg/L	pH
26 Apr		31	9	15	46	8.5
13 May	A,B	21	4	15	61	7.0
4 Jun		27	7	15	73	7.0
30 Jun		31	6	10	55	7.0
19 Jul		33	8	15	60	8.0
10 Aug		28	11	5	56	8.5
24 Aug	C	28	9	10	55	8.5
17 Apr	E	15	9	5	-	6.5
18 May		29	5	10	-	7.0