

Effects of Toxic Substances on Growth, Mortality, and Pathology of Larval Fishes in the River Raisin, Michigan

## Prepared by

Laura A. Fay, Mary Gessner, Paul C. Stromberg, and John Hageman

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# The Ohio State University Lake Erie Programs

Karataga Araba Kabupatèn

Center for Lake Erie Area Research (CLEAR)
Ohio Sea Grant Program
F.T. Stone Laboratory

1314 Kinnear Road Columbus. Ohio 43212 614/292-8949

P.O. Box 119 Put-in-Bay, Ohio 43456 419/285-4754

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### EXECUTIVE SUMMARY

- 1. Gizzard shad were the predominant larval fish collected during the 1983/1984 River Raisin study. Although the number of larval fish collected during 1984 was almost 10,000 greater than in 1983 the relative abundance of gizzard shad was similar (73%) for the two years.
- 2. The apparent relative abundance of the remaining species varied during the 2 year study as a result of temporal differences in the initiations of the field programs. The 1983 field program began on May 30th and ended on September 12th, while the 1984 program was initiated on April 2 and lasted until July 19. Many of the sport fish and early spring spawners (white suckers) were caught as a result of the early Spring sampling program as anticipated.
- 3. The major storm event of February 14, 1984 had no apparent adverse effect on the fish spawning habitat based on the increased numbers of larval fish captured during 1984.
- 4. Simple growth rates (d1/dt) for gizzard shad were the highest at station 3 (adjacent to the Monroe Sewage Treatment plant). Gizzard shad growth rates at the remaining stations were substantially lower (0.25 0.78 mm/day). This increased growth rate may be due to an increased availability of food (i.e. plankton).
- 5. There is a discrepancy between results obtained for growth when comparing the simple growth rates and the growth rate coefficients for both gizzard shad and emerald shiners. The reason for this disparity has not been resolved.
- 6. The instantaneous mortality rates were much higher during 1984 (stations 1 through 4). Increased mortality may be explained by something routine like weather, water temperature, lake level, food availability, or any one of the numerous variables accounting for natural mortality or by something more unorthodox like introduced chemicals or toxic contamination. Food availability (phytoplankton and zooplankton) and contamination level data for the two years should be compared.
- 7. Real lesions compatable with acute toxicity were observed in organs in contact with the environment (sensory organs, oropharynx, proximal esophagus, and gills) as well as for the intestine and the kidney.
- 8. Lesions appeared to affect primarily gizzard shad in all size classes.
- 9. Lesions were observerd on gizzard shad from all sampling stations.

- 10. Lesions, similar to those known to be caused by toxic chemicals were observed in gizzard shad and alewife from the control station.
- 11. Lesions observed in gizzard shad at the control station were identical in quality, distribution, and range of severity to those found in gizzard shad from the river stations. These lesions most likely indicate similar, adverse environmental conditions at both the riverine and lake control station.
- 12. The attempt to reproduce lesions experimentally in fathead minnows was not successful. However, differences in metabolism between species, bioavailability of toxic substances and duration of the exposure might account for such failure and does not mean toxicants were absent. The length of the fathead minnow exposure experiments was limited to 70 days.
- 13. It is possible that a serious health problem exists for gizzard shad in Lake Erie, based on the number of lesions observed in gizzard shad at the control station.
- 14. Larval gizzard shad from the River Raisin contained total PCB residues ranging from 0.056 to 2.9 mg/kg. PCB residues in 10 of 11 samples exceeded the 0.1 mg/kg objective for protection of aquatic life set by the Great Lakes Water Quality Agreement of 1978. Three of the 11 samples exceeded the U. S. FDA action level of 2 mg/kg PCB, established for edible portions of fish.
- 15. Young-of-the-Year emerald shiners, collected from the River Raisin contained total PCB residues ranging from 0.48 to 3.7 mg/kg. All samples exceeded the 0.1 mg/kg objective for the protection of aquatic life, and five of nine samples exceeded the 2 mg/kg PCB action level set by the U.S. FDA.

#### RECOMMENDATIONS

- 1. Future studies of larval fish growth rates involving gizzard shad, should use data from only the largest larvae collected during each sampling period due to the ability of gizzard shad to spawn over wide temporal ranges. This ability results in a continual influx of newly hatched larvae skewing the growth rate downwards (Gordon 1982).
- 2. Comparisons should be initiated for the 2 year database to define the probable cause for both the increased density observed in 1984 and for the increased mortality rates.
- 3. Future studies of larval fish involving histopathological evaluation should be limited to fish between 20 mm and 50 mm. Fish smaller than this are not sufficiently differentiated to allow complete analysis of tissues. Fish larger than 50 mm create technical problems resulting in poor specimen quality.
- 4. Consideration should be given to an investigation of spontaneous lesions in gizzard shad from multiple Lake Erie localities. Correlate observed lesions with water chemistry data and toxicologic analysis of whole gizzard shad.

### INTRODUCTION

#### BACKGROUND

Although the role of marine estuaries as spawning and nursery areas for economically important fish populations has been the subject of considerable research during the last 20 years, investigation of the role of riverine habitats in the Great Lakes has long been neglected. Half of the approximately 175 fish species occurring in the Great Lakes basin are believed to be dependent on riverine habitats as spawning, nursery, or adult concentration areas. Approximately 50 of these species are currently important as commercial, recreational, or forage species. Few of the species residing in the Great Lakes (as opposed to those restricted largely to tributaries) are thought to be independent of riverine, coastal wetlands, or coastal shallows as spawning and nursery areas (Trautman, 1981; Hubbs and Lagler, 1964; Van Meter and Trautman, 1970). The riverine areas of Lake Erie have long been recognized as major breeding grounds for many species of fishes. These areas have traditionally exhibited greater species diversity and numbers of fishes, especially larval fishes, than the remainder of the lake (Wickliff, 1931; White et al., 1975; Cooper et al., 1981a, b, c,; Mizera, et al., 1981).

The cultural stresses placed on river mouth areas are quite intense. These areas are subject to inputs of toxic substances from agricultural, industrial, and municipal sources. Alterations in the flow of tributary water into the nearshore area by agricultural and storm water runoff can significantly affect the characteristics of the mixing zone ecosystem. The Lake Erie Basin is the most densely populated and heavily industrialized area within the Great Lakes Basin and therefore the most seriously impacted.

In 1981, the International Joint Commission's Water Quality Board identified 39 "Areas of Concern" within the Great Lakes Basin. The River Raisin was identified as an area "exhibiting signficant environmental degradation and impairment of beneficial uses." This designation of the River Raisin was based on:

- a substantial number of violations of water quality objectives
- sediments highly polluted by heavy metals, and
- high concentrations of PCB's and industrial and agricultural organic chemicals in fish.

### PROGRAM OBJECTIVES

In the spring of 1983, the U. S. Environmental Protection

Agency's Large Lakes Research Station at Grosse Ile, Michigan, selected the River Raisin as a site to address the issue of transport, exposure and effects of contaminants in the tributaries and nearshore areas of the Great Lakes. The primary objective of the study was to develop a predictive capability whereby effects of contaminants could be estimated, given their loadings, transport and fate characteristics. Secondary objectives of the study were: 1) to investigate the longevity and importance of in-place pollutants, 2) to provide input to surveillance databases, and 3) to develop a protocol for assessing ecological effects of toxic substances.

In order to address these objectives, an integrated analysis and modeling framework was developed which included: 1) exposure modeling (via fate and transport), 2) food chain modeling (in the form of bioaccumulation/bioconcentration) and 3) toxicity modeling (based on correlations between chemical concentrations and bioassay results). The field and laboratory research, which was designed to provide input into model development and calibration included analysis of selected chemical residues in water, sediment and biota and measurement of toxic effects on various components of the ecosystem.

### STUDY OBJECTIVES

As part of the biological effects work, we undertook a study to investigate the effects of toxic substances on growth, survival and pathology of larval fishes. The primary objectives of this work were:

- to identify species of larval fish present in the River, and determine spatio-temporal differences in density and species composition of the ichthyoplankton of the River;
- determine the spatio-temporal dose patterns of toxic substances in fish larvae;
- determine spatio-temporal differences in instantaneous growth and mortality rates of the most abundant species of fish larvae and relate those to exposure and dose patterns; and
- determine the incidence of pathologic lesions in the most abundant species.

Inasmuch as excessive concentrations of toxic substances are a major problem in the waters, sediments, and biota of the Great Lakes, they are particularly so in rivers due to source proximity and lack of open lake dilution. Moreover, the coincidence of high ambient environmental concentrations of toxic substances with the early life history stages of many fish species (some of considerable economic importance) represents a potential hazard

to the growth, survival, and health of those stages and ultimately to recruitment and maintenance of adult populations. This is particularly true in view of the rapid growth, cell proliferation, and cell differentiation which occurs during egg, larval, and juvenile stages. An initial approach to field determination of the biological effects of toxic substances on larval fishes in polluted riverine ecosystems is to determine spatio-temporal exposure (i.e. concentrations in water and food organisms) and dose patterns (i.e. residues in larval fish) and attempt to relate these to instantaneous growth and mortality rates of larval fishes of different species present at various points along environmental and toxicity gradients in the river system.

#### SITE DESCRIPTION

To accomplish our objective, seven (7) sampling stations were established, five (5) in the river and two (2) in the nearshore areas of western Lake Erie (Figure 1). Station descriptions for the two sampling seasons are described below.

In 1984, 4 minute circular oblique tows were made at the stations listed. Evaluation of the data indicated the largest numbers of larvae were collected in the first tow with decreasing density in subsequent tows. Averaging the 3 replicate tows obscured this decline. However, this evaulation necessitated the change in sampling procedures described for 1984.

## Raisin River Station Locations (1983)

- 300 meters downstream of the Route 50 dam, midstream, 100 meters upstream of the northwest tip of Sterling Island. Average depth during the study was 2 meters. (Figure 2a).
- 2. Approximately 50 meters downstream of the River Front Marina, at the electrical substation. Approximately 200 meters upstream of the I-75 overpass. Average depth during the study was 3 meters. (Figures 2a and 2b).
- 3. Midstream, even with the mouth of a cove slightly downstream of the Monroe wastewater treatment plant. Approximately 340 meters downstream of the I-75 overpass. Average depth during the study was 4 meters. (Figure 2b).
- 4. Midstream, downstream of the ship turning basin, near the Port of Monroe Terminal building. Near buoy #11. Average depth during the study was 8 meters. (Figures 2c and 2e).
- 5. Midstream at the Monroe power plant intake canal. Average depth during the study was 8 meters. (Figures 2d and 2e).

- 6. In the River Raisin mouth's outermost region. Approximately 150 meters outbound of buoys #9 and #10 and 225 meters from the mouth. Average depth was 8 meters. (Figure 2e).
- 7. 200 meters beyond cans #7 and #8 in the shipping canal. Average depth was 9 meters.

River Raisin Station Locations (1984)

In 1984, longitudinal, oblique tows were taken for: replicate A at 0.3 of river width, replicate B at 0.5 river width, and replicate C at 0.7 of river width. The transects consistently covered approximately the same distances in each 6 minute tow.

- Station 1 Tow started at the downstream tip of Sterling Island and ended in the vicinity of the upstream tip of the same island. Average water depth was 2 meters.
- Station 2 Tow started at a lighted, white garage on the south side of the river near the downstream edge of the boat slips and and ended in the vicinity of the electrical substation on the south side of the river across from the Riverfront Marina. Average water depth was 3 meters.
- Station 3 Tow started under the overhead high tension wires between the turning basin and the Monroe wastewater treatment plant and ended approximately 50 meters downstream of the I-75 bridge over the river. Average water depth was 4 meters.
- Station 4 Tow started at the ship mooring post on the south side of the river and ended in the vicinity of buoy #11. Average water depth was 8 meters.
- Station 5 Tow started at a cove across from the Monroe Power Plant and ended in the vicinity of wood posts protruding from water on north side of river. Average water depth was 8 meters.

The station pattern during both years of study is comparable, the only difference being that in 1983 circular tows were made and hence the station location was more resricted than in 1984 when a range was sampled.

Collections for body burden of River Raisin endemic spottail shiners was undertaken in the late summer of 1984 to permit

comparison to body burdens in spottails from other stations within the Great Lakes (Suns et al., 1983). due to the paucity of spottail shiners during the first collection period (8/17/84) and because of conversations with Dr. Suns, the second collection period focused on both spottail and emerald shiners. The two species are similar in uptake of organochlorine contaminants. The drawback in using emerald shiners for pinpointing contaminant problems is that they move more throughout the river system. Spottails were collected as a first priority; emerald shiners as second choice and the two were not mixed in any compositve sample. Due to specific habitat preferences of these fish, new stations were selected to permit seine collections.

#### BASIN DESCRIPTION

The River Raisin drains an area of 1,070 square miles (2,771 square km) and discharges into the western basin of Lake Erie at Monroe, Michigan (Figure 3). A portion of Michigan's southeastern lower peninsula and the northeastern portion of Fulton County, Ohio lie within the boundaries of the basin. The drainage basin narrows down to a 2.5 mile (4 km-wide) strip for the last 15 miles (24 km) of the river. The area consists of clay till reworked by glacial lake water and veneered by lacustrine sands, silts, and clays. Two-thirds of Monroe County is covered by a layer of this glacial drift that is less than 50 feet (15 m) in thickness. The underlying bedrock is mostly carbonate in composition (Mozola, 1970).

### HYDROLOGY

Monroe County is essentially flat terrain. There is a gentle slope southeastward from a maximum elevation of 730 feet (223 m) in the northwest corner to 572 feet (174 m) at Lake Erie. This gradual decline of only 158 feet (48 m) in nearly 26 miles (42 km) explains the low velocities of streams located in the county (Mozola, 1970).

Runoff in the drainage basin is signficant due to the clay till. The runoff during rain events creates rapid stream fluctuations and very turbid waters. Relative to other areas in Michigan, erosion in the River Raisin basin is considered to be high. The U. S. Department of Agriculture estimated that 8.3 to 10.8 tonnes of topsoil per hectare per year are lost (Michigan DNR, 1979). The U. S. Department of the Interior (1967) reported that the average annual precipitation for the drainage basin area is 31.52 inches (80.1 cm). Of this amount, approximately onethird runs off through the river system.

Much of the area adjacent to the River Raisin is prone to flooding. A large portion of the eastern fringe of the city of Monroe was once marshland. Over the last thirty years, approximately 80% of the marshlands have been filled in for

industrial and recreational uses. The river banks and surrounding areas at the mouth of the River Raisin are man-made (Monroe County Drain Commission, 1984).

The U. S. Geological Survey (USGS) maintains a stream flow gauge (Station #04176500) in the River Raisin near Monroe. It is located in Monroe County, 1.3 km downstream from the bridge on the Ida Maybe Road, at latitude 41 57' 38" and longitude 83 31'52". The drainage area above this point in the river is 1,042 square miles (2,699 square km). The average discharge for the period of record 1937-1981 was 709 cubic ft/sec (19.9 cubic m/sec). The maximum and minimum discharge for the period of record was 14,500 cubic ft/sec (407.3 cubic m/sec) and 2 cubic ft/sec (0.06 cubic m/sec), respectively (U.S.Geological Survey, 1982). River flows for an 11-year period are displayed in Figure 4. Peak flow frequencies for the period of record since 1938 are presented in Figure 5.

The City of Monroe maintains a stream flow gauge in the River Raisin at Dam #1 (second low head dam relative to the river mouth). This guage is located in the City of Monroe approximately 152 m downstream from Maple Avenue (Petty, 1984). Daily readings are recorded by the Monroe Waste Treatment Plant.

The lake level is monitored hourly by a National Oceanographic and Atmospheric Administration (NOAA) guage located near the study area. Water stage readings for Gage 3087 in the turning basin (station 4) are presented in Figure 6 (January 1, 1975 to March 31, 1983).

The port of Monroe is served by a dredged shipping canal 15,800 feet (4.8 km) long, 300 feet (91.2 m) wide and 21 feet (6.4 m) deep from Lake Erie to the mouth of the River Raisin. From the river mouth to the turning basin, there is a dredged channel 8,100 feet (2.5 km) long and 200 feet (60.8 m) wide (Michigan DNR, 1979).

### INDUSTRIAL DEVELOPMENT

Most of the River Raisin is in areas of agricultural production. Over 70% of Lenawee and Monroe Counties is farmland. Urban development of the basin is centered around three cities: Monroe, Adrian, and Tecumseh. Monroe, at the river mouth is the most populous and industrialized city in the basin. Much of the industry is associated with automobile manufacturing in nearby Detroit. Additional industries in the area are primary metals, fabrication of metal products, machinery and transportation equipment, manufacture of paper products, chemicals, furniture, food processing and dairy related industries (Michigan DNR, 1979).

Several paper product companies are located on the River Raisin within the study area. Consolidated Packaging

Corporation, South and North Plant closed on February 1978 and July 1975, respectively, produced corrugated and solid fiber containers. Time Container Company, a paper products industry, is located upstream of the study site near the Chesapeake and Ohio Railroad. Union Camp Corporation on the north shore of the River Raisin produces corrugated paper board and containers. The effluents from the primary treatment facilities of both Time Container and Union Camp are sent to the Monroe WWTP for secondary treatment (Michigan Department of Public Health and the Michigan Water Resources Commission, 1969).

The Detroit Edison Monroe electric generating plant, located near the mouth of the River Raisin, is the largest coal-burning plant in the United States. Up to 85 cubic m/sec of river/lake water is pumped for cooling purposes. During spring runoff, the River Raisin makes up more than 95% of the cooling water. However, during low flow in the summer, the river makes up less than 5%, the balance of water coming from Lake Erie. enters the cooling system through a 100-meter long intake canal that is located about 650 meters upstream from the river mouth. The water passes through a condenser and is then released into a 350-meter long, concrete conduit where water velocities are approximately 1 m/sec at full operation. The water is then discharged through a rock-walled 175-meter wide canal. Plum Creek joins the discharge canal, but contributes less than 1% of the volumetric flow to Lake Erie. The average annual river discharge is equivalent to 20% of the total cooling water demand - the rest is drawn from Lake Erie (Cole, 1978). In essence almost all the river water is funnelled through the power plant.

The Monroe Metropolitan Pollution Control Facility is an activated sludge treatment plant with a design capacity of 24 MGD (90,800 cubic m/d). The plant receives raw wastewater from the City of Monroe and the Frenchtown and Monroe townships. Industrial dischargers contribute approximately 70% of the daily flow (Horvath, 1985). The treated effluent is discharged into the River Raisin. Under severe runoff conditions, high flows in the collection system exceed plant capacity. During this time, untreated wastewater is pumped directly into the river from the flood pumping station.

The Ford Motor Company Stamping Plant at Monroe draws its process and cooling water from Lake Erie. The water is treated with chlorine, lime and ferric sulfate prior to being used (Boerson, 1984). Waste cooling and process waters and sanitary wastewaters are treated by the company. The combined wastewaters are discharged to a polishing lagoon, with overflow discharged to the River Raisin (Horvath, 1985).

#### CONTAMINANT SOURCES

Both toxic contaminant reserves in sediments and current toxic industrial, munincipal and landfill effluent loadings to

the River Raisin were considered as potential sources of toxins in the Monroe Harbor study.

Copper, chromium, and zinc were analyzed during this study because of the relatively high concentrations of these materials found in sediments in the River Raisin and because of the toxic nature of these metals to cladocerans and other freshwater invertebrates. Relatively high levels of toxic heavy metals in the navigation channel have been reported in the literature. U.S. Environmental Protection Agency (1975) recommended that the contaminated dredged sediments from the navigation channel should not be disposed in the open lake. Analysis of contaminants, revealed high levels of copper (1450 mg/kg), zinc (970 mg/kg), and chromium (530 mg/kg). Based on atomic absorption spectroscopy (AAS) by Cranbrook Institute of Science and neutron activation analysis by the University of Michigan's Phoenix Memorial Laboratory (Jones, 1983), concentrations of these metals were relatively high when compared to mean sediment levels in southern Lake Huron. Concentrations of some other metals were also found to be relatively high in these studies, but their toxicity at the current levels to freshwater biota was negligible or unknown.

In addition to reserves of metals in the sediment, there is an existing potential for heavy metal discharge from primary metal production, plating, and metal machining industries in the Monroe Harbor area.

Polychlorinated biphenyls (PCB's) were included in the study of Monroe Harbor because high levels of PCB's in fish were found in the area. In 1971, the Michigan Department of Natural Resources collected fish in the River Raisin and found up to 6.45 mg/kg of Aroclor 1254 in northern pike (wet weight) and up to 3.08 mg/kg of Aroclor 1254 in carp (wet weight). The results of a 1979 survey included a single carp with 77.2 mg/kg of total PCB (Burby et al, 1983)

PCB's have been linked to industrial activity that use the persistant compounds in lubricants and coolants for electrical equipment. PCB's have also been found to be a by-product in paper recycling plants. These industrial uses and processes exist (or existed) in the River Raisin study area; therefore, it is possible that the sediment and fish contamination observed originated from local industrial activity.

#### **METHODS**

### FIELD METHODS

## Sampling Plan

Larval fish samples were collected at night (45 minutes after sunset) twice weekly, towing a .75 meter diameter conical oceanographic plankton net of .571 millimeter mesh behind an outboard motor-powered boat travelling at 4-5 knots. Flow rates (i.e. volume of water sampled) were measured via a center mounted General Oceanic Model MKII flowmeter. From 30 May to 12 September 1983, 7 stations in the lower Raisin River and adjacent Lake Erie were sampled using 4 minute circular, oblique tows. Raisin River water temperature data was obtained from the Monroe Wastewater Treatment Plant and the Detroit Edison Monroe Power Plant. From 2 April to 19 July 1984, stations 1-5 in the lower Raisin River were sampled. In an attempt to insure parity among replicates by sampling "new" water during each tow, and to increase the number of species that could potentially be statistically analyzed, tow times were increased to 6 mintues and were made travelling upstream longitudinally at .3, .5, .7 of the width of the river. While returning downstream to begin the next replicate, special effort was made to travel around the pending transects. Refer to Figures 1 and 2 for the locations of the 1983 and 1984 stations. In 1984, Raisin River surface water temperature was measured at each station with a VWR Scientific thermometer.

In both years, three replicate samples were collected from each station. In the field, replicates "A" and "B" were preserved with a 5 percent volume of 37 percent buffered formaldehyde solution, and replicate "C" was preserved with a 100 percent volume of Dietrick's fixative for future pathologic analysis. Dietrick's fixative was made using the following recipe:

30 parts distilled water

15 parts 95 percent ethyl alcohol

5 parts 37 percent buffered formaldehyde solution

1 part concentrated, glacial acetic acid

When larvae were determined to be sufficiently abundant, additional weekly samples were collected for body burden assessment. A single 10 minute tow was made at each body burden station, but was not chemically preserved. The target species of gizzard shad (Dorosoma cepedianum) and emerald shiner (Notropis atherinoides) were each:

- 1. Separated from the rest of the raw sample
- 2. Patted dry
- 3. Frozen whole for pick-up by Cranbrook Institute

Refer to Appendix A for a list of fish larvae provided for body burden analysis. Additionally, 3 replicate 4 liter amber bottles were filled with 1 liter the surface water from each body burden station (4, 5, 7, in 1983; 4, 5 in 1984) using the following procedure for pick-up and analysis by Cranbrook Institute:

- 1. Rinse bottle with station water; Discard rinse
- 2. Submerge bottle and fill to 1 liter
- 3. Add 100 ml methylene chloride to bottle
- 4. Cap and shake vigorously for 3 minutes

Water samples were not collected on nights when larval abundance was too low for body burden analysis.

## Flow Calibration

Before the first larvae sampling date, once each month, and after the completion of the sampling season, the flowmeter was calibrated by towing the meter on the net frame (without the net) for a known distance (500 meters) for 10 repetitions (Appendix B).

In order to sample all levels of the water column, our oblique tows were adjusted to conform with water depth, as determined with a weighted depth chain as follows:

Station (#)	Depth (m)	Rope Length (m)	Time (min)
1 2	2	2	4 4
3	4	2 + 4 2 + 4 + 6 + 8	2 + 2 1 + 1 + 1 + 1
5	8	2 + 4 + 6 + 8	1 + 1 + 1 + 1
6 7	8 9	2 + 4 + 6 + 8 2 + 4 + 6 + 8	$1 + 1 + 1 + 1 + 1 \\ 1 + 1 + 1 + 1$

#### LABORATORY METHODS

## Larval Fish Sorting

Each sample was recorded in a sample log as it was sorted along with the sorter's initials and processing data book. The entire sample was poured through a USGS #40 sand sieve and rinsed with low pressure tap water to eliminate fine sediments. A small aliquot (1 cubic cm) was removed from the sieve, placed in an enamel or pyrex pan and diluted with tap water. The pan was then placed in the lighted sorting chamber. A Luxo Magnifier (combination light and magnifying lens) was used to facilitate recognition of fish larvae from othe recovered material. The larvae were carefully removed using fine point tweezers and placed in a vial containing 70% Ethanol. A label with the appropriate station and date was inserted in the vial. The

sample remaining in the sieve was treated accordingly. All extraneous zooplankton, invertebrates and detritus were disposed of. The seive was rinsed thoroughly between samples. A note was added to the sorting log indicating the presence or absence of larvae for every sample. The samples were stored by sampling date for identification and enumeration.

Samples for pathological analyses were sorted in a similar fashion but were transferred to vials containing Dietrick's preservative instead of 70% alcohol.

## Larval Fish Identification

Using a Bausch and Lomb stereo dissecting microscope with a polarized stage, rheostatic light source, and magnification range of 6x to 100x, larval fish were identified to species (when possible), developmental stage noted (as defined by Snyder, 1976), and total length measured to the nearest 0.5 mm. Gross morphology was examined for pathological defects using the criteria of Drummond(undated) The following taxonomic keys, relevant papers, and the Ohio State University's Center for Lake Erie Area Research (CLEAR) larval fish archive collection were utilized to facilitate identification.

- 1. Auer, N.A. (ed.) 1982. Identification of Larval Fishes of the Great Lakes Basin with Emphasis on the Lake Michigan Drainage. Great Lakes Fishery Commission, Ann Arbor, Michigan 48105. Special Publication 82-3:744 p.
- 2. Hogue, J. J., R. Wallus, and L. K. Kory. 1976.
  Preliminary Guide to the Identification of Larval
  Fishes in the Tennessee River. Tennessee Valley
  Authority, Div. of Forestry, Fisheries, and
  Wildlife Dept., Norris, TN. 67 p.
- 3. Nelson, D. Working Key to the Larval Fishes Discovered Near the West Shore of Lake Erie. Michigan State University, Dept. of Fisheries and Wildlife. Unpublished. 12 p.
- 4. Norden, C. R. Key to Larval Fishes from Lake Erie.
  University of Southwestern Louisiana, Lafayette.
  Unpublished. 4 p.
- 5. Olney, J. E., G. C. Grant, F. E. Schultz, C. L. Cooper, and J. Hageman. 1983. Pterygiophore-Interdigitation Patterns in Larvae of Four Morone Species. Trans. Amer. Fish. Soc. 1983, No. 4: 525-531.
- 6. Siefert, D. E. 1976. Terminologies for Intervals of Larval Fish Development. Pages 41-60 in Borrman (ed.); Great Lakes Fish Egg and Larvae

Identification. U. S. Dept. of the Interior, Fish and Wildlife Service, Washington, D.C., FWS/OBS-76/23.

Upon completion of the identification of a sample, the final columns of the sample log were filled with date of identification, identifiers initials, and number of the sample's vials. All fully processed samples were preserved with 70 percent ethanol and stored in the CLEAR biological archive. Additionally, one voucher specimen of each species encountered at each developmental stage (I-IV) observed were archived in the CLEAR reference collection (Appendices C and D).

## Identification Problems

As noted above, all larvae encountered were identified to species when possible (Appendix E), but several closely related species among families are difficult or impossible to positively identify while in the early larval stages. Problem families were treated in the following manner:

### CLUPEIDAE

Alewife (Alosa pseudoharengus) and gizzard shad (Dorosoma cepedianum) are separated only by meticulous measurements and/or muscle segment (myomere) counts. Gizzard shad overwhelmingly dominated our catch, thus whenever damaged CLUPEIDAE were encountered, they were expressed as gizzard shad. Specimens in good condition were always keyed to proper species.

### CATOSTOMIDAE/CYPRINIDAE

Poor specimen condition occasionally called for an individual to be expressed as "Unidentified Catostomidae" or "Unidentified Cyprinid". Carp/Goldfish were expressed as carp due to the difficulty of separating wild caught specimens of these species made worse by their propensity to hybridize with each other (Crunkilton, 1977, personal communication)

#### CYPRINODONTIDAE

Fundulus spp. are poorly represented in the literature, thus no attempt was made to assign our wild caught, Fundulus specimens to species.

#### PERCICHTHYIDAE

Morone spp. cannot be separated using morphological features until anal ray pterygiophores become evident at approximately 13 mm, thus Morone spp. less than 13 mm are usually expressed as Morone spp. and those over 13 mm in good condition were separated to White perch (Morone americana) or White bass (Morone chrysops).

### CENTRARCHIDAE

Lepomis spp. are virtually impossible to separate while in their early life stages due to similar morphology and widespread hybridization, thus almost always were expressed as Lepomis sp. Pomoxis spp. are also difficult, but attempts were made when possible to separate the two species using Seifert (1969), otherwise were expressed as Pomoxis sp.

### PERCIDAE

There were occasionally darters, Etheostoma spp. that could not be assigned to species.

## Pathology

Fish preserved in Dietrichs fixative were delivered to a certified histology technician. These fish were dehydrated and embedded in paraffin blocks. Smaller larvae (4-10 mm) were embedded at a density of five fish per block. Larger fish (12-25 mm) were embedded one per block. Fish were oriented so that longitudinal, mid-line sections, cut at 5u could be produced. Sections were mounted on glass slides and stained with hematoxylin and eosin. The following tissues were examined for histologic lesions: skin, oral epithelium, bronchial epithelium, gills, thymus, brain, spinal cord, eye, otolith organ, thyroid, interrenal organ (adrenal), pancreatic islets, heart, skeletal muscle, excretory kidney, urinary bladder, head kidney (hemopoietic organ), liver, exocrine pancreas, stomach, intestine, peritoneal fat, air bladder, cartilage and bone.

A list of observed lesions from each fish examined was kept and a table of lesion frequencies was generated. This data was analyzed and compared to a table of lesions generated from similar fish larvae from the control collection site.

Several larval fish that had observed spinal deformities or tumored growths were sent directly to the pathologist for

observation before they were prepared for histological analysis.

## Laboratory Exposure - Fathead Minnows

The Fathead minnows used in this laboratory induced exposure experiment were hatched on October 16, 1984. At the initiation of the experiment on October 18th the fish were 48 hours old. Sediment and water for the experiment were collected on October 16th from Station 4 (in the turning basin and the upstream control Station 12.

Covered five gallon aquariums were filled with a combination of:

Station 4 water and Station 4 sediment

Station 12 water

Station 12 water and Station 12 sediment

Water was replaced as needed dur to evaporation. The tanks with sediment were too cloudy to determine if all the fish were alive on two different dates, December 13 (56 day exposure) and December 27 (70 day exposure). Eight larvae were removed from each aquaria during each sampling and placed in formaldehyde prior to transport to the Ohio State University Laboratory. Upon arrival they were transferred to Dietrick's solution and sent to the pathologist for analysis.

## Chemical Extraction Procedures

Biological tissue (approximately 20 g) was mixed with anhydrous sodium sulfate and Soxhlet extracted for 48 hours with a 1:1 mixture of n-hexane and dichloromethane. When less than 20 g of tissue was available, the total sample was extracted. (Composite larval fish samples ranged in weight from approximately 5 g to 47 g). The extract was partitioned into n-hexane and its volume reduced to 10.0 ml over a steam bath. A one ml sample was air dried in a tared aluminum weighing dish for lipid determination. Details of the extraction procedure may be found in Rathbun (1985).

## Chemical Clean-Up

Extracts were cleaned of lipids and other interferring compounds with Florisil. [Details are provided in Smith  $\underline{et}$  al. (1985)]. Columns were packed with 20 grams of Florisil; rinsed with 50 ml n-hexane; 1 ml of extract was injected onto the column followed by 250 ml of 4% DCM in n-hexane. The solvent volume was reduced over a steam bath to less than 10 ml, and to 1.0 ml under a stream of dry N2 gas. The extract was sealed in a glass ampule until analysis.

## Chemical Analysis and Quantitation

High resolution fused silica capillary gas chromatography was performed on a VARIAN Model 3700 gas chromatograph equipped with a 63Ni electron capture detector (ECD). A 50 m fused silica column (0.2 mm i.d.) coated with SE-54 (Hewlett-Packard) was used to separate the PCB congeners. The oven temperature was programmed at a rate of 1.0 degrees C min-1 from 100 to 270 degrees C and held at 270 degrees C for ten minutes. The injector and detector temperatues were 270 degrees C and 320 degrees C, respectively. The sample volume, 4.5 uL, was injected by an automatic sampler using a splitless injection technique (10:1 split ratio, vented from 0.75 to 1.75 minute). The hydrogen carrier gas was held at a constant pressure of 2.25 kg cm-2 to give the optimized velocity (u) 50 cm sec-1 (at 100 degrees C).

The chromatograph data were acquired using a Hewlett-Packard 3354 Laboratory Automation System (LAS) and transferred to a Digital PDP-11/45 computer via magnetic tape.

Once transferred, each raw file was subjected to a series of programs for data analysis:

- (1) Attenuate (ATN) Expands the scale of the chromatogram.
- (2) PLOT (PLT) Establishes a chromatogram base line.
- (3)  $\overline{PEAKS}$  (PKS) Determines peak height.
- (4) Mean Standard Deviation (MSD) Determines baseline noise mean and the standard deviation used in the 'Peaks' program.
- (5) PUP Compares the sample file to a library file for retention time and names PCB or Pesticide peaks detected.
- (6) <u>UPDATE</u> (UPD) Updates the library with the calibrating standard.
- (7) Sample Final Concentration (SFC) Gives the total PCB or Pesticide sample concentration, the number of peaks accepted in the analysis and the homolog distribution.
- (8) <u>COMSTAR</u> (CMS) A multiple regression program that fits observed congeners in sample with a linear combination or Aroclors. Any peaks not fit are considered outliers and marked for rejection.

#### DATA ANALYSIS

## Growth and Mortality Calculations

Seven calculation steps were performed on the ichthyoplankton database obtained from the 1983-1984 Raisin River Study. See Appendix F for the computer program documentation to achieve the following seven steps.

- Step 1: Calculate larval fish density (#/1000 cu meter) for all samples (A, B, and C) for each station and sampling period. See Figure 7 for density calculation procedure.
- Step 2: Average A, B, and C density replicates by species, size, station, and sampling period.
- Step 3: Sum each species total density by station on an individual sampling period basis and over the total season.
- Step 4: Calculate the average length (mm) of each species by station and sampling period.
- Step 5: Calculate the date when each species population length (TL) by station equals 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, and 70 mm.
- Step 6: Calculate the instantaneous growth rate coefficient (G) for each species at each station.
- Step 7: Calculate mortality (Z) for each species at each station.

Instantaneous Growth Rate Coefficients were calculated for all fish that appeared for a sufficient period of time during the sampling season to acquire a rate (i.e. n > 3). The instantaneous growth rate equation utilized was

Lt = Lto e G (t - to) where:

L(to) = Length of larval fish at an intial time

Gt = Growth rate

t = time final

to = time zero

Lt = Length of larval fish at final time

Length (Lo) and Growth (G) were determined by regression techniques using time (t) for x and length (L) for y. This

procedure is outlined by Hackney and Webb (1978) in the Proceedings of the National Workshop on Entrainment and Impingement.

Mortality was calculated using the equation

Nt = N(to) e -z(t - to) where:

N(to) = Numbers of larval fish at an initial time

Z = Mortality rate

t = time final

to = time zero

Nt = Number of larval fish at final time

Further information on the development and use of this equation can be seen in Hackney and Webb (1978).

### RESULTS

## DISTRIBUTION/ABUNDANCE

A total of 46 species of larval fish were collected and identified during the two year field study (Table 1). The predominant species of larval fish found during the 1983/1984 EPA survey are reported in Tables 2 (1983) and 3 (1984). A total of 15,849 larval fish were collected from May 30th to September 12th, 1983. A substantially larger population (25,583) was collected from April 2, to July 19,1984. A feasible explanation for the increased larval fish catch is the improved sampling design utilized in 1984 (see Methods). The most abundant fish captured during both field seasons was the gizzard shad (11,410 in 1983 and 18,853 in 1984). The major portion of the 1984 larval fish increase (n = 9734) is accounted for by gizzard shad, however gizzard shad represented approximately 73% of the entire larval fish population collected during each field season.

The predominance of the remaining species varied slightly between the two field years with the largest shift found in the Morone spp. population (13th in 1983 and 3rd in 1984). This is explained by the spawning season of Morone spp. which extends from late April to June. Remember that the field season in 1983 did not begin until May 30th.

Analysis of the distribution of larval fish indicated some conflicting results between the two years. For example, the ten most predominant fish were generally collected at all stations in 1983 with the exception of freshwater drum and yellow perch. Freshwater drum larvae were not collected from either station 1,2 or 3 in 1983 and yellow perch were not observed at either station 2 or 3. However both species were represented at all stations during the 1984 field season (Tables 4 and 5). Once again this might be explained by our improved sampling design in 1984 and the initiation of spring sampling in 1984.

Jude et al. 1983, studied the Monroe Power Plant from February of 1982 thru February of 1983 to assess the entrainment and impingement of fish larvae. The abundance of each species collected during the study has been ranked and compared to the two separate field seasons of the present study (Table 6). The major differences in species predominance are as follows:

Alewife White Bass	not	found	bу	Jude
	not	found	рy	Jude
Brook silverisde	not	found	bу	Jude
Rock Bass	not	found	bу	Jude
Largemouth Bass	not	found	bу	0 S U
Northern Hogsucker	not	found	bν	0.811

Estimated abundance of the predominant species and relative

percentages were calculated for Jude's 1982 study (Table 7). As observed in the power plant study, gizzard shad were the most abundant (4.08 and 109) for a total of 86.8 % of the fish population (Table 7). This percentage slightly exceeds the percentage calculated (Tables 2 and 3) by Ohio State University (73%).

#### GROWTH RATES

Two different calculations were performed to assess the growth rate of pre- and post- larval fish from the River Raisin study area. The first method involved the simple ratio of differences in length (dl (mm)) to differences in time (dt (days)). The second calculation (instantaneous growth rate coefficient) involved the use of the linear differential equation  $\text{Lt} = \text{Lto} \ e \ G(t-to). \quad \text{Each of the methods employed in these calculations are described previously in this report.}$ 

The 1983/1984 data for simple growth rates (d1/dt) are shown in Table 4 for gizzard shad. Values for the two years range from 0.27 to 0.98 mm/day. A summary of the 1983/1984 rates can be seen in Figures 8 and 9, respectively. Individual plots of simple growth rates by station can be seen in Figures 10-20. Different growth rate slopes are shown in these figures to demonstrate the variability in growth estimates. Slopes of three or more points, initiating with newly hatched larvae are projected.

Simple growth rates were also calculated for emerald shiners at all stations in both 1983 and 1984 (Table 9). Summary plots of the 1983 and 1984 rates can be seen in Figures 21 and 22 respectively. The individual plots of the simple growth rates by station can be seen in Figures 23-33.

The instantaneous growth rate coefficients for gizzard shad and emerald shiners are presented in Tables 10 and 11 and Figures Growth is represented by the variable L1 and ranges from 0.016 to 0.125. The 1983 rates for both gizzard shad and emerald shiners are summarized in Table 12. Each station was ranked giving the lowest ranking (r=1) to the station with the highest instantaneous growth rate coefficient and highest ranking to station with the lowest instantaneous growth rate coefficient The rankings for both species were combined and then reranked. The initial rankings for both species were similiar for except station 2. Station 2 gizzard shad exhibited the lowest instantaneous growth rate while station 2 emerald shiners exhibited the highest instantaneous growth. This discrepancy will be discussed later in this report. The 1984 rates for gizzard shad and emerald shiners are ranked in Table 12, similiar to the method previously described. However, the 1984 data did not exhibit the discrepancy found in the 1983 station 2 data.

## MORTALITY RATES

Mortality rates were calculated according to the equation proposed by Hackney and Webb (1978) and outlined in the methods section. The mortality equation involves the use of the initial and final density of larval fish resulting in the determination of Z, the mortality rate. The mortality rates for 1983 and 1984 are presented in Tables 14 and 15, respectively. Z should be positive under normal circumstances, indicating decreasing larval fish density thru time. Several species have negative estimates of the variable Z, indicating increasing larval fish density. The initial larval fish population size (No) in these cases is always small (<10 fish per 1000 cubic meters). These values should not be considered in further discussion. Values based on data from species when the initial population densities (No) are sufficiently large have mortality rates (Z) which range from 0.011 to 0.200 (Tables 14 and 15).

#### PATHOLOGY

Station 4 fish were selected for histopathological analysis because it had been selected by the USEPA as a master station and therefore would have a corresponding weekly database of organics and metals. Gizzard shad were selected from station 4 because of their high density and frequency. Gizzard shad were selected from each sampling period beginning on June 16 and ending on September 8. A list of samples taken for pathological analyses is included in Appendix G. In addition to station 4 gizzard shad, 15 samples were sent to the pathologist because of observed spinal defects or possible internal tumors (Table 16) Gizzard shad from every station on August 8 were analyzed due to tumors observed on August 4 and 8. Finally, at least one specimen from every species found at station 4 was analyzed.

The results of the first group of fish revealed that the smaller fish (i.e. <15 mm) were too difficult to interpret. The second set of samples were selected so that the size exceeded the  $15\ \text{mm}$  limitation.

A total of 104 blocks of fish collected from six stations between June and September 1983, were evaluated for histopathologic lesions. Twelve different species of fish were submitted but only gizzard shad were numerous enough for significant analysis (Table 17). The majority of fish were collected from stations 4 and 5. The quality of fixation of the specimens was generally good with autolysis impeding histopathologic interpretation in only a few cases. The quality of the prepared slides was excellent. Twenty three organs and tissues were present with sufficient frequency to permit significant analysis.

A total of 46 fishes were received for histopathologic diagnosis from the control station (#7). There were 39 gizzard

shad, 4 alewife, and 3 yellow perch collected from the control lake station to be compared to gizzard shad from the riverine stations (1-6). In addition, 18 fathead minnows were submitted which were exposed to potentially toxic substances from station 4. The technical quality of these specimens was fair to good. While consistent evaluation of 24 tissues was possible, fixation of tissues was clearly less satisfactory than the previous lot of fish. Many tissues had autolyzed or were distorted due to the fact that the samples were shipped to Ohio State University in formaldehyde and then transferred to Dietrich's fixative. On the whole, however, a significant number of specimens of good quality permitted adequate interpretation of lesions with sufficient consistency to validate the results.

Lesions were consistently observed only in gizzard shad from the river stations. Basically, lesions consisted of acute epithelial necrosis characterized by picnosis, coagulation and separation of cells from the basement membrane and often accompanied by sloughing into the lumen of the organ. These changes ranged from mild to severe and from a focal to diffuse distribution. Acute epithelial necrosis was observed with a high frequency in the olfactory organ (94.6%), lateral line organs (94.4%), the oropharyngeal epithelium (96.2%), esophagus (91.5%), gills (91.5%), renal tubules (94.3%) and intestine (70.4%) (Table 18).

Two of the most severely affected, important organs were the gills and kidney. Gill tissue was present in 47 of the 77 (61%) gizzard shad. Besides frank necrosis of branchial epithelium, a high percentage (80.8%) of gizzard shad had separation and ballooning in the gill tissue interpreted to be branchial Seventy two percent of gizzard shad had gill parasites. The most common parasites were the protozoans Ichthyophirius sp. and Trichodina sp. In addition, agents compatable with Epistylus, monogenetic trematodes and glochidia of fresh water mussels were occasionally observed. In all cases, the branchial epithelial changes associated with these agents were localized. The kidney was evaluated in 53 of the 77 (69%) gizzard shad. addition to acute tubular epithelial necrosis observed in 94.3% of gizzard shad kidneys, there was a significant number of kidneys (32.1%) with hyaline droplet degeneration. The lesion was manifest as one to several circular, eosinophilic inclusions in the cytoplasm of renal tubular epithelium.

Sample sizes of the other species of fish were not large enough to provide signficant interpretation. However, carp, logperch, catfish, yellow perch and walleye had no lesions. The spottail and emerald shiners, troutperch and Morone sp. were too small for significant analysis. One freshwater drum was normal and one had a questionable lesion in the olfactory organ and intestine. The single specimen of alewife had lesions similar to those in gizzard shad.

Table 19 compares the distribution of affected gizzard shad

(exhibiting histopathological lesions) among four different size groups and five collection stations. Although the majority of fish were collected from stations 4 and 5, fish from all stations had lesions. Fish in all size classes had significant histopathological lesions. The relatively low percentage of fish less than 20 mm long exhibiting lesions is an artefact. Many fish in this size class were too small or not sufficiently differentiated to permit pathological evaluation.

Several specimens were submitted with severe spinal curvature but no histological basis for this lesion was observed. In addition, several fish specimens were submitted with grossly evident tumors. Histological evaluation revealed these to be non-neoplatic, microsporidian cysts, probably of the genus Glugea.

A large number of fish from the control station had lesions (Table 20). Acute coagulation necrosis of epithelial cells was observed in 11 of 12 olfactory organs, 1 of 28 otolith organs, 10 of 11 lateral line organs, 37 of 40 oropharynxs, 31 of 38 esophaguses, 41 of 43 gills and 15 of 16 intestines. Acute renal tubular epithelial necrosis was observed in 35 of 42 fish. Hyaline droplet degeneration occured in the kidneys of 16 of 42 Thymic lymphoid necrosis occured in 2 of 29 fish. parasites were observed on 10 of 43 (23.3%) of these fish. gizzard shad evaluated had lesions. The most consistent lesion was epithelial necrosis in the gills and kidney, observed in 35 of 35 fish. Four of four alewives had gill and kidney lesions similar to gizzard shad but less severe. Three alewives had necrosis of the oropharyngeal epithelium. Only one yellow perch had lesions. Mild necrosis was observed in the olfactory and lateral line organs as well as the oropharynx and gills.

## <u>Laboratory Exposure - Fathead Minnows</u>

A total of 18 fathead minnows subjected to laboratory exposures of sediments and water from the River Raisin and control site were evaluated for histopathologic lesions. Twenty-three tissues were examined but no lesions or abnormalities were noted. The three different groups of fish (station 4 sediment and water, station 12 water, and station 12 sediment) could not be distinguished in any way by microscopic evaluation (Table 21). One mortality was observed from the station 4 sediment/station 4 water exposure on December 6, 1984 (49 day exposure). This specimen was not submitted for pathological analysis due to the broken condition.

BODY BURDENS

## Larval Gizzard Shad

The only larval species that was collected in sufficient numbers, throughout the sampling period, to provide enough tissue

for residue analyses was gizzard shad. Eleven samples were analyzed for total PCBs and chlorinated pesticides. In 1983, samples from Stations 4, 5 and 7 were analyzed for contaminant body burdens. Total PCB and pesticide body burden concentrations are presented in Table 22. Larval gizzard shad collected from Station 4 on 7/14, 7/21, and 9/1/83 contained 0.056, 2.9 and 0.91 mg/kg total PCB, respectively. The single control station sample, which was analyzed for chemical contaminants, was collected on 8.18.83 and contained 0.40 mg/kg total PCB. PCB homolog concentrations are presented in Appendix H.

During the 1984 sampling season, only larvae from Station 4 were retained for contaminants analyses. Total PCB body burdens for these samples ranged from 0.26 to 2.5 mg/kg (Table 22). During both years, larval gizzard shad from Station 4 exceeded the 2.0 mg/kg U. S. FDA action level for PCB's. Conversely, residue levels for all pesticides analyzed were less than 0.1 mg/kg throughout the two year study.

## Young-of-the-Year Emerald Shiners

During the 1984 sampling season, young-of-the-year emerald shiners were collected from Stations 1, 4, and 45 (a new station established mid-way between Stations 4 and 5). These were the only locations at which sufficient numbers were collected to allow for residue analyses. Nine samples were analyzed for PCB's and chlorinated pesticides. Body burden concentrations are presented in Table 23. Emerald shiners from the upstream reference site (Station 1) contained total PCB concentrations ranging from 0.48 to 0.79 mg/kg. Significantly higher (P <.01) PCB levels were seen at stations 4 (1.7-2.9 mg/kg) and (P <.005) 45 (2.4-3.7 mg/kg). Young-of-the-year emerald shiners collected at station 4 and station 45 did not exhibit significantly different body burdens (P > .2) All the samples from Station 45 and two of three samples from Station 4 exceeded the U.S. FDA action level of 2 mg/kg PCB. PCB homolog concentrations are presented in Appendix H. Chlorinated pesticide concentrations for all samples analyzed were less than 0.1 mg/kg (Table 23).

#### DISCUSSION

## DISTRIBUTION/ABUNDANCE

Average density for 1983 gizzard shad larval fish ranged from a low at station 2 (49.6 fish/1000 cubic meters) to a maximum at station 7 (828.2 fish /1000 cubic meters) (Table Miller (1960) reported that gizzard shad were abundant throughout the western basin of Lake Erie particularly in protected bays and at the mouths of tributaries. Gizzard shad are particularly attracted by warm water flowing from industrial plants and able to withstand temperatures up to 35 C. The River Raisin should be an ideal location for gizzard shad due to the heat introduced from the once-thru cooling power plant located at the mouth of the river. However, the densities found in the River Raisin and the surrounding portion of the western basin are low compared with densities of peak abundance reported in the literature for Lake Erie. Literature values for gizzard shad peak abundance (Table 25) for the Maumee River were recorded at 16,349 fish /1000 cubic meters (Snyder, 1978). The peak abundance of larval gizzard shad was recorded at station 6 at 5,596 fish /1000 cubic meters (Table 24) or approximately 35% of the peak density at the Maumee. Literature values for the open lake area surrounding Davis Besse fluctuated greatly (1104 - 10,369 fish 1000 cubic meters) over a 3 year period (Gordon, 1982). from Sandusky Bay (3812/1000 cubic meters) seems to be more in the range of the values reported for the open lake area near Monroe and at the mouth of the River Raisin (Snyder, 1978).

Data provided by the Michigan Department of Natural Resources based on a qualitative sediment survey undertaken in the 1983 field year indicates that station 4 and 5 both represent poorer sediment quality due to the presence of oil or oil odors (Table 26). This is incongruous with the larval fish density data reported for these stations. Average densities for fish <5 mm (indicating they were hatched within the immediate area) indicate that station 4 and 5 contribute 10.9% and 15.2%, respectively of the system's larval gizzard shad. Stations 1-3 contribute less than 10% combined (Table 27). By far, stations 6 and 7 produce the major portion (31.9% and 30.7%) of the population.

A spring rain event on February 14, 1984 resulted in the river stage level rising 6.2 feet above the previous day (577.20). Although the river level had subsided by February 16th to 578.3 feet (+1.1 feet), it took over one week for the river to return to the level prior to the rain storm. The river level was accentuated during this storm due to large chunks of ice blocking the river mouth. It was believed that this event would have disrupted much of the spawning habitat but it appears to have had no negative effect.

#### GROWTH RATES

#### <u>Gizzard Shad</u>

Simple growth rate data for gizzard shad collected by Carlander (1970) demonstrate that throughout their distribution, shad exhibit a higher growth rate in Lake Erie (1.0 mm/day) than elsewhere. These rates are presented in Table 28 (Carlander,1970 and Bodola,1955). Growth data from the recent River Raisin study and surrounding lake area ranged from 0.25 to 2.20 mm/day (Table 8). The highest growth rates (0.94 - 2.20 mm/day) occurred at station 3 , adjacent to the Monroe sewage treatment plant. Growth rates from the remaining stations are substantially lower (0.25 - 0.78 mm/day).

Growth rates following yolk sac absorption are dependent on food abundance and availability, ability of the larvae to capture food and water temperature (Gordon, 1982). Gizzard shad larvae are planktivores, switching from zooplankton to phytoplankton after the first few weeks (Miller, 1960). Possibly the differences in simple growth rates between stations can be explained by the analysis of the distribution and abundance of plankton.

The growth rate coefficient data presents a different picture than that of the simple growth rates (Tables 12 and 13). The highest gizzard shad growth rates predicted from growth rate coefficient data occurred at station 1 during both 1983 and 1984. The second most productive station was station 3 in 1983 and station 2 in 1984. The discrepancy obtained from utilizing the results of the two different growth rate techniques has not been resolved to date.

Part of the problem with utilizing the Hackney and Webb (1978) equation to calculate growth rate coefficients is that gizzard shad are wide temporal spawners and the presence of newly hatched larvae over several months biases the actual growth rate. In the future, calculations for wide temporal spawners might be calculated by simply using data limited to the largest larvae captured as suggested by Gordon, 1982. The differences in rate coefficients that she obtained when using the entire population (0.028) was lower than that obtained when data for only the largest larvae was utilized (0.034).

The growth rate coefficients found by Gordon (1982) for 1978 – 1980 gizzard shad at Davis Besse (0.028 – 0.034) are within the range of those found for the River Raisin 1983 – 1984 study (0.017 – 0.090).

## Emerald Shiners

Simple growth rates (dl/dt) calculated for emerald shiners (Table 9) ranged from 0.19 to 1.06 mm/day with the greatest value occurring at station 3 in 1984. Similiar data presented in

Carlander (1970) indicated growth for emerald shiner larvae was 5.6 mm/week or 0.8 mm/day, higher than the average simple growth rate calculated for this study (0.48 (mm/day). Growth rate coefficient data again indicates different results when compared to simple growth rate results. The 1983 coefficient data shows that station 2 has the fastest growth and station 5 the slowest. The results for 1984 indicate a wider range of growth rate coefficients (-.009 to 0.142) with station 4 having the highest rate and station 3 the lowest.

Once again the discrepancy between the two methods may be explained by the a temporal spawning range. If the number of days over which 5 mm larvae were collected is used to define the seasonal range for spawning, length of the spawning season in days for 1983 gizzard shad and emerald shiners is as follows:

STATION		SPECIES
	Gizzard Sha	d Emerald Shiner
1	35	22
2	14	45
3	39	31
4	49	43
5	56	42
6	46	25
7	73	6

The number of potential spawning days ranges from 6 to 73. This variability results in an irregular flux of newly hatched larvae masking the actual growth rate results.

#### MORTALITY RATES

### Gizzard Shad

Data resulting from instantaneous mortality calculations is more difficult to interpret than the growth data. The value of Z, the estimated mortality coefficient should be positive under normal circumstances. On an average of the two years, 30% of the mortality coefficients are negative indicating a decrease in density with time. Many of these negative coefficients exist for species with small initial populations (i.e. number is < 10 fish /1000 cubic meters). One case of negative mortality (an increase in population size through time) occurred for gizzard shad at station 3, 1983, with an initial population density of 45.5 fish per 1000 cubic meters. This data will be deleted from further discussion due to a lack of sufficient density.

The 1983/1984 mortality results for all species are presented in Tables 14 and 15 respectively. Mortality data for gizzard shad are as follows:

# Gizzard Shad Mortality Coefficients (Z)

YEAR	STATION	Z
83	1 2 3 4 5 6 7	.018 .001 010 .043 .044 NA
84	1 2 3 4	.073 .082 .049 .049

Gizzard shad mortality rate coefficients ranged from -.010 to .082. As with the instantaneous growth rates, station 2 mortality rates were the most inconsistent between the 2 years ranging from a low mortality (.001) in 1983 to the highest mortality rate observed in 1984 (.082). Station 2 growth data demonstrated the lowest growth (.017) in 1983 and the highest in 1984. In summary, 1983 station 2 data had the lowest growth rate and also the lowest mortality. In 1984, when the growth rate was high, mortality was also high. Station 1 gizzard shad mortality data also presented a dichotomy between the two field years due to the large increase in mortality during 1984.

Little is written in the literature about the calculation of larval fish instantaneous mortality data. Hackney and Webb (1978) present only one example of instantaneous mortality for larval crappie in which Z=0.1067. This represents higher mortalities than those observed during this study. Hackney and Webb were also dealing with much more dense fish populations (i.e. No = 7.6 x 10 ) which exceeds any of the population sizes encountered in the River Raisin.

In general, mortality rates are much higher for the 1984 field season and unless data can be correlated on a yearly and a station basis for food availability and toxic contamination it will be difficult to interpret the significance of these results.

#### PATHOLOGY

Although the preliminary analysis did not include any fish from the control station the histological alterations observed were determined to be real and in most individuals, severe. The lesions of acute epithelial necrosis in tissues in contact with environmental water (sensory organs, oropharynx, proximal esophagus and gills) are compatable with acute toxicity due to the direct action of an environmental contaminant. Similar

lesions in the intestine and excretory kidney are compatable with concentrations of a toxic substance at sites of absorption, metabolism and/or excretion. There was no observable evidence of carcinogenicity.

Probably the most significant lesions were those observed in the gills and kidney. Significant necrosis in these tissues may impair gas exchange, electrolyte concentration, nitrogen metabolism and osmotic regulation, which might adversely affect performance. Hyaline droplet degeneration in renal tubular epithelium is often correlated with excessive proteinuria. The extent, frequency and severity of these lesions in the gizzard shad might reasonably be expected to have a negative effect on the exposed local fish population. In addition, if gizzard shad retain any toxic substances, predation by piscivorous fish, birds and mammals might cause accumulation and potentially cause lesions at higher trophic levels.

The histological changes observed in the gizzard shad from the control station are interpreted to be real and significant pathological lesions. The lesions were characteristic of acute coagulation necrosis and ranged in severity from mild to These lesions are almost identical to those found in the river shad, and while not diagnostic, are compatable with toxic The tissue distribution of lesions is strikingly similiar to that observed in the gizzard shad from the river stations. As observed in the river shad, the tissue pattern is consistent with an environmental toxicant which is concentrated or transported in the intestine and kidney. Although the numbers of alewife and yellow perch are too few to draw conclusions, it appears that alewife were similiarily but less severly affected than gizzard shad. Likewise, the data suggests that yellow perch seem more resistant.

The finding of fish with lesions at the control station similiar to those from the river was unexpected (Table 29). There are several possible explanations. One explanation is that the fish move between the two localities and that the two samples represent a single fish subpopulation. An argument against this hypothesis may be found in the histologic observations of the two groups of shad. Gill parasites were observed on 34 of 47 (72.3%) of the river shad. However, only 10 of 36 (27.8 %) shad the control lake station had gill parasites. This seems to be a large difference and suggests that the samples are drawn from either separate shad subpopulations or that exchange between the two localities is very slow. A second explanation for the pathologic changes in the control shad is that the control station is contaminated with similiar toxicants to those in the river system. Comparison of water chemistry data from the two localities not only will be helpful in answering this question but may also suggest which substance(s) may be involved. A third explanation is that the lesions might be caused by an unaccounted for variable common to both localities but unrelated to pollution

(i.e. viral disease). In the authors opinion, the most likely explanation is contamination of the control station. If this is correct, it might indicate that a serious health problem exists for gizzard shad and perhaps alewife over a wide range of environments in Lake Erie.

Failure to observe lesions in the experimentally exposed fathead minnows might be explained by any of several hypotheses. It is possible that the fathead minnows are either more tolerant or resistant to the exposed toxic material than the naturally exposed fish (gizzard shad). Alternatively, there may have been an insufficient level of toxic material in the experimental system or low bioavailability of material which was present. It is also possible that there was insufficient time for lesion development.

### **BODY BURDENS**

#### Gizzard Shad

The limited number of samples (n = 11) that were analyzed for contaminants prohibits any statistical interpretation of the data. The majority of samples (n = 8) were collected at Station 4 with only two samples from Station 5 and one from Station 7 were analyzed, making it very difficult to draw any conclusions regarding spatial variability of contaminant levels. these data, however, larvae collected at Station 4 did accumulate higher levels of PCBs than those collected at either Station 5 or 7. Larvae collected on three different dates from Station 4 had PCB concentrations in excess of the 2 mg/kg U. S. FDA "action level" for PCBs in edible portions of fish (Table 22). This level of PCB accumulation has not previously been reported in larval fish, and is probably indicative of exposure to very high levels of environmental contamination. Other data generated by the Monroe Harbor study indicates very high levels of PCBs in River Raisin sediments (Filkins, et al., 1985). The Michigan Department of Natural Resources has issued a health advisory against eating fish taken from the River Raisin, based on PCB levels in fillets (James Rossio, pers. comm., MI Dept. of Nat. Res.). All but one of the samples analyzed exceeded the aquatic life objective of 0.1 mg/kg PCB established by the 1978 Great Lakes Water Quality Agreement.

## Young-of-the-Year Emerald Shiners

Mean total PCB residues in young-of-the-year emerald shiners were 0.66, 2.46 and 3.16 mg/kg at Stations 1, 4, and 45 respectively (Table 23). Samples from Station 1, the upstream reference site, had significantly (P <.01) lower levels of PCB than the two downstream stations. Similarly, Station 1 water and surficial sediment samples contained lower levels of PCBs than the downstream stations (Smith et al., 1985 and Filkins et al., 1985).

All of the emerald shiner samples contained PCB residues in excess of the 0.1 mg/kg PCB objective, established by the 1978 Great Lakes Water Quality Agreement, for the protection of aquatic life. Five of nine emerald shiner samples also exceeded the 2 mg/kg U. S. FDA action level for PCBs in edible tissues of fish. Because of the low mobility of young-of-the-year emerald shiners, these residue levels do indicate substantial exposure to PCBs, in the River Raisin, over the first few months of the shiners' lives.

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TABLES

TABLE 1
Larval Fish Species for the River Raisin
1983-1984

CDECIEC " AASE		
SPECIES # CODE	COMMON NAME	SCIENTIFIC NAME
101 102 103 104 105 106 107 108 109	carp goldfish shiner or minnow spottail shiner emerald shiner central stoneroller bluntnose minnow golden shiner creek chub silverjaw minnow	Cyprinus carpio Carassius auratus Cyprinid Notropis hudsonius Notropis atherinoides Campostoma anomalum Pimephales notatus Notemigonus crysoleucas Semotilus atromaculatus Ericymba buccata
201 202 203 204	white sucker lake chubsucker quillback carpsucker unidentified sucker	Catostomus commersoni Erimyzon sucetta Carpiodes cyrpinus Catostomus sp.
301 302	alewife gizzard shad	Alosa pseudoharengus Dorosoma cepedianum
401 402 403 404 405	channel catfish stonecat madtom yellow bullhead tadpole madtom unidentified catfish	Ictalurus punctatus Notorus flavus Ictalurus natalis Notorus gyrinus Ictalurus sp
501	trout-perch	Percopsis omiscomaycus
601 602 603	wh.bass or wh.perch white bass white perch	Morone sp. Morone chrysops Morone americana
701 702 703 704	green sunfish unidentified sunfish white crappie rock bass	Lempomis cyanellus Lepomis sp. Pomoxis annularis Ambloplites
705 706	wh. or bl. crappie bluegill	rupestris Pomoxis sp. Lepomis macrochirus

TABLE 1 (Continued)

SPECIES # CODE	COMMON NAME	SCIENTIFIC NAME
801 802 803 804	yellow perch logperch sauger walleye	Perca flavescens Percina caprodes Stizostedion canadense Stizostedion v. vitreum
805 806	johnny darter	Etheostoma nigrum
	perch or darter	Percidae
901	freshwater drum	Aplodinotus grunniens
1001	rainbow smelt	Osmerus mordax
1101	brook silverside	Labidesthes sicculus
1201	killifish or topminnow	Fundulus sp.
1301	northern pike	Esox lucius
1401	Brook stickleback	Culaea inconstans
1501	lake whitefish	Coregonus clupeaformis
1601	Burbot	Lota lota
1901	Unidentified	Unidentified

Abundance of Larval Fish Collected in the River Raisin, 1983

TABLE 2

	(	Total # Larvae Collected, 1983	% of Total Collected, 1983
1	Gizzard shad	11,440	72.1
2 3 4 5 6 7	Emerald shiner	919	5.8
3	Carp	814	5.1
4	Morone sp.	701	4.4
5	Freshwater drum	512	3.3
6	Spottail shiner	345	2.2
/	Channel catfish	245	1.5
8 9	Yellow perch	215	1.4
	Lepomis spp.	114	0.7
10	Unidentified Cyprini		0.6
11	Alewife	63	0.4
12	Walleye	67	0.4
13	White bass	49	0.3
14	Logperch	44	0.28
15	Brook silverside	49	0.27
16 17	Rock bass	29	0.18
18	Rainbow smelt	25	0.17
	White sucker	16	0.101
19	Trout-perch	16	0.100
20 21	Bluntnose minnow	14	0.088
22	Pomoxis sp.	12	0.080
23	Tadpole madtom	9	0.057
24	White crappie	8	0.050
25	Sauger	7	0.040
26	Stonecat madtom	6	0.040
27	Johnny darter	4	0.020
28	Unidentified	5	0.020
29	Unidentified percid	2	0.020
30	Silverjaw minnow	3	0.019
31	Quillback carpsucker Golden shiner	2	0.013
32	Green sunfish	2	0.013
33	White perch	6 4 5 2 3 2 2 2 2 1	0.010
34	Goldfish	<u> </u>	0.010
35	Central stoneroller		0.006
36	Lake chubsucker		0.006
37	Creek chub	1 1	0.006
38	Unidentified catosto		0.006
39	Yellow bullhead	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	0.006
40	Ictalurus sp.	1	0.006
41	Bluegill	1	0.006
42	Unidentified Fundulu:		0.006
	on denotified tuniquitu	3 <u>1</u>	0.005
	TOTAL	15,849	99.834

TABLE 3
Abundance of Larval Fish Collected in the River Raisin, 1984

		Total # Larvae Collected, 1984	% of Total Collected 1984
12345678901121345678901223456789012345678901223456	Gizzard shad Carp White bass Morone sp. Channel catfish Freshwater drum Lepomis sp. Emerald shiner Spottail shiner Rainbow smelt White sucker Yellow perch Walleye Trout-perch Logperch Tadpole madtom Pomoxis sp. White crappie Rock bass Stonecat madtom Lake chubsucker Alewife White perch Johnny darter Unidentified Northern pike	18,853 18,853 1,849 976 952 907 532 457 365 225 124 83 44 40 39 29 25 12 10 10 9 8 8 5 5	73.7 7.3 3.8 3.7 3.5 2.1 1.8 1.4 0.9 0.5 0.3 0.17 0.16 0.15 0.11 0.10 0.047 0.039 0.039 0.039 0.039 0.035 0.0195 0.0195
27 28 29 30 31 32	Green sunfish Unidentified cyrpinion Bluntnose minnow Brook stickleback Lake whitefish Yellow bullhead	2	0.0156 0.0078 0.0039 0.0039 0.0039 0.0039
	TOTAL	25,583	100.0134

 $$\operatorname{\textsc{TABLE}}$4$$  Distribution of Larval Fish Collected in the River Raisin, 1983

				s	ta ti on	#		
		1	2	3	4	5	6	7
1	Gizzard shad	х	x	x	x	x	Х	x
2	Emerald shiner	Х	Х	X	Х	Х	Х	X
3	Carp	Х	Х	Х	Х	Х	X	X
4	Morone sp.	Х	Х	X	Х	Х	х	X
5	Freshwater drum				Х	X	Х	х
6	Spottail shiner	X	Х	X	X	Х	Х	Х
7	Channel catfish	X	X	Х	Х	Х	Х	
8	Yellow perch	Х			Х	Х	Х	X
9	Lepomis sp.	X	Х	Х	Х	X	Х	X
10	Unident. cyprinid	Х	X	Х	X	X		
11	Alewife	Х	Х	Х	X	X	Х	Х
12	Walleye	Х	Х			Х	Х	
13	White bass	Х			Х	Х	Х	х
14	Logperch				Х	X	Х	Х
15	Brook silverside	· X	Х	X	Х		Х	X
16	Rock bass	X	Х	Х	Х	Х	Х	
17	Rainbow smelt					X	Х	х
18	White sucker	X	Х	Х	Х	Х	Х	
19	Trout-perch		X	Х	Х	Х	Х	
20	Bluntnose minnow	Х	Х	Х	Х			
21	Pomoxis sp.		X		Х			Х
22	Tadpole madtom		Х	X	Х	X		
23	White crappie	Х	X	Х	Х	Х		
24	Sauger	Х	Х	Х				
25	Stonecat madtom	Х	Х	X				
26	Johnny darter	Х					X	Х
27	Unidentified	Х			Х	Х	X	
28	Unident. Percid	Х				Х		
29 30	Silverjaw minnow						Х	
31	Quillback carpsucker					Х		
31 32	Golden shiner			Х				
	Green sunfish				X			
33	White perch					X	X	
34	Goldfish				Х			
35	Cent. stoneroller	Х						
36	Lake chubsucker					Х		
37	Creek chub				Х			
38	Unident. catostomid					X		
39 40	Yellow bullhead			X				
40 41	Ictalurus sp.							Х
41	Bluegill		Х					
42	Unident. Fundulus	Х						

TABLE 5
Distribution of Larval Fish Collected in the River Raisin, 1984

			Stat	ion#	
		1	2	3	4
1	Gizzard shad	х	Х	Х	х
2	Carp	х	Х	X	X
2 3 4 5 6 7 8	White bass	X	Х	Х	X
4	Morone sp.	X	Х	Х	Х
5	<u>C</u> hannel catfish	X	Х	X	X
6	Freshwater drum	Х	Х	Х	X
7	Lepomis spp.	х	X	Х	X
8	Emerald shiner	X	Х	X	X
9	Spottail shiner	х	X	Х	Х
10	Rainbow smelt	х	х	X	X
11	White sucker	X	Х	X	X
12	Yellow perch	x	Х	X	Х
13	Walleye	x	Х	X	X
14	Trout-perch	X	X	X	
15	Logperch	х	Х	X	х
16	Tadpole madtom	Х	Х	X	•
17	Pomoxis sp.	X			х
18	White crappie	х	х	X	• • •
19	Rock bass	Х		X	
20	Stonecat madtom	X	Х	X	
21	Lake chubsucker	x		X	х
22	Alewife	x	Х	х	X
23	White perch				X
24 25	Johnny darter	X	Х		×
25	Unidentified	х	X	х	X
26	Northern pike			X	X
27	Green sunfish	X			• • •
28	Undentified cyprinid			Х	
29	Bluntnose minnow			X	
30	Brook stickleback			X	
31	Lake whitefish			- <del>-</del>	Х
32	Yellow bullhead	T.		Х	••

TABLE 6

Ranking of Species Abundance Determinined in the River Raisin 1983/1984 Study Compared to Jude's 1982 Study

SPECIES	Jude 1982	0SU 1983	0SU 1984
Gizzard shad Emerald shiner Carp Morone sp. Freshwater drum Spottail shiner Channel catfish Yellow perch Lepomis spp. Cyrpinid (unid) Alewife Walleye White bass Logperch Brook silverside Rock bass Rainbow smelt White sucker Trout-perch Bluntnose minnow Pomoxis sp. Tadpole madtom Damaged larvae Quillback carpsucker Burbot Largemouth bass Lake whitefish	1 7 5 3 2 9 11 4 17 12 NF 18 NF 18 NF 18 14 22 24 6 10 13 12 12	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 NF NF NF NF	1 8 3 4 6 9 5 1 2 1 3 1 5 1 9 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
Northern hogsucker	22	NF	ΝF

NF = not found

TABLE 7

Estimated Numbers of Fish Larvae Entrained from February 13, 1982 through February 12, 1983 at the Monroe Power Plant (Data Taken from Jude, et al., 1983)

Species	Total Impinged	% of Total
Gizzard shad	4.08 x 10 <sup>9</sup>	86.8
Freshwater drum	1.58 x 10 <sup>8</sup>	3.4
White bass and White perch	1.56 x 108	3.3
Yellow perch	1.28 x 10 <sup>8</sup>	2.7
Common carp	$8.0 \times 10^{7}$	1.7
Damaged larvae	$3.8 \times 10^{7}$	0.8
Emerald shiner	$2.3 \times 10^{7}$	0.5
Rainbow smelt	1.1 x 10 <sup>7</sup>	0.2
Spottail shiner	5.0 x 10 <sup>6</sup>	0.1
Quillback carpsucker	4.9 x 10 <sup>6</sup>	0.1
Channel catfish	4.1 x 10 <sup>6</sup>	0.09
Unidentified Cyprinid	2.8 x 10 <sup>6</sup>	0.06
Burbot	2.8 x 10 <sup>6</sup>	0.06
Trout-perch	$2.4 \times 10^6$	0.05
Walleye	$2.1 \times 10^6$	0.04
White sucker	$1.2 \times 10^6$	0.03
Lempomis spp.	9.2 $\times 10^5$	0.02
Logperch	6.0 $\times 10^5$	0.01
Largemouth bass	6.0 x 10 <sup>5</sup>	0.01
Pomoxis spp.	5.8 x 10 <sup>5</sup>	0.01
Unidentified Coregonid	$1.9 \times 10^5$	0.004
Northern hogsucker	$1.2 \times 10^5$	0.003
TOTAL	$4.7 \times 10^9$	99.987
	43	

TABLE 8

Gizzard Shad Simple Growth Rates
River Raisin 1983/1984

Species	Station	Year	Initial Day	Final Day	Initial Size (mm)	Final Size (mm)	dl (mm) dt (day)
Gizzard shad	1	1983a b c d	199 202 192 171	220 244 237 181	40.5 31.2 14.7 4.0	49.2 48.2 35.3 6.5	0.41 0.40 0.46 0.25
	2	1983a b c	160 171 174	209 227 230	3.5 3.5 4.3	41.6 40.4 33.5	0.78 0.66 0.52
	3	1983a b c	160 164 167	195 209 216	3.5 3.9 3.8	38.0 47.4 49.9	0.98 0.97 0.94
	4	1983a b	160 209	223 251	3.0 12.3	45.3 41.7	0.67 0.70
	5	1983a b c d e	150 160 171 188 209	234 227 230 241 216	6.7 6.8 6.0 10.1 13.9	37.3 31.5 27.2 30.0 16.4	0.36 0.37 0.36 0.38 0.36
	6	1983a b c d e	150 167 174 160 199	227 234 230 181 213	8.0 8.9 9.7 4.0 11.7	32.9 30.8 28.3 9.7 16.9	0.32 0.33 0.33 0.27 0.37
	7	1983a b c d e	164 167 171 195 199	216 227 213 220 234	7.8 7.5 5.2 11.4 6.8	24.9 26.0 19.8 20.4 17.8	0.33 0.31 0.35 0.36 0.32

TABLE 8 (Continued)
Gizzard Shad Simple Growth Rates

Species	Station	Year	Initial Day	Final Day	Initial Size (mm)	Final Size (mm)	$\frac{d1}{dt} (\frac{mm}{day})$
Gizzard shad	1	1984a b c d e	145 159 163 166 170	201 194 191 180 184	3.3 3.1 3.3 3.3 3.3	43.4 29.0 23.2 9.9 11.2	0.72 0.74 0.71 0.47 0.56
	2	1984a b c d	163 166 173 170	187 184 198 194	3.5 3.4 7.6 3.4	21.6 16.1 24.6 19.8	0.75 0.70 0.68 0.68
	3	1984a b c d e f	170 180 184 170 163 159	176 187 194 191 198 187	3.8 8.6 13.4 3.8 3.4 3.2	16.4 23.7 32.8 18.4 25.8 23.7	2.10 2.20 1.90 0.70 0.64 0.73
	4	1984a b c d e	149 156 166 170 170	201 180 198 194 198	3.3 3.2 3.9 3.9 3.9	35.4 18.1 22.6 15.2 22.6	0.62 0.62 0.58 0.47 0.68

TABLE 9

Emerald Shiner Simple Growth Rates,
River Raisin 1983/1984

Species	Station	Year	Initial Day	Final Day	Initial Size (mm)	Final Size (mm)	$\frac{d1}{dt} (\frac{mm}{day})$
Emerald shiner	1	1983a b c d e	171 192 171 192 174	230 234 216 241 223	5.9 6.0 5.9 6.0 6.0	31.3 14.0 31.0 33.8 30.8	0.43 0.19 0.56 0.57 0.51
	2	1983a b	171 195	241 255	6.0 6.5	34.3 36.0	0.40 0.49
	3	1983a b c d	160 171 174 227	230 209 251 255	6.5 7.5 5.5 11.1	39.9 22.9 38.4 34.9	0.48 0.41 0.43 0.85
	4	1983a b c d e	167 171 171 206 209	202 241 192 244 227	5.0 5.0 5.0 11.8 7.8	23.1 31.8 11.0 25.2 13.5	0.52 0.38 0.29 0.35 0.32
	5	1983a b	181 164	230 241	14.0 4.0	26.0 20.5	0.24 0.21
	6	1983a b c	185 181 206	195 216 230	17.3 5.5 11.0	19.4 20.2 19.0	0.21 0.42 0.33
	7	1983a b c	185 202 206	195 251 230	13.4 13.3 13.0	19.0 32.7 23.0	0.56 0.40 0.42
Emerald shiner	1	1984a b c	166 163 184	198 187 191	5.5 7.0 8.2	25.8 16.5 12.5	0.63 0.40 0.61
	2	1984a b	176 170	198 194	13.5 5.5	29.0 23.3	0.70 0.74
	3	1984a	166	201	3.8	40.9	1.06
	4	1984a b	163 173	180 191	5.5 6.3	14.4 16.5	0.52 0.57

Larval Fish Growth Rate Coefficients, River Raisin 1983

TABLE 10

STATION #	SPECIES CODE	Lo	Li	Correlation Lo:Li	CONVERGENCE
1 1 1	105 301 302 1101	3.5 55.7 4.3 30.7	0.037 0.032 0.051 0.022	9919 9074 9923 9399	yes yes yes yes
2	105	2.7	0.048	9926	yes
2	302	15.5	0.017	9826	yes
3	105	3.0	0.036	9892	yes
3	301	52.0	-0.006	8354	yes
3	302	5.4	0.036	9920	yes
3	1101	29.0	0.016	8943	yes
4 4 4 4 4 4	101 103 105 302 702 802 901	8.6 3.0 10.1 10.0 2.8 11.9 10.0	0.017 0.023 0.023 0.024 0.031 0.053 0.047	9620 9735 9551 9724 9787 7970 9306	yes yes yes yes yes
55555555	105 302 702 801 802 901 1001	9.2 10.8 0.5 7.0 4.1 12.9 18.6	0.016 0.019 0.092 0.037 0.023 0.038 0.013	9324 9488 9961 9209 9850 8923 9081	yes yes yes yes yes yes yes yes
6	105	13.8	0.022	7701	yes
6	302	23.4	0.017	8542	yes
7	105	12.1	0.021	8939	yes
7	302	14.1	0.025	9401	yes

Species Code:

Lo:

For species identity see Table 1 Length at initial time fish observed Growth rate coefficient = Slope of Growth L1:

Lo:L1: Correlation Convergence: Yes or No

TABLE 11 (Continued)

STATION #	SPECIES CODE	Lo	Li	CORRELATION Lo:Li	CONVERGENCE
4 4 4 4 4 4 4 4	101 105 302 601 602 603 702 801 802 901 1001	10.93 16.57 6.84 3.12 16.25 14.47 15.35 2.03 11.68 4.75 33.62	0.039 0.142 0.056 0.064 0.031 0.051 131 0.062 0.007 0.098 176	9804 9683 9735 9824 8666 8942 6710 9927 9004 9752 7241	yes yes yes yes yes yes yes yes

Species Code:

For species identity see Table 1 Length at initial time fish observed Growth rate coefficient = Slope of Growth Lo:

L1:

Lo:L1: Correlation Convergence: Yes or No

TABLE 12
Ranking of 1983 Larval Fish Growth Rate Coefficients

Species	Stati on	L1	Growth Rank
Gizzard Shad (302)	1 2 3 4 5 6 7	0.051 0.017 0.036 0.024 0.019 0.017 0.025	1 7 2 4 5 7 3
Emerald Shiner (105)	1 2 3 4 5 6 7	0.037 0.048 0.036 0.023 0.016 0.022 0.021	2 1 3 4 7 5 6
Combination GS + ES (302 + 105)	1 2 3 4 5 6 7		3 8 5 8 12 12 9

Li: Growth Rate Coefficient = Slope of Growth

TABLE 13

Ranking of 1984 Larval Fish Growth Rate Coefficients

Species	Station	L1	Growth Rank
Gizzard Shad (302)	1 2 3 4 5	0.090 0.059 0.057 0.056	1 2 3 4
Emerald Shiner (105)	1 2 3 4	0.125 0.081 -0.009 0.142	2 3 4 1
Combination GS + ES (302 + 105)	1 2 3 4 5		3 5 7 5

L1 = Growth Rate Coefficient = Slope of Growth

TABLE 14
River Raisin 1983 Larval Fish Mortality Coefficients

Station	Species Code	Estimate No	Estimate Z	Correlation No:Z	Convergence
1	105 301 302 1101	4.63 4.74 40.65 1.38	039 .138 .018 007	0.9905 0.6805 0.9314 0.9028	yes yes yes yes
2	105 302	1.73 10.36	048 .001	0.9924 0.9568	yes yes
3	105 301 302 1101	10.04 1.16 45.54 5.00	007 027 010 .049	0.9673 0.9435 0.9657 0.6882	yes yes yes yes
4	101 103 105 302 702 802 901	1072.56 292.97 33.46 269.04 239.41 3.31 5.28	.102 .068 .032 .043 .075 .063	0.9909 0.9872 0.9376 0.9052 0.9966 0.5106 0.7824	yes yes yes yes yes yes
5	105 302 702 801 802 901 1001	32.80 1916.35 2.66 16.47 98.72 73.10 1.54	.024 .044 035 .012 .060 .207 006	0.9606 0.9740 0.9935 0.7682 0.9887 0.9614 0.8795	yes yes yes yes yes yes
6	105 601 602 702 801 802 804 901	53.80 821.00 2.91 11.84 18.25 6.71 6.84 6.32 2.36	.037 .094 .054 .106 .013 .018 130 350 430	0.9352 0.9666 - 0.1043 0.8770 0.7798 0.9837 0.9700 0.9867 0.9499	yes yes yes yes yes yes yes yes

TABLE 14 (Continued)

Station	Species Code	Estimate No	Estimate Z	Correlation No:Z	Convergence
7	301 302 601 602 702 802 901 1001	4.07 6355.70 48.01 7.27 0.62 5.73 12.15 1.40	.043 .072 .110 .062 191 .018 076 006	0.6613 0.9868 0.9669 0.7963 0.9826 0.8740 0.8800 0.7384	yes yes yes yes yes yes yes

No: Initial density over day by station and species

Z: Estimated mortality rate

No:Z: Correlation

Convergence: Yes or No

TABLE 15
River Raisin 1984 Larval Fish Mortality Coefficients

Stati on	Species Code	No	Z	Correlation No:Z	Convergence
1	105	.26	128	0.9972	yes
	302	737	0.073	0.6541	yes
	404	6.29	0.027	0.6507	yes
	601	18.1	0.058	0.6574	yes
	602	62.1	0.096	0.9639	yes
2	104	2717	0.184	0.9997	yes
	105	7.8	0.005	0.9638	yes
	302	2182	0.081	0.9202	yes
	602	36.2	0.023	0.9167	yes
3	104 105 201 302 601 602 801 802	7.23 18.3 10.2 987 7.1 18.15 3.97 1.18	022 005 0.104 0.049 - 0.108 - 0.007 0.052 - 0.004	0.9559 0.411 0.451 0.769 0.9931 0.9713 0.541 0.7746	yes yes yes yes yes yes yes
4	101	107	0.078	0.6676	yes
	302	2709	0.049	0.7023	yes
	901	198	0.074	0.5414	yes

No: Initial density over day by station and species

Z: Estimated mortality rate

No:Z: Correlation

Convergence: Yes or No

TABLE 16

Macroscopically Observed Deformities in Larval Fish from the River Raisin during 1983

Julian Date	Station	Name	Age	Length (mm)	Deformi ty
185	4 <b>-</b> A	Gizzard shad	III	19.0	irregular spine curvature
167	4-C	Yellow perch	III	12.5	spinal deformity
202	4-C	Gizzard shad	ΙV	36.0	abnormal growth mass on stomach
220	3-A	Gizzard shad	ΙV	54.0	stomach tumor
174	5-C	Gizzard shad	ΙΙ	11.5- 15.0	6 specimens with severe spine curvature
185	5 <b>-</b> A	Gizzard shad	III	17.0	severe spine defect
185	5-C	Gizzard shad	III	15.5- 22.0	many with spine curvatures
216	2 <b>-</b> A	Rock bass	ΙΙ	7.0	tumor near tail
171	4-C	Gizzard shad	ΙΙ	15.0	2 specimens with severe spine curvature
188	4-C	Gizzard shad	II	14.0	spinal deformity

TABLE 17

Larval Fish By Species and Station Evaluated Pathologically

Species	Station ,					Total
	1	2	3	4	5	
Gizzard shad	2	-	3	48	24	77
Yellow perch	_	-	-	4	4	8
Spottail shiner	_	-	_	1	_	1
Emerald shiner	_	-	-	1	-	1
Carp	-	_	-	2	-	2
Logperch	_	-	_	1	_	1
Trout-perch	-	-	-	-	1	1
Channel Catfish	_	-	_	2	-	2
Alewife	_	_		1	-	1
Freshwater drum		-	-	2	2	4
Walleye	-	-	_	-	3	3
Morone sp.	-		-	-	3	3

Total = 104

TABLE 18
Lesions in Gizzard Shad from the River Raisin, 1983

Tissue	Lesion	No. Affected	% Affected
Eye Brain Spinal Cord Olfactory Organ Otolith Organ Lat. Line Organ Oropharynx Esophagus (anterior)	Epithelial necrosis Epithelial necrosis Epithelial necrosis Epithelial necrosis Epithelial necrosis	0/50 0/59 0/53 35/37 3/46 34/36 50/52 43/47	0.0 0.0 0.0 94.6 6.5 94.4 96.2 91.5
Gills Gills Gills Heart Stomach Intestine Liver Pancreas Excretory Kidney	Edema Epithelial necrosis Parasites Epithelial necrosis - Tubular epithelial necrosis	38/47 43/47 34/47 0/42 0/47 38/54 0/53 0/51 50/53	80.8 91.5 72.3 0.0 0.0 70.4 0.0 0.0 94.3
Excretory Kidney	Hyaline droplet degeneration	17/53	32.1
Hemo. Kidney Spleen Swim Bladder Thymus Skin Skeletal Muscle Cartilage Bone	-	0/50 0/26 0/54 0/41 0/60 0/71 0/64 0/45	0.0 0.0 0.0 0.0 0.0 0.0

TABLE 19 Gizzard Shad Larvae By Size and Station Indicating Lesions.

Collection Station	Fish Length								
	< :	< 20 mm		21-30 mm		31-40 mm		> 41 mm	
	С	_ <u>A</u> _	_ <u>C</u>	_ <u>A</u> _	<u>C</u>	_ <u>A</u> _	<u> </u>	_A	
1	0	0	1	1	0	0	1	1	
3	0	0	1	1	0	0	2	0	
4	14	1	13	13	14	13	7	7	
5	14	5	3	3	7	7	0	0	
Total	28	6	18	18	21	20	10	8	

C = No. fish collected A = No. fish with lesions

TABLE 20
Histopathological Lesions in Gizzard Shad from the Control Station (#7)

Tissue	Lesion	No. Affected	% Affected
Eye	_	0/23	0.0
Brain	-	0/34	0.0
Spinal cord	<b>-</b> '	0/32	0.0
Olfactory organ	Epithelial necrosis	10/11	90.9
Otolith organ	Epithelial necrosis	1/24	3.6
Lat. Line organ	Epithelial necrosis	9/10	90.0
Oropharynx	Epithelial necrosis	33/34	97.1
Esophagus	Epithelial necrosis	31/34	91.2
Gills	Epithelial necrosis	36/36	100.0
Gills	Parasi tes	10/36	27.8
Heart	-	0/30	0.0
Stomach	_	0/30	0.0
Intestine	Epithelial necrosis	15/16	93.8
Liver	-	0/36	0.0
Pancreas	-	0/27	0.0
Excretory kidney	Tubular epithelial necrosis	35/35	100.0
Excretory kidney	Hyaline droplet degeneration	13/38	34.2
Hemo. kidney	-	0/34	0.0
Spleen	—	0/13	0.0
Swim Bladder	-	0/25	0.0
Thymus	Lymphoid necrosis	2/28	7.1
Skin	-	0/37	0.0
Muscle	-	0/37	0.0
Cartilage	-	0/38	0.0
Bone	-	0/26	0.0

TABLE 21
Lesions in Fathead Minnows, River Raisin 1984

	STA 4	STA 12	STA 12	No.	%
	H2O + sed	H20	H2O + sed	Affected	Affected
	(n = 6)	(n=6)	(n=6)	(n=18)	
Eye Brain Spinal cord Olfactory org Otolith org. Lat. Line org Oropharynx Esophagus Gills Heart Stomach Intestine Liver Pancreas Ex. Kidney Hemo. Kidney Spleen Swim Bladder Thymus Skin	0/1	0/4 0/6 0/5 0/3 0/1 0/0 0/5 0/1 0/6 0/6 0/6 0/6 0/6 0/6 0/6 0/6	0/4 0/6 0/5 0/5 0/2 0/0 0/6 0/6 0/6 0/6 0/6 0/6 0/6 0/6	0/10 0/17 0/15 0/12 0/4 0/0 0/16 0/17 0/0 0/2 0/16 0/18 0/12 0/17 0/17 0/17 0/13 0/2 0/18	000000000000000000000000000000000000000
Skeletal Mus.	0/6	0/6	0/6	0/18	0
Cartilage	0/6	0/6	0/6	0/18	0
Bone	0/6	0/6	0/6	0/18	0

TABLE 22

PCB and Pesticide Concentrations (mg/kg) in Larval Gizzard Shad from the River Raisin, 1983-1984

STATION	1983	8/18	4.	0.40		000	0	2	00	$\frac{1}{2}$	0	O	00	00	00	00	00	00	X	ΑN	AN	AN	AN	Z Z	N N	N A
0N 5 83 8/18	Τ.	1.10	Q.	001	0	002	2			0	003	90	012	002	007	N Q	NA	Ϋ́	Ä	N A	AN	\ Z	A N	Y N		
STATI	19	7/14	0	0.44	000	000	00	002	Q.N	000	00	001	002	003	008	001	007	000	Ϋ́	ΑN	N N	Υ Z	₹ Z	A N	ΥN	NA
		7/21	∞.	2.5	001	00	002	≪	ΑN	0	ND	003	00	019	019	002	019	001	000	000	038	00	001	000	000	0
		7/18	∞.	2.5	00	0	001	⋖	⋖			002	007	025	020	002	023	001	001	001	015	0.1	001	ON.	01	.0013
	1984	6/28	9	0.91	00	0.1	2	⋖	⋖	00	.0005	00	00	00	-	00	0	00			01		00	00	N O N	QN
		6/21	ω,	0.44	000	00	002	=	⋖	QN		001	0.1	002	900	000	04	001	000	_	02	0	01	00	00	00
ATION 4		6/21	9	0.26	00	000	Δ.	AN	ΝA	0	.0001	000	00	003	04	000	001		00	S	0	2	.0002	O N	ON	ND
ST/		9/1				N (	9:	2	Ó		Z	0	00	08	0	00	01	00								
	1983	7/21	•	g, c	$\supset$	70		⊇ ;	QN		.0013	03	900	03	013	00	18	0	NA	ΥN	ΑN	ΑN	NA	ΑN	ΑN	N A
		7/14	$\sim$	0.5	(	3	1000.		2	Q N		.0002	00	00	001	00	00	Q	e NA	ΝA	ΝA	ΝA	ΝA	ΝA	A A	NA
		DATE COLLECTED	LIPID	otal	ipna -brc	exacni	a mma - b	อเลาหม	elta-BHC	eptachlor	eptachlor Epoxid	amma Chlor	Ipha Chlordan	rans-Nonachlo	4 . DDE	0N-S 1	4. DD	4. 001	entachlorobenzen	ipha-Chlorden	ldrin	amma-Chlo	kychlordan	eldri	ıdri	41

ND = None detected NA = None analyzed

TABLE 23

PCB and Pesticide Concentrations (mg/kg) in Young-of-the-Year Emerald Shiners from the River Raisin, 1984

				2	ALION	4	9	NOT I W	45
		1984			1984			1984	
COLLECTED 9	9/6	9/6	9/6	8/17	9/6	9/6	8/17	9/6	9/6
n	9.	0	3	4.	•	, →	Φ,	-	5
0	•	7.	4.	•	∞.	ο.	4.		•
0.	00	01	001	00	01	01	01	0	001
enzene .0	0.1	001	00	01	01	001	001	2	001
0.	00	0	000	Z	0	0.1	00	00	
	Q.	000	2	02	12	N <sub>D</sub>	001	Z	002
poxide.	00	2	2	2	Z	2	Q	000	2
dane .	01	002	001	02	02	003	003	02	01
ane .	0	04	003	14	0	05	05	00	2 O
lor.	08	012	007	013	19	027	032	020	4
0.	47	053	059	44	28	037	053	022	02
	0	002		00		001	002		
•	15	028	018	90	13	40	25	014	017
°.	00	00	000	$\frac{1}{2}$	Z	2	QN	Z	Z
zene.	00	00	000	00	00	00	000	Z	000
ne .	00	00	000	$\frac{1}{2}$	00	000	00	00	00
•	03	04	900	$\sim$	17	21	055	29	028
lordene .0	00	00	00		0	01	0.1	0	0
•	0.1	02	00	00	01	01	02	0.1	01
0.	00	00	00	0	2	2	$\frac{2}{2}$		2
0.	015	.0024	01	01	.0020	.0043	.0030	.0020	.0017
o 0	00	00					001		$\mathbf{z}$

ND = None detected

TABLE 24

Gizzard Shad Larval Fish Density of the River Raisin, 1983

# All Sizes

	Station	Species	Date of First Capture	Date of Last Capture	Period of Peak Abundance	Minimum Mean Density #/1000 m	Maximum Mean Density #/1000 m	Average of Density Means	Relative Abundance
	п	Gizzard Shad	171	244	199	9.2	280.0	74.0	3.1
	2		160	255	199	9.5	414.7	49.6	2.1
	က		160	255	199	8.7	844.4	129.4	5.4
63.	4		160	255	195	10.2	662.6	119.1	5.0
	5		150	255	202	8.5	4723.0	406.4	17.0
	9		150	251	202	7.5	5595.7	781.7	32.7
	7		164	251	188	8.5	2101.8	828.2	34.7
	Total							2388.4	100%

TABLE 25

Historical Lake Erie Gizzard Shad
Larval Density, Peak Abundance Data

Date	Location	Density #/1000 m	Study
June 8,78	Davis-Besse	1104.4	Gordon, 1982
May 31,79	Davis-Besse	2004.4	Gordon, 1982
June 6,80	Davis-Besse	10369.3	Gordon, 1982
June 3,76	Maumee River	16348.9	Snyder, 1978
May 31,76	Sandusky River	3811.7	Snyder, 1978
June 4,77	Western Basin	8000.0	Cooper et al,1981c
June 19,78	Central Basin	1070.0	Cooper et al,1981c

TABLE 26

# River Raisin Qualitative Sediment Survey (Data Supplied by Michigan Department of Natural Resources)

STATION	TRANSECT	SEDIMENT DESCRIPTION
1	1	No description
2	7	Hard rocky bottom along the central portion .Fine gravel/sand along north shore.Silt along south shore.
3	10	Hard rocky bottom along south shore. Silt sand and gravel along central and north portion.
4	43	Silt dark gray color, slightly oily odor, some detritus, rocky along the north shore.
5	48	Black silt,oily, some detritus, sandy silt along north shore.
6	50	Silt,gray-brown,some detritus, no unusual odor
7	NS	Not sampled

TABLE 27

Gizzard Shad Larval Fish Density of the River Raisin, 1983

(< 5 mm)

· <del>-</del> · ·	Species Gizzard Shad	Date of First Capture 171	Date of Last Capture 206	Date of of Peak Abundance 174	No. of Spawning Days 35	Minimum Density #/1000 m 9.2	Maximum Density #/1000 m 101.4	Avge of Density Means 38.0	Relative Abundance 5.3
		160	174	160	14	10.3	. 16.5	12.5	1.7
		160	199	199	39	8.4	51.4	31.2	4.3
		160	209	174	49	10.2	285.2	78.2	10.9
		150	206	174	56	7.8	590.1	109.7	15.2
		160	206	202	46	18.0	543.3	230.1	31.9
		164	237	206	73	10.7	1140.7	221.3	30.7
								721.0	100%

TABLE 28
Review of Fish Growth Rates

SPECIES	SIZE RANGE	GROWTH RATE	LOCATION	AUTHOR
Gizzard Shad	6.2 - 29.0mm	0.034 (1)	L.Erie	Gordon
Gizzard Shad		1.01mm/day	L.Erie	Bodola
Gizzard Shad	36 - 185 mm	0.99mm/day	L.Erie	Carlander
Gizzard Shad	4.0-49.2 mm	.25-2.2mm/day	L.Erie	Fay
Yellow Perch	pro larvae	0.018 (I)	L.Erie	Gordon
Yellow Perch	post larvae	0.038 (1)	L.Erie	Gordon
Emerald Shiner		0.80mm/day	L.Erie	Carlander
Emerald Shiner	4- 40.9 mm	.1985mm/day	L.Erie	Fay
Smelt	5.3 - 15.7mm	0.35mm/day	L.Michiga	ın Tin
Smelt	5.3 - 41.1mm	0.39mm/day	L.Michiga	in Tin

I = Instantaneous Growth Rate

TABLE 29

Comparison of Histopathologic Lesions in Larval Gizzard Shad at Station 4 and the Control Station (#7)

Tissue	Lesion	% Affe	cted
		STA 4	STA 7
Olfactory organ	Epithelial necrosis	95	91
Lateral line		94	90
Oropharynx		96	97
Esophagus (ant.)		92	91
Gills		92	100
Excretory kidney	Tubular epithelial necrosis	94	100
Excretory kidney	Hyaline droplet degeneration	32	34
Intestine	Epithelial necrosis	70	94
Gills	Parasites	72	28

APPENDIX A

APPENDIX A

# Larval Fish Body Burden Samples Collected

19	83
----	----

1303								
	14	July					Gizzard Gizzard	
	21	July					Gizzard Gizzard	
	28	July	Stati	on	5		Emerald Emerald Emerald	shiner
	4	August					Emerald Gizzard	
	18	August					Gizzard Gizzard Emerald	shad*,
	1	September	Stati	on	4	_	Gizzard	shad*
	8	September					Gizzard Emerald Gizzard Emerald	shiner shad,
1984								•
	21	June					Gizzard Gizzard	
	28	June					Gizzard Gizzard	

<sup>\*</sup> Body burden samples analyzed by Cranbrook Institute

APPENDIX B

APPENDIX B Raisin River 1983 Flowmeter Calibration

DATE	REPLICATES	REVOLUTIONS	DISTANCE	REV/METER
5-30-83	1 2 3 4 5 6 7 8 9 10 X	17988 17724 18787 18886 18828 18581 18293 18283 18045 17522 18293	500 m 500 m 500 m 500 m 500 m 500 m 500 m 500 m 500 m	36.6
7-07-83	1 2 3 4 5 6 7 8 9 10 X	21184 18621 19855 18623 20667 18962 19996 18239 19631 18545 19432	500.m 500.m 500.m 500.m 500.m 500.m 500.m 500.m 500.m	38.9
8-04-83	1 2 3 4 5 6 7 8 9 10 X	18273 17542 17315 16682 17558 17439 17595 16811 16560 9422 17308	500 m 500 m 500 m 500 m 500 m 500 m 500 m 500 m 500 m	34.6
9-08-83	1 2 3 4 5 6 7 8 9 10 X	18886 18813 16501 5070 15996 17740 18378 17707 18402 18248 17852	500 m 500 m 500 m 500 m 500 m 500 m 500 m 500 m 500 m	35.7
OVERALL		18225	500 m	36.4

APPENDIX C

APPENDIX C
List of River Raisin Vouchers by Stage

			STAGE		
	I	ΙΙ	III	ΙV	ADULT
Alewife Gizzard Shad	x	x x	x x	X X	
	^				
Lake Whitefish	X	X			
Smelt		x	x	х	х
Pike				х	
Silverjaw Minnow				Х	
Central Stoneroller Goldfish	x		Х		
Carp	X	Х	×	х	
Emerald Shiner	x		х	X	x
Spottail Shiner	X	x	Х	X	
Bluntnose Minnow		X	X	X	
Fathead Minnow					Х
Spotfin Shiner Creek Chub			x		
Quillback	X			· · · · · · · · · · · · · · · · · · ·	···
White Sucker	x	x	X	x	
Lake Chubsucker	х	X			
Yellow Bullhead				×	
Channel Catfish			X	Х	
Tadpole Madtom Stonecat			X X		
Burbot	X				
Troutperch	x	x			х
Brook Silverside			Х	х	

# APPENDIX C (Continued)

# List of River Raisin Vouchers by Stage

### STAGE

	I	11	III	ΙV	ADULT
Brook Stickleback	·			x	х
White Perch Morone sp.				x	х
White Bass	х			x	
Rock Bass		×	х		
Green Sunfish				х	
Bluegill White Crappie	x		x	x	
Yellow perch	×	х	x	х	
Logperch	X	Х	X		
Sauger Walleye	X	v	v	v	
Johnny Darter	X X	X X	X X	X X	
Freshwater Drum	х	х	х	х	
Fundulus sp.		х			
Mudminnow					х

APPENDIX D

River Raisin Larval Fish Vouchers by Capture Date and Length

APPENDIX D

STATION/DATE	SPECIES	STAGE	LENGTH
7-16-84 3/A	Smelt	ΙΙ	17.0
7-16-84 3/A	Smelt	III	22.5
7-16-84 3/A	Smelt	ΙV	28.0
7-16-84 3/A	Gizzard Shad	III	17.0
7-16-84 3/A	White Bass	ΙV	26.5
7-16-84 3/A	Morone sp.	III	11.5
7-16-84 3/A	Morone sp.	ΙΙ	9.0
7-16-84 3/A	Freshwater Drum	ΙΙ	8.0
7- 5-84 3/B	Emerald Shiner	ΙV	19.5
7- 5-84 3/B	Gizzard Shad	I	5.0
7- 5-84 3/B	Carp	I	7.0
7- 2-84 3/B	Gizzard Shad	ΙV	30.5
7- 9-84 3/B	Gizzard Shad	ΙΙ	13.0
7- 2-84 3/B	Emerald Shiner	III	15.0
7- 9-84 3/B	Spottail Shiner	I	6.0
7-25-84 5/A	Spottail Shiner	ΙΙ	8.5
UNKNOWN	Mottled Sculpin	I	6.5
7-16-84 5/A	Morone sp.	I	7.5
7 -9-84 5/A	Freshwater Drum	I	5.5
6-24-84 5/B	Morone sp.	I	4.5
6-24-84 5/B	Logperch	ΙV	21.5
6-24-84 5/B	Logperch	III	10.5
6-24-85 5/B	Logperch	ΙΙ	8.0
6-28-84 5/B	Carp	III	14.5
6-28-84 5/B	Carp	ΙΙ	13.0
6-28-84 5/B	Lake Chubsucker	I	7.0
6-18-84 5/A	Freshwater Drum	I	4.0
6-18-84 4/A	Yellow Perch	ΙV	20.0
7 -5-84 5/C	Spottail Shiner	ΙV	17.0
6-28-84 5/A	Channel Catfish	IV	19.0
6-28-84 5/A	White Bass	III	16.5
6-28-84 5/A	White Bass	IV	22.5
6-21-84 4/A	Carp	ΙV	22.0
6-21-84 4/A	Yellow Perch	ΙΙΙ	17.5

APPENDIX E

### APPENDIX E

### Larval Fish Identification Characteristics

SPECIES	OCCURRENCE IN WESTERN LAKE ERIE	HELPFUL CHARACTERS
Major		
Morone sp.	Early to mid	
Yellow Perch	Early	
Freshwater Drum	Mid to late Low myomere count, Big head	
Walleye	Early	
Logperch	Early to mid	Thick gut, several large chromato-phores behind vent Two lines chromato-phores on ventrum
Gizzard Shad	Early to late	•
Alewife	Early to late	
White Sucker	Early season	
Troutperch		
Rockbass	Mid to late	
Sunfish-Other	Mid to late	
Pomoxis	Mi d	
Carp	Early to late	Y pigmentation Large bulbous yolk
Spottail Shiner	Mid to late	Ventrum widely pigmented in gut less pointed snout
Emerald Shiner	Mid to late	Ventrum linearly pigmented, more pointed snout
Smelt .	Mid to early season	<pre>1 line chromato- phores on ventrum conspic curbled</pre>

### APPENDIX E (Continued)

### Larval Fish Identification Characteristics

SPECIES OCCURRENCE IN HELPFUL CHARACTERS

WESTERN LAKE ERIE

Channel catfish Mid to late

Minor

Darters Mid

Bluntnose Minnow Mid to late

Whitefish Early

Brook Silverside Mid to late

Madtoms

APPENDIX F

### APPENDIX F

### Documentation for Fish Growth Mortality

### I. Brief Interpretation Of This Program

- (1) Lines 1-7 are the job control statements (JCL).

  In these lines we input two data files: RAISIN83.XFRO and RAISIN3B.XFRO, then we rename them as PETER and CHOKE respectively
- (2) Lines 11-73 are for the step of input and proof data.
  - (a) Purpose: In these lines we want to input the data sets and transform all lengths into standard lengths 0, 5, 10, . . ., or 70 mm. Also, we calculate the difference of final flow and initial flow for our density calculation.

### (b) Procedures:

\*Lines 11-39 -- We form the SAS data set JJ1 by using PETER as the input data file and drop some useless variables from the input data file. Note that we compute the difference of final flow and initial flow at line 21 and convert the lengths into the standard integer lengths 0, 5, 10, . . . or 70 mm denoted by SYMBOL, at line 24, e.g. all lengths in the interval 2.6-7.5 are denoted by SYMBOL = 5 and so on. As to these lines 25-39 we assign to each standard length SYMBOL from 5 to 70 a corresponding notation SIZE from A to N and '\*' for otherwise standard lengths.

\*Lines 40-42 -- We convert all missing data (values) in the variables DIFFLOW and F into SAS standard form '.'. Then we define the obtained new data set as J1.

\*Lines 43-73 -- We repeat the same procedures as we did in lines 11-42 for the input data file 'CHOKE' and denote the obtained new data set as J2.

### (c) Some variable notations:

DAY = Julian day
PD = Period of station (e.g. in station 3-A
we mean station = 3 and PD = A)
SP = Species Codes
ST = Larval Stage (1-4)

L = Length (mm)

INFLOW = Intitial flow (revolutions)
OUTFLOW = Final flow (revolutions)
DIFFLOW = OUTFLOW - INFLOW
F = Ave per stage = Frequency

### (3) Lines 79-95 are for step 1.

(a) Purpose: In these lines we perform the procedures of data reduction for data sets J1 and J2 and obtain a new data set COMB1 which is going to be used to compute larval density.

### (b) Procedures:

- \*Lines 79-86 -- We perform data reduction and merge related data sets together.
- \*Lines 87-95 -- We compute the larval density and obtain a new data set COMB.
- (c) Some variable notations:

SV = sample volume (m3) FACTOR = 1000 m3/Sample volume (m3)1 DENS = Density/1000 m3 = Factor x Ave per stage TOTAL = Ave. per stage = variable F

# (4) Lines 102-116 are for step 2.

(a) Purpose: We average replicates A, B, and C densities in these lines by station, species code, and size, then we plot the density vs. Julian day.

### (b) Procedures:

\*Lines 102-104 -- We average A, B, and C density to form a new data set TEMP2.

\*Line 105 -- We delete those data with SP = 0.

\*Lines 115-116 -- We plot the density vs. Julian day.

### (c) Some variable notations:

DENS = The density obtained by DAY STATION PD SP and SIZE MDEN = The mean density obtained over PD by STATION DAY and SP SIZE

# (5) Lines 123-124 are for step 3A.

(a) Purpose: We want to average A, B, and C density by station and species code.

### (b) Procedures:

- \*Lines 123-126 -- We perform data reduction to obtain a new data set TEMP3.
- \*Lines 127-131 -- We calculate A, B, and C density separately by station and species code.
- \*Lines 132-134 -- We average A, B, and C density to obtain a new data set TEMP5.
- \*Lines 143-144 -- We plot average A, B, and C density vs. Julian day.

### (c) Some variable notations:

TOT1 = Total frequency by DAY STATION PD and SP DEN1 = Density by DAY STATION PD and SP

AVG1 = Average density by DAY STATION and SP

### (6) Lines 151-175 are for step 3B.

Purpose: We want to calculate the total seasonal (a) density by station and species code.

### (b) Procedures:

- \*Lines 155-159 -- We calculate the density for each Julian dav.
- \*Lines 160-162 -- We average A, B, and C density for each Julian day to obtain a new data set TEMP7.
- \*Lines 163-165 -- We obtain total seasonal density by summing up all Julian days' density.
- \*Lines 174-175 -- We plot total seasonal density vs. station.

### (c) Some variable notations:

= Total frequency by DAY STATION SP and PD TOT3

= Density by DAY STATION SP and PD

= Mean density over PD by DAY STATION and AVG7

SP

TOT4 = Season total density by STATION and SP

### (7) Lines 181-191 are for step 4.

Purpose: We calculate average length by (a) STATION SP.

### (b) Procedures:

- \*Lines 181-183 -- We average all sizes of larval fish by DAY STATION SP and ST (stage). These results, denoted by AVG, form the new data set COUNT.
- \*Lines 184-186 -- We average A, B, and C density by DAY STATION and SP.
- \*Lines 190-191 -- We plot average length vs. Julian day by station and species code.

### (c) Some variable notations:

AVG = Mean length over SIZE by DAY STATION PD

AVG3 = Mean length over SIZE by PD by DAY STATION and SP

### (8) Lines 198-219 are for step 5.

Purpose: We calculate the date when total length (a) of the population is 5, 10, 15, . . . to 70 mm.

### Procedures: (b)

- \*Lines 198-200 -- We sum the frequencies by STATION SP SYMBOL for every Julian day, denoted as T, and form the data set CH1.
- \*Lines 201-203 -- We sum the total seasonal frequencies by STATION SP SYMBOL, denoted as TT. and form the data set CH2.
- \*Lines 204-207 -- We calculate the relative frequency for each Julian day by STATION SP and SYMBOL, denoted as AVERAGE.
- \*Lines 208-210 -- We calculate the mean Julian day by using the relative frequency (AVERAGE), denoted as TL, and form the data set CH4.
- \*Lines 218-219 -- We plot the SYMBOL vs. TL (mean Julian day) by STATION SP.

### (c) Some variable notations:

= Frequency by DAY STATION SP and SYMBOL TT

= Total frequency over DAY by STATION SP

SYMBOL

Prob = Relative frequency (i.e. T/TT) by STATION SP SYMBOL

### (9) Lines 226-238 are for step 6.

(a) <u>Purpose</u>: We use the non-linear regression method to estimate the growth for specified STATION and SP.

### (b) Procedures:

\*Line 226 -- Suppose that we want to estimate the slope of Growth for STATION = 1 and SP = 302, i.e. We specify STATION = 1 and SP = 302.

\*Lines 229-235 -- We use the MARQUARDT method as our tool for the non-linear regression. This method represents a compromise between the linearization (or Taylor series) method and the steepest descent method and appears to combine the best features of both while avoiding their most serious limitations. It is good in that it almost always converges and does not "slow down" as the steepest descent method often does.

\*Line 237 -- We plot SYMBOL vs. TL.

### (c) Some variable notations:

LO = Initial Length
L1 = Slope of Growth
YHAT = Y = Estimated Length
YRESID = Y - Y = Residual for Y

\*We note that SYMBOL = L0\*EXP(L1\*(TL-150)) at line 232 means L = L eG(t - to) as it appears in Hackney and Webb's paper where hatching date to = 150.

- (d) How to find the estimated slope of growth for Station A Species B? We first replace 1 by A and 302 by B at line 226. Then we examine whether 150 (lines 232-234) is a suitable initial value (Julian day) if it is not, replace all 150 in lines 232-234 by a suitable initial value. Initial values can be determined by presence of larval fish in earlier plots.
- (e) If we want to obtain the results for more combinations of stations and species simultaneously, we can copy whole lines 226-238 repeatedly for the desired number of stations and species and then follow Step (d) to make suitable modifications for stations, species and/or initial (Julian) day.

# (10) <u>Lines 244-263 are for step 7.</u>

(a) Purpose: We use a non-linear regression method to estimate the mortality for specified STATION and SP.

### (b) Procedures:

\*Line 245 -- We specify STATION = 1 and SP = 105.

\*Lines 254-260 -- We use the MARQUARDT method as the tool for analyzing non-linear regression.

\*Line 262 -- We plot TT vs. TL.

### (c) Some variable notations:

NO = Initial (frequency) number over DAY by STATION SP SYMBOL Z = Mortality rate

- (d) How to find the mortality rate for station A species B? First, we replace 1 by A and 105 by B at line 245. Next, we examine whether 173 is a suitable initial Julian day. If it is not, replace all 173 in lines 257-260 by a suitable initial value, determined by initial presence of larval fish in previous plots.
- (e) Using the same steps as (9)(e) we can obtain results for more combinations of stations and species simultaneously.
  - \*Note that TT = N0\*EXP(-Z(TL-173)) means this formula Nt = Nto e-Z(t to) which appears in Hackney and Webb's paper.

# II. How To Use This Program.

- (a) We can use this program to obtain the results for separate steps or some combination of steps. The basic procedures for establishing a desired subprogram are as follows:
  - (i) Use CHENPJ1 on IRCC93.
  - (ii) Lines 1-73 must be included in any subprogram(s).
  - (iii) Keep those lines for corresponding steps desired in the subprogram and delete the rest of the lines.

(iv) Substitute suitable station, species, or initial values when subprogram contains step 6 or step 7. Therefore, the diagram for above procedure is:

lines lines for 1-73 + corresponding steps

examine ST SP
initial values if =
subprogram
steps 6 or 7 is
is concerned

# (b) Some Examples:

- (i) Suppose we want to get the results of step 3. The subprogram should contain lines 1-73 and 123-175 (lines for step 3) only, so we delete the other lines from main program and then run this subprogram.
- (ii) Suppose we want to get the results of step 4 and step 6 simultaneously and consider station Al species B1 and station A2 species B2 instead of station 1 species 302 in step 6. In order to obtain the subprogram we first keep lines 1-73 and lines 181-191 (for step 4) and lines 226-238 (for step 6) then delete other lines, since we consider two combinations of station and species (A1,B1) (A2,B2), we need to  $\underline{\text{copy}}$  lines 226-238 Suppose these latter lines are renumbered as 239-251 (Note that these lines  $239-\overline{251}$  are not the original lines 239-251 in our main program). Now, we replace 1 by A1 and 302 by B1 at line 226 and examine the initial values for lines 232-234 to see whether the value 150 is suitable; replace 1 by A2 and 302 by B2 at line 239 and again examine the value 150 in lines 245-247. After doing these, we finally obtain our subprogram which includes lines 1-73 and 181-191 and 226-238 and the new lines 239-251.

# (c) Remark:

If we choose different initial values for the same station and species in step 6 and step 7, the estimated values may be different but the estimated growth rate and mortality rate are still the same, i.e., the estimated values depend on the choice of the initial values (it's not important since we consider their corresponding confidence intervals) while the estimated growth rate and their mortality rate do not depend on the choice of the initial values. However, if we can choose a good initial value, the iteration times will be reduced.

APPENDIX G

APPENDIX G

River Raisin Larval Fish Submitted for Pathological Examination

Comments		tage tage ge	Stage 2 Stage 2 Stage 2
Size (mm)	6	740648	33333333333333333333333333333333333333
Species	izzard sha izzard sha izzard sha izzard sha izzard sha izzard sha	izzard sha izzard sha izzard sha izzard sha izzard sha izzard sha	a a a a a a a a a a a a a a a a a a a
Station	444444	4 4 4 4 4 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	44444444444444
Collection Date	116 116 116 116 116 116 116 116 116 116		7-07-83 7-07-83 7-07-83 7-07-83 7-07-83 7-11-83 7-14-83 7-14-83 7-14-83 7-14-83 7-14-83 7-14-83
Sample #	1112223	r 4 τι ο / α * *	2222209876555432110999

APPENDIX G (Continued)

River Raisin Larval Fish Submitted for Pathological Examination

Comments	Abnormal growth mass; on stomach																			pinal deformit	evere spine curvature - vouche	ere spine curvature - vouch	evere spine curvature - vouche			
Size (mm)	35.0 35.0 35.0	4. 4	2	٠. د د	/	4.	_ <	ນ ແ	• •	4.	5	9	9	2	0	÷	ნ	<b>.</b>		ო	ij	• 	ς.	۲,	4.	5
Species	Gizzard shad Gizzard shad Gizzard shad	izzard sha	izzard sha	izzard sha izzard sha	izzard sha	izzard sha	izzard sha	izzard sha izzard sha	izzard sha izzard sha	izzard sha	ellow perc	izzard sha	izzard sha	izzard sha	izzard sha	izzard sha	izzard sha									
Station	4 4 4 0 0 0																									
Collection Date	7-18-83 7-21-83 7-21-83	-25-8 -25-8	-25-8	-28-8 -28-8	-28-8	-01 - 8	-04-8	-041 -081 8	-11-8	-15-8	-18-8	-18-8	-18 - 8	-22-8	-25-8	-01 - 8	-08-8	-08-8	-08-8	-16 - 8	-23-8	-23-8	-23 - 8	-23-8	-23-8	-23-8
Sample #	25* 26 27																							0		2

APPENDIX G (Continued)

River Raisin Larval Fish Submitted for Pathological Examination

Commments	tage 3 - stained w/Rose	tage 3 - stained w/Rose	Stage 3 - stained w/Rose Bengals; many with spine curvatures																izzard shad selected fro	8th ed on	agast tell a ot				
Size (mm)				•	6	9	33.	ъ •	ъ.	6	о О	6	0	0	0	6	2	ä			ъ.	7	48.0	۲,	4.
Species	Gizzard shad	Gizzard shad	Gizzard shad	izzard sha	Gizzard shad	izzard sha		izzard shad I	izzard shad I	Gizzard shad IV	izzard shad I	izzard shad I													
Station	25	50	5C	50															10		10				
Collection Date	7-04-83	7-04-83	7-04-83	7-14-	-14-8	-14-8	-14-8	-14-8	-14-8	-21 - 8	-21-8	-21-8	-21-8	-21-8	-21-8	-21 - 8	-21-8	-21-8	-08-8		8-80-	-08-8	8-08-83	-08-8	-08-8
Sample #	53. *	54*	လ လ *	56																	70				

APPENDIX G (Continued)

River Raisin Larval Fish Submitted for Pathological Examination

Comments	
Size (mm)	29.0 65.0 7.7.0 7.0 18.5 12.0 17.0 19.0 19.0 10.0 10.0
Species	Gizzard shad IV Spottail shiner Spottail shiner Carp Carp Carp Logperch IV Emerald shiner Channel catfish IV Carp Carp Carp Carp Carp Carp Carp Carp
Station	
Collection Date	8 6-233-6 6-223-83 6-223-83 6-223-83 6-223-83 6-223-83 7-04-833-83 7-21-833 7-21-833 7-21-833 7-21-833 7-21-833 6-06-833 6-06-833 6-06-833
Sample #	77777777777777777777777777777777777777

APPENDIX G

River Raisin Larval Fish Submitted for Pathological Examination

Comments		ne with spinal de evere spinal defe	internal t formity
Size (mm)	6.0 19.0 13.5 6.0 7.0 7.5 13.5 7.5		097655
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APPENDIX G

River Raisin Larval Fish Submitted for Pathological Examination

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APPENDIX G (Continued)

Fish Suhmitted for Pathological Examination Raisin Larval

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Raisin Larval	Station																															2/	
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APPENDIX G (Continued)

River Raisin Larval Fish Submitted for Pathological Examination

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Station	70					H20/4Se	0/4	H20/4Se	2H2	2H2	2H2	2H20/12Se	2H20/12Se	0/1	2H20/12Se	2H20/12Se	2H20/12Se	2H2	<b>2H2</b>	<b>2H2</b>	H20/4Se	20/	H20/4Se
Collection Date	7-04-83	-04-8	-04-8	-08-8	8-80-	2 - 13 - 8	2 - 13 - 8	2 - 13 - 8	2 - 13 - 8	2 - 13 - 8	2 - 13 - 8	2 - 13 - 8	2 - 13 - 8	2 - 13 - 8	2-27-8	2-27-8	2-27-8	2-27-8	2-27-8	2-27-8	2-27-8	2-27-	2-27-8
Sample #	118	$^{\circ}$	$\sim$	$^{\circ}$	$\sim$	2	$\sim$	$\sim$	$\sim$	$^{\prime}$	2	$\sim$	$\sim$	2	$\sim$	$\sim$	$^{\sim}$	$^{\circ}$	$\sim$	$\sim$	$\alpha$	$\sim$	$\sim$

\*Samples sent directory to Paul Stromberg (n = 15)

Station 4 was selected for pathology because it was the EPA Master Station and has weekly organics and metals data

APPENDIX H

APPENDIX H

PCB Homologs, as Percent of Total PCBs, for Larval Gizzard Shad and Young-of-the Year Emerald Shiners from the River Raisin, 1983-1984

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FIGURES

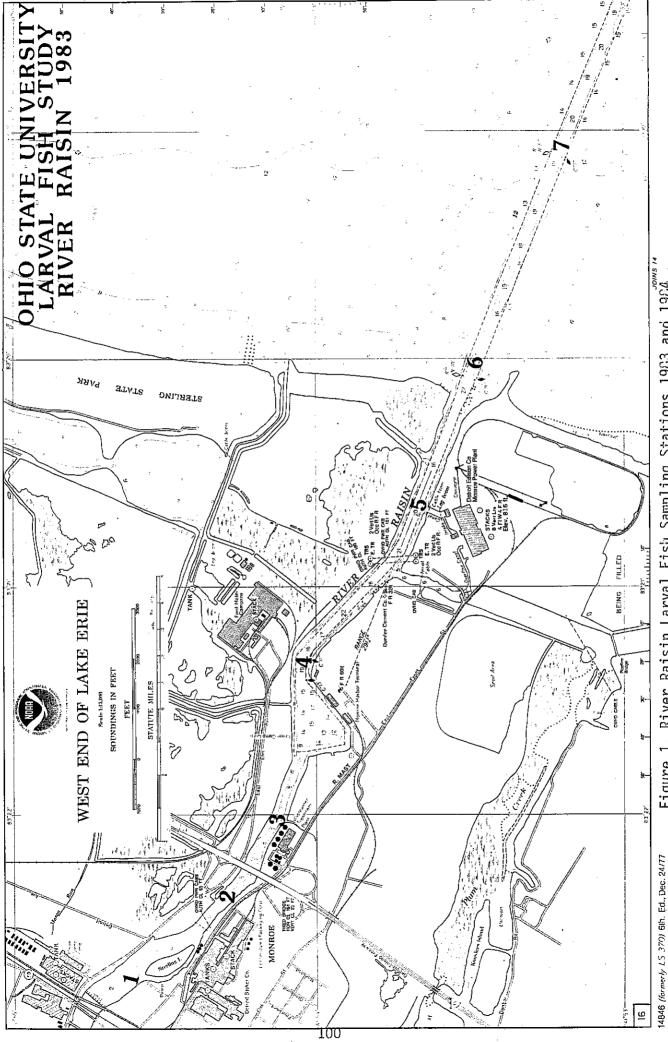


Figure 1. River Raisin Larval Fish Sampling Stations 1983 and 1984 .

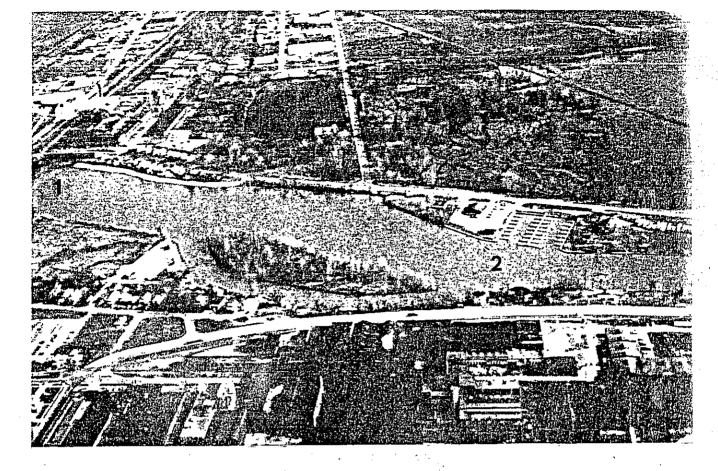


Figure 2a. Aerial View of Station 1 and 2, River Raisin.

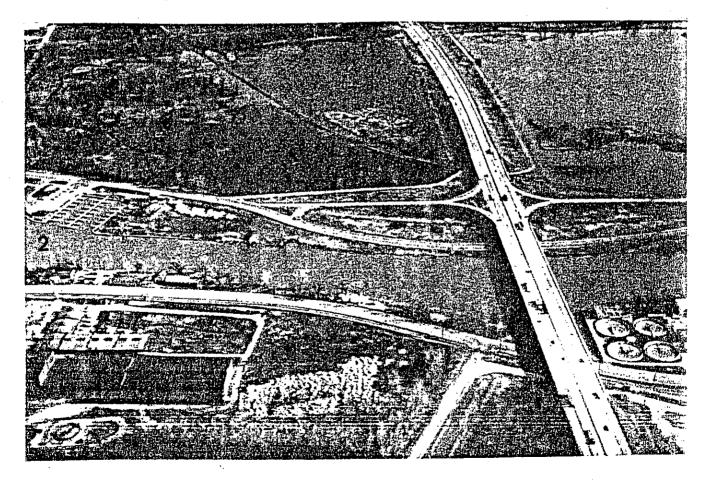


Figure 2b. Aerial View of Station 2 and 3, River Raisin.
101

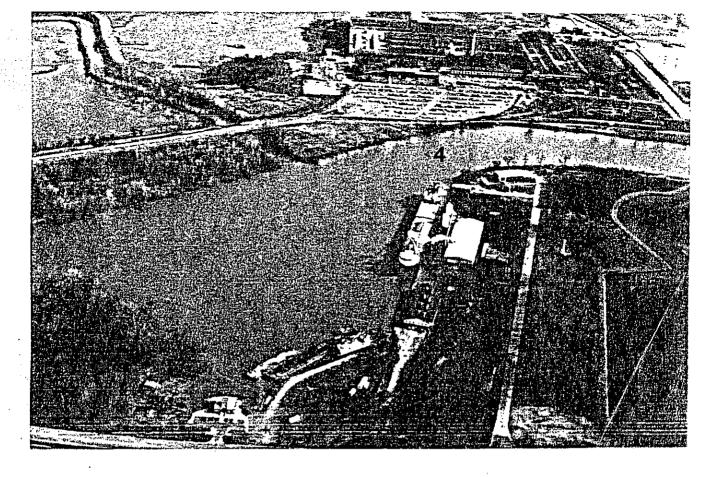


Figure 2c. Aerial View of Station 4, River Raisin.

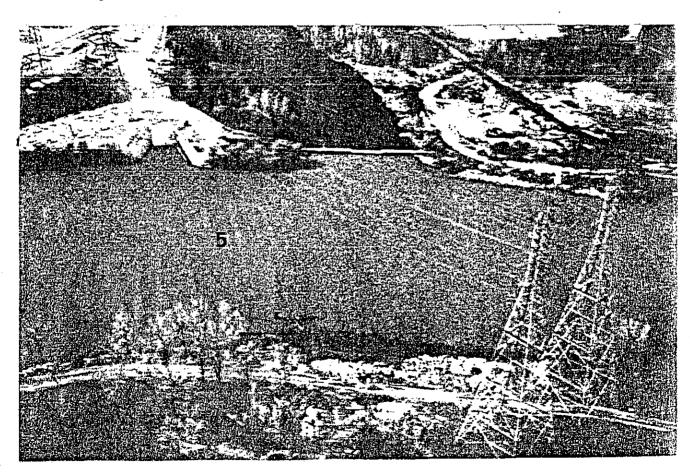
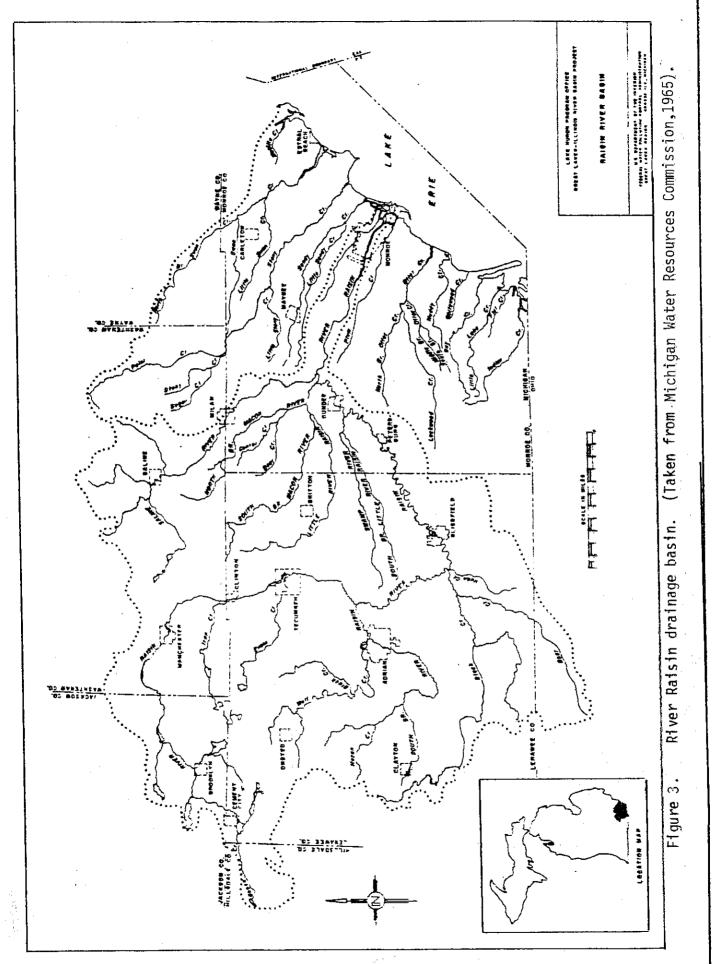
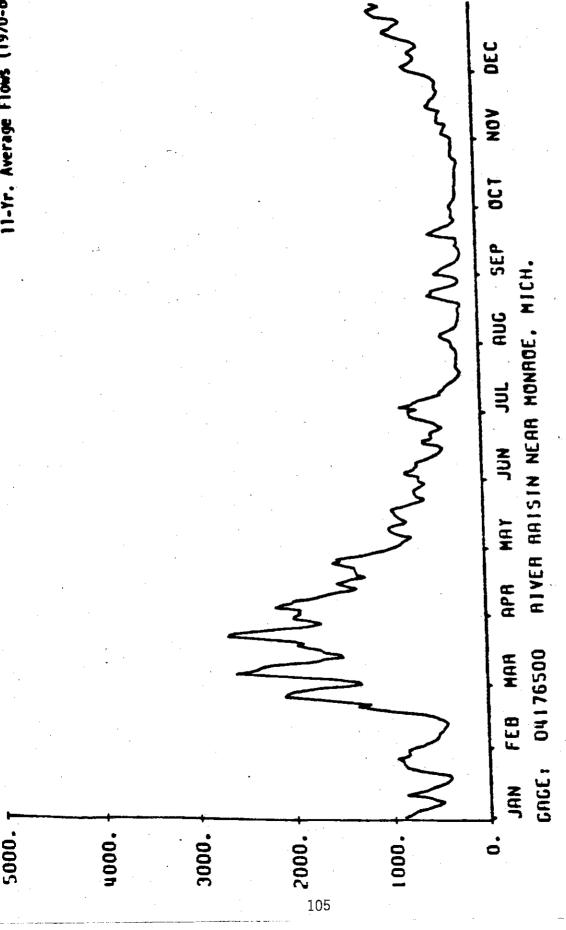


Figure 2d. Aerial View of Station 5, River Raisin.

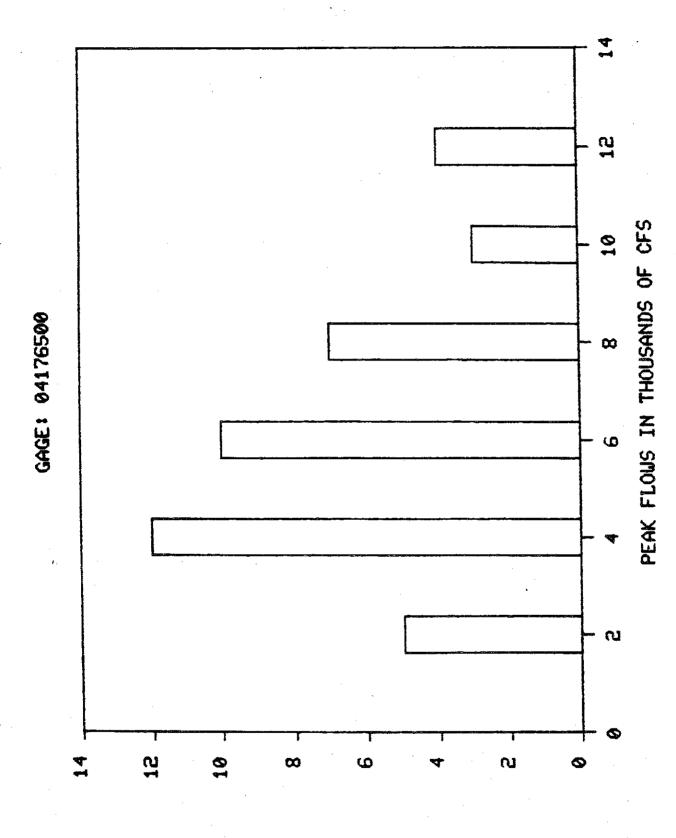


Figure 2e. Aerial View of Stations 4,5 and 6, River Raisin.

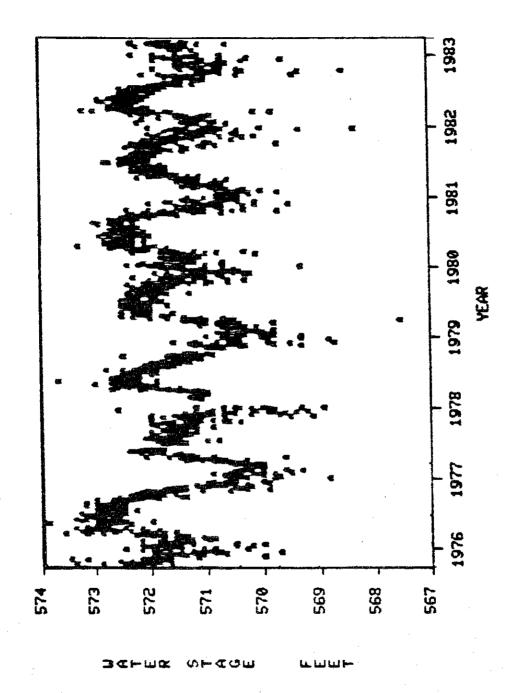




River Raisin 11-year average daily flow (cfs) 12 km upstream from Lake Erie (USGS data). Figure 4.

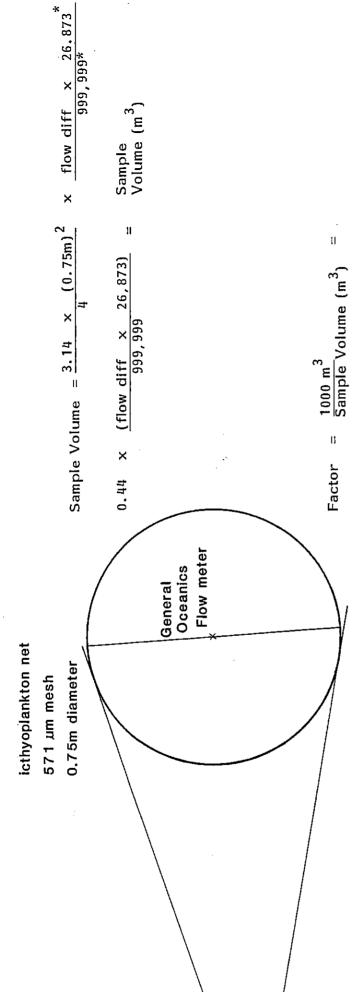


Peak flows in the River Raisin at Monroe since 1938. (Data from the U.S.G.S.) ນ Figure



Lake Erie level at gage 3087. Data is from NOAA.

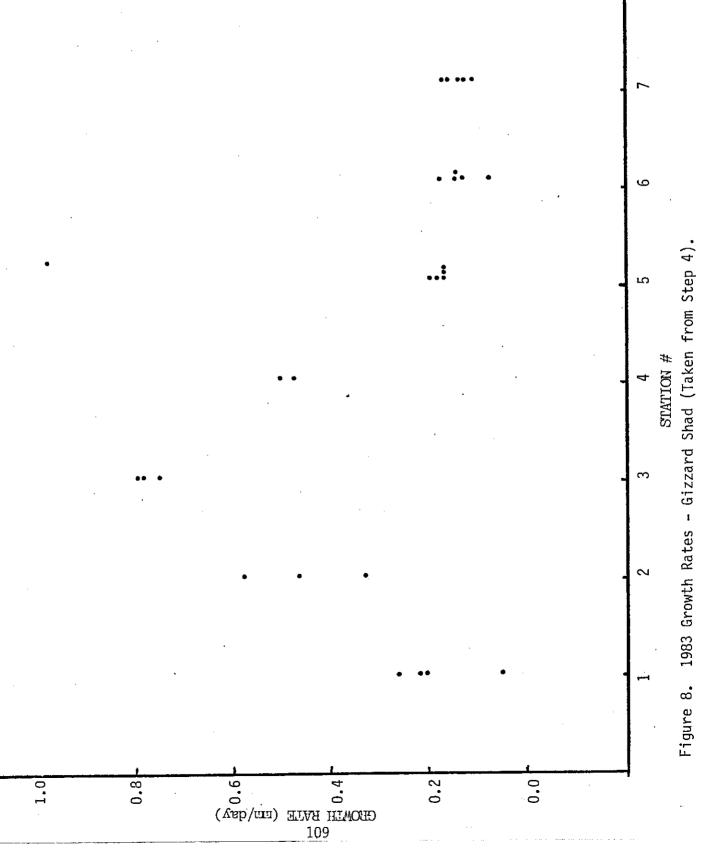
107



To calculate density of fish as Density/1000 m  $^3$  = Factor x Average Number per stage (I-IV) for all 3 replicates

Figure 7. Larval Fish Density Calculation Procedure.

\*(Constants supplied by General Oceanics)



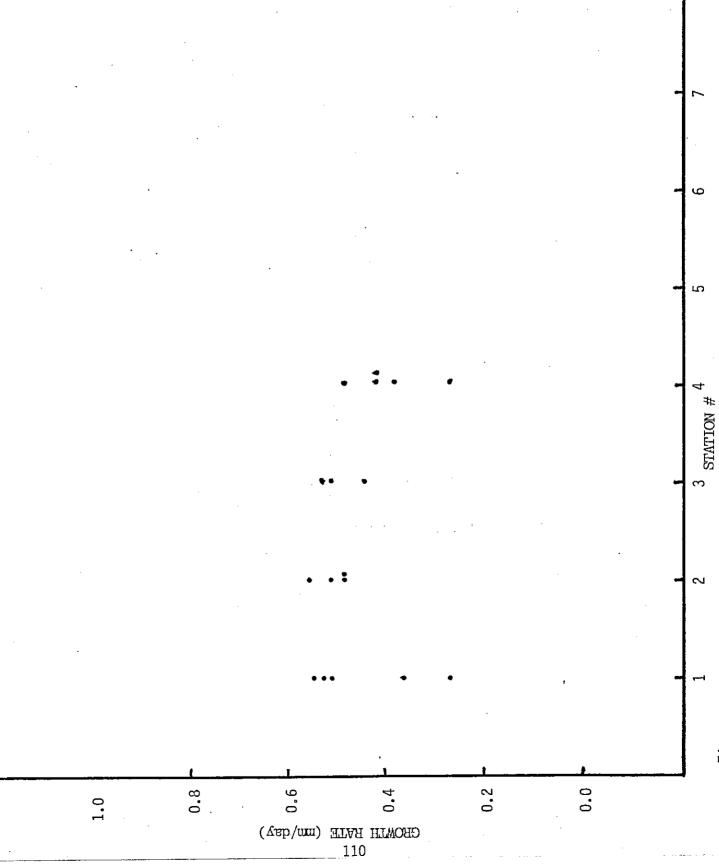
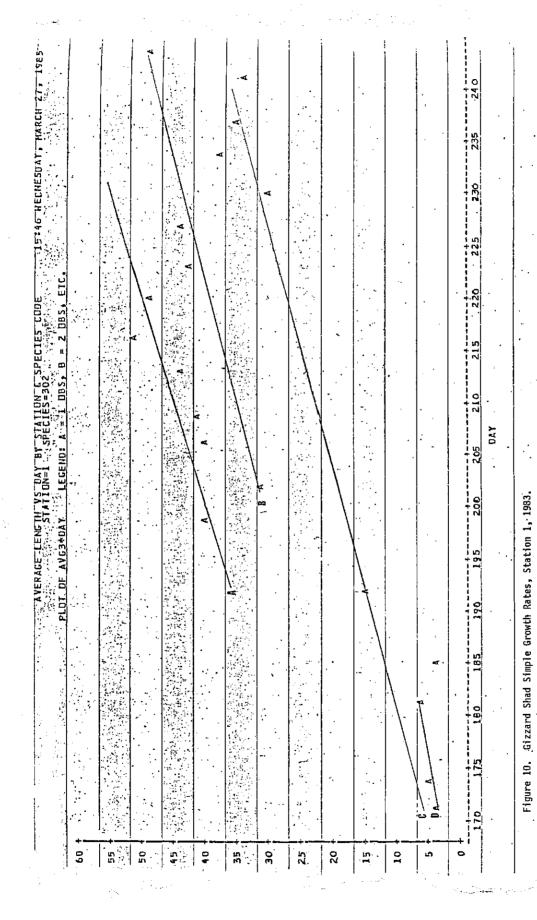
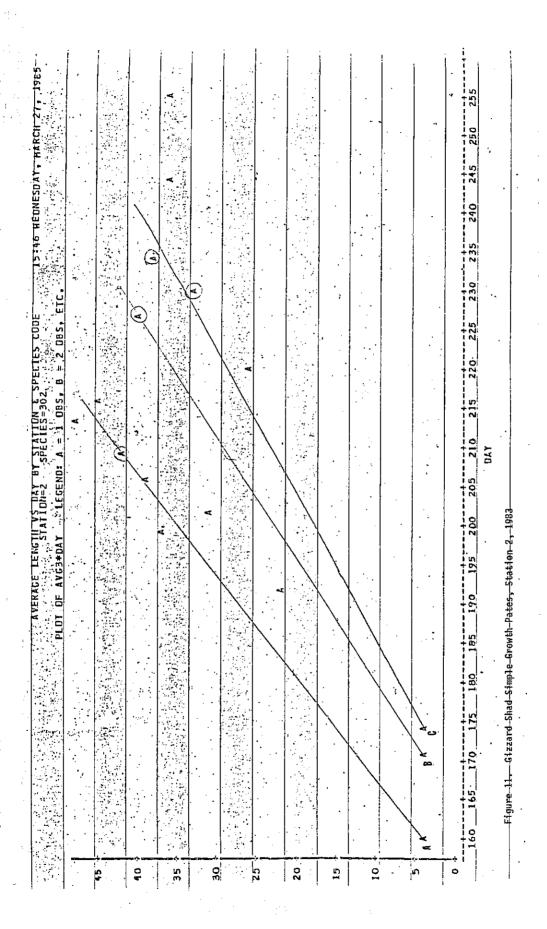
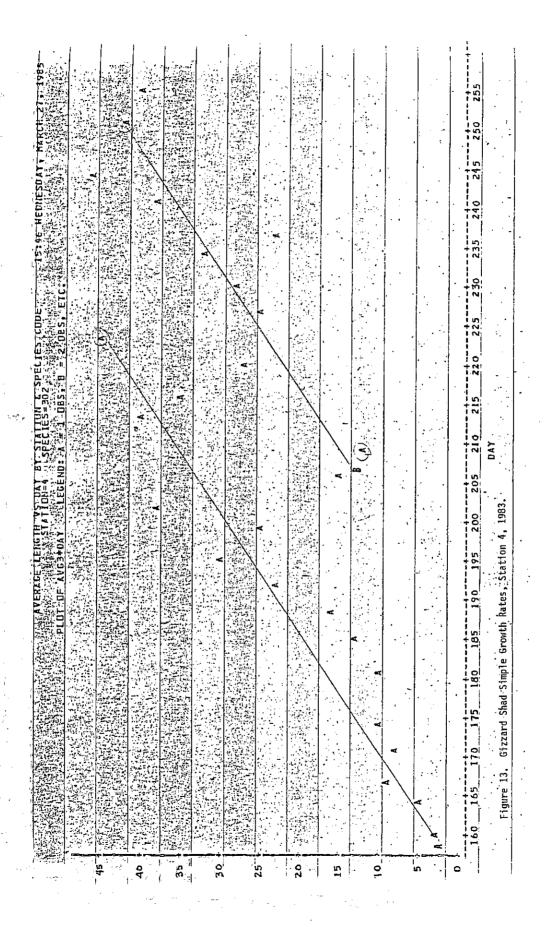


Figure 9. 1984 Growth Rates - Gizzard Shad (data taken from computer Step 4).

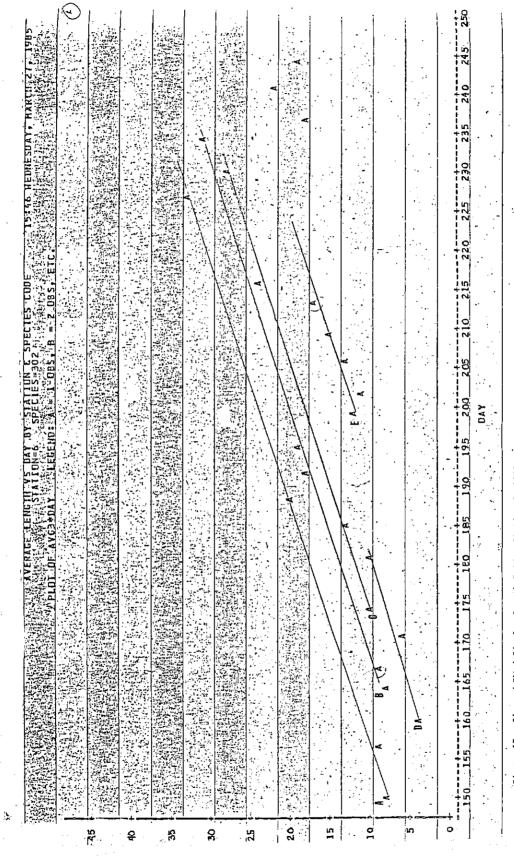




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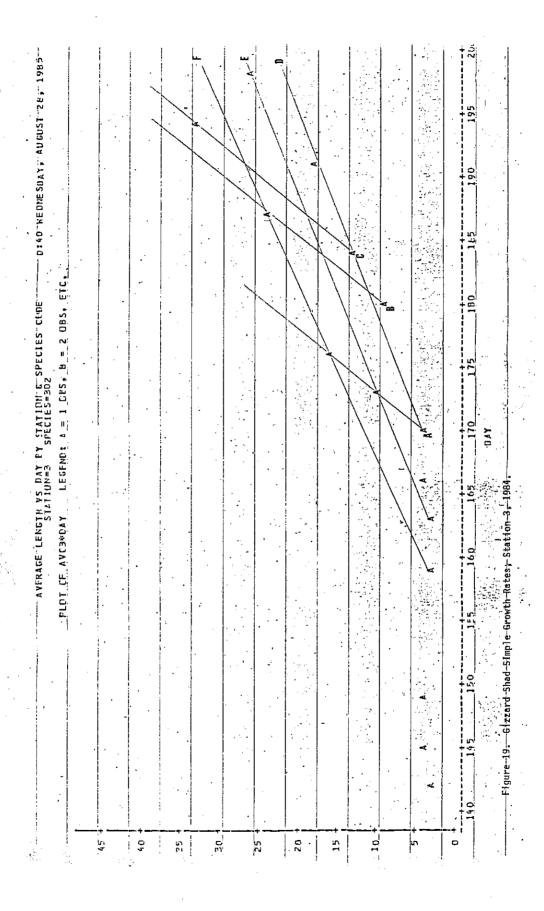


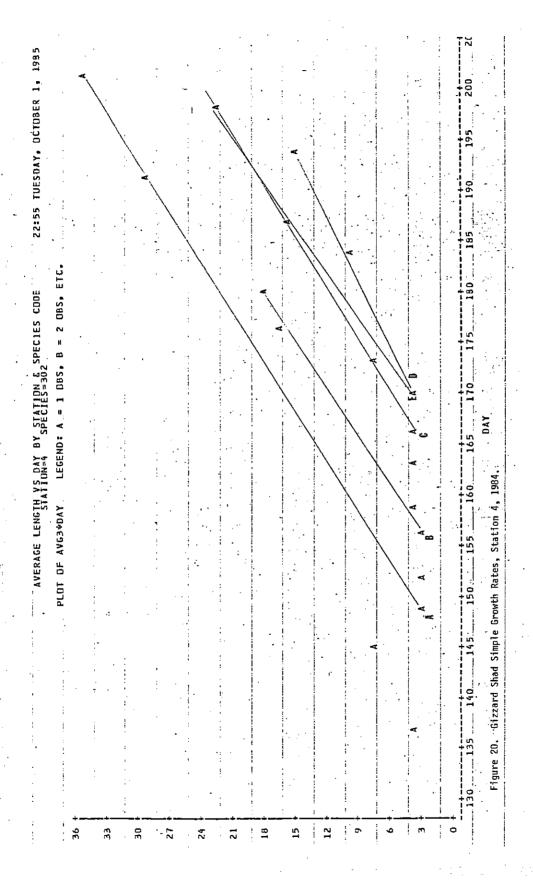
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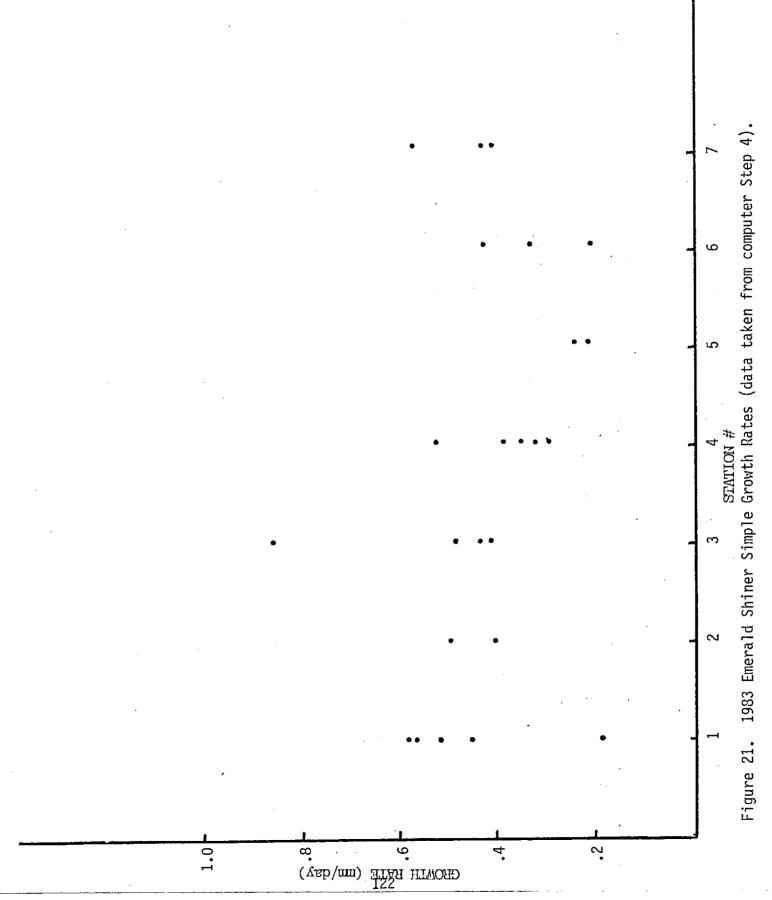
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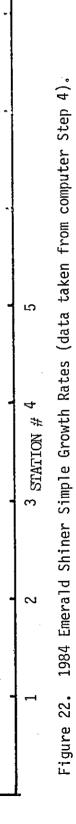
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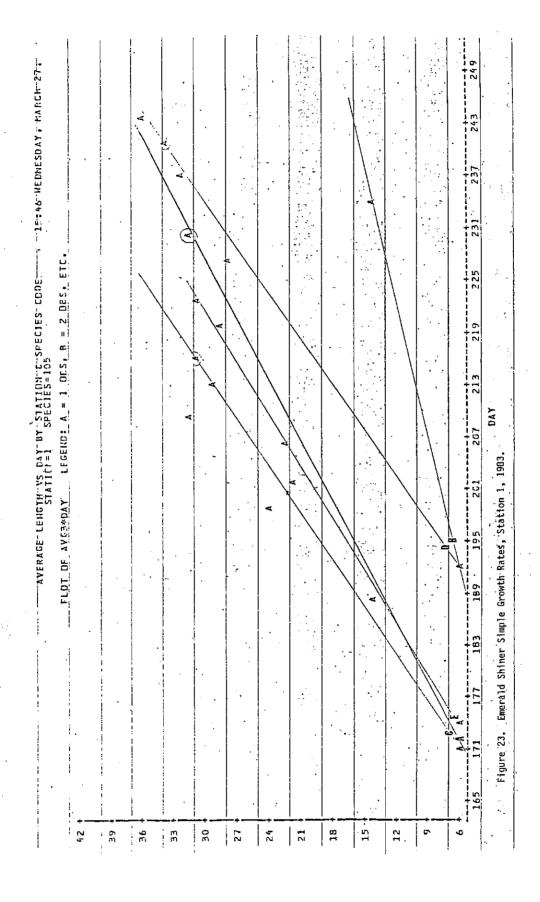


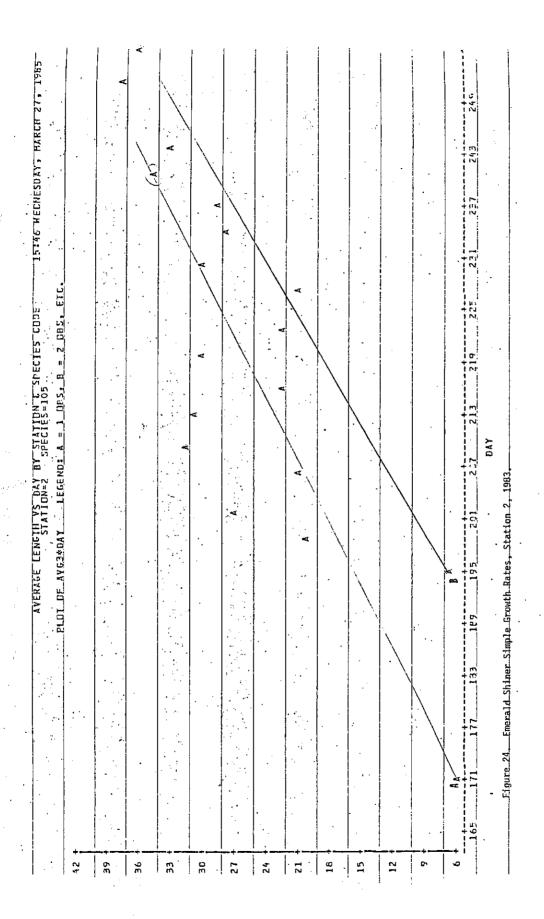


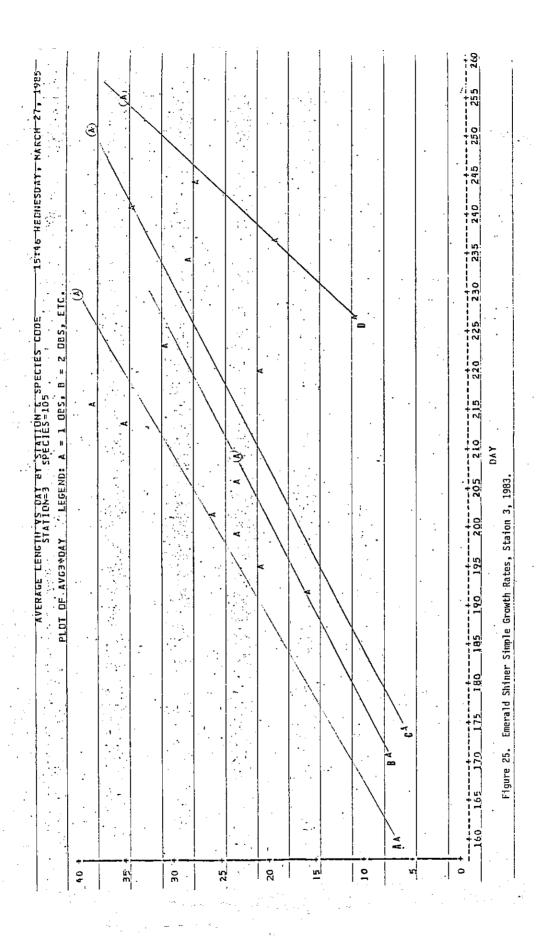


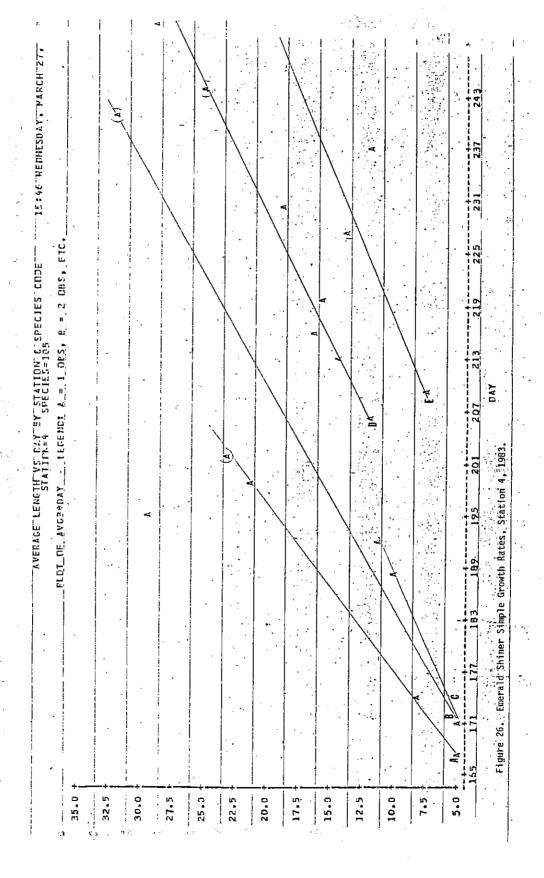
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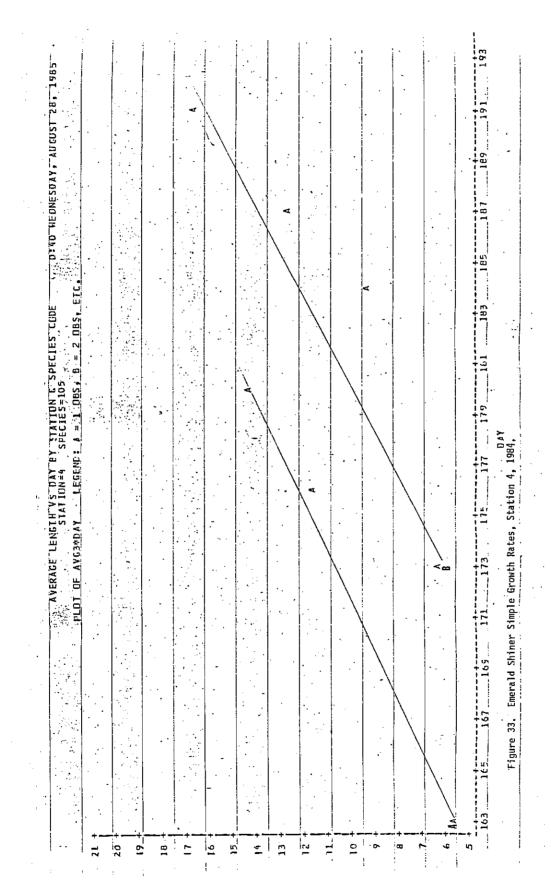
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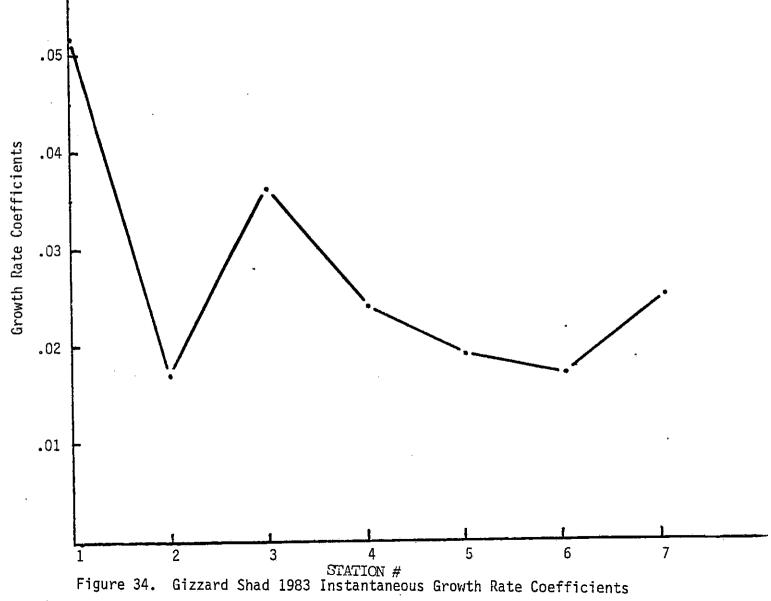
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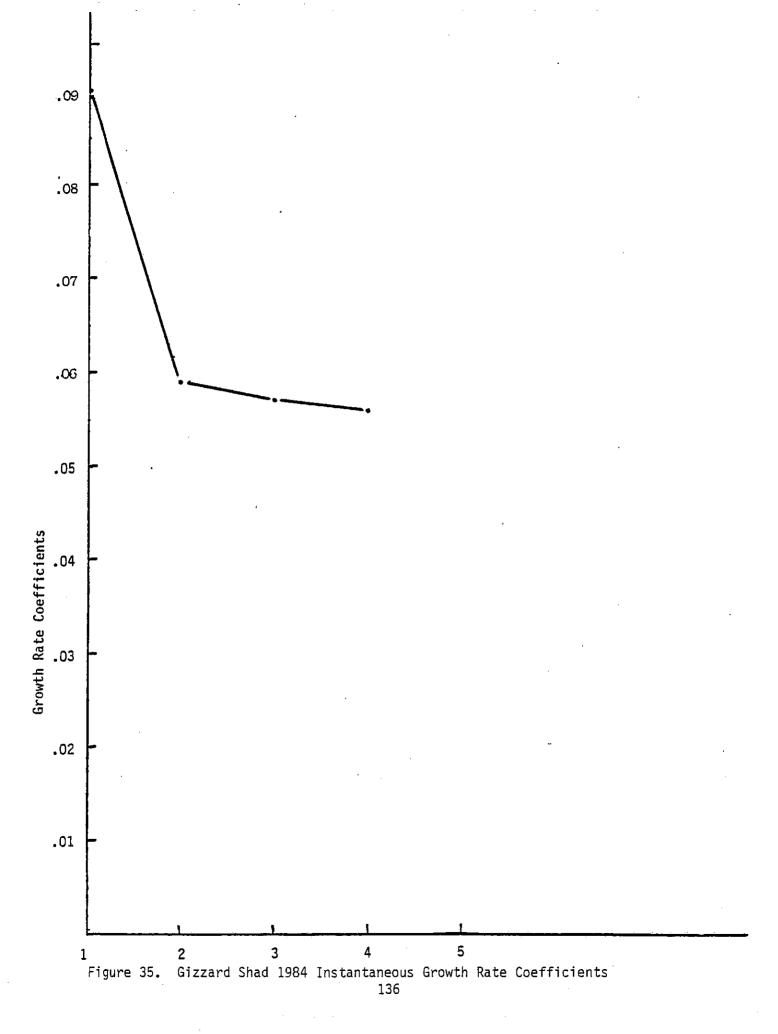
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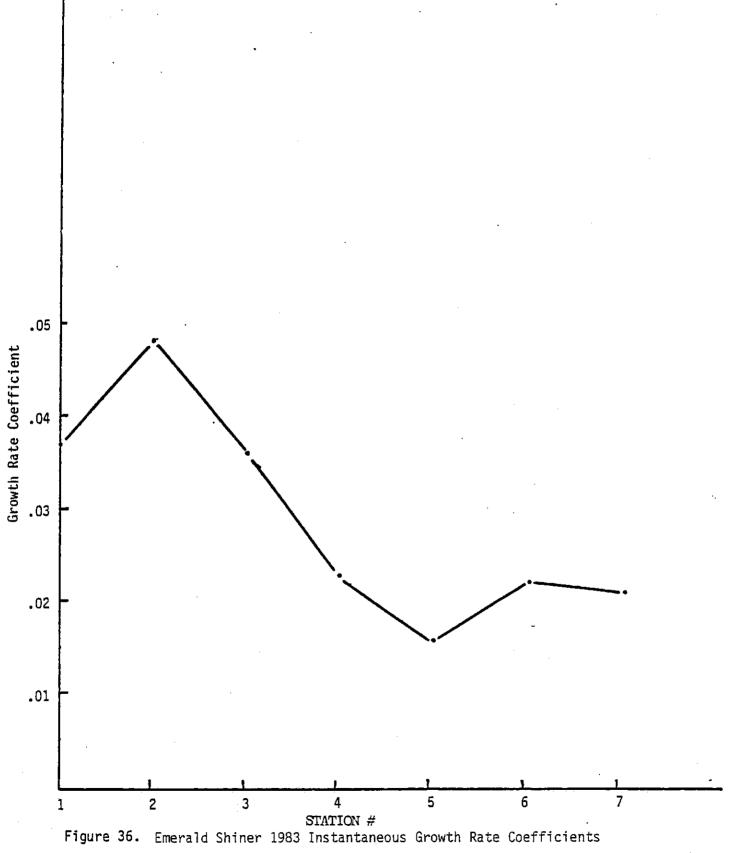
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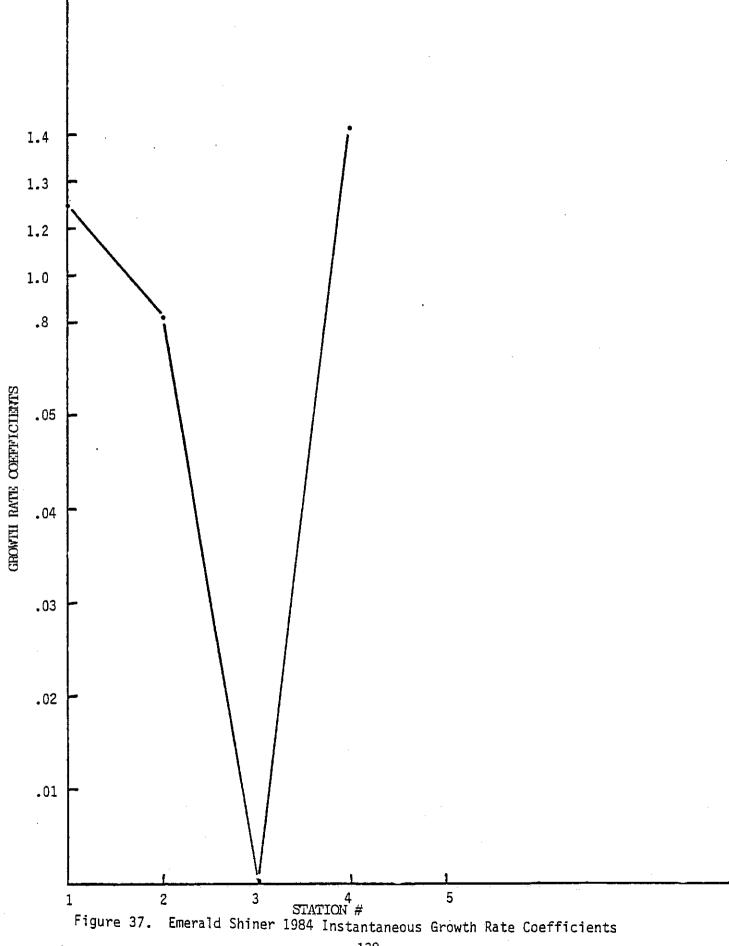


Figure 37. 138

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