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RESPONSE OF MOSQUITOFISH (*Gambusia affinis*) POPULATIONS TO SEASONALLY UNPREDICTABLE PERTURBATIONS[1]

M. J. Horn[2] and A. J. Stewart

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[1] Submitted as a thesis by M. J. Horn to the Graduate Council of the University of Tennessee, Knoxville, in partial fulfillment of the requirements for the degree of Master of Science.

[2] Present address: Department of Zoology, Arizona State University, Tempe, Arizona 85287

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ABSTRACT

Many questions remain unresolved about the linkages between life history attributes of fishes and the tactics that these organisms employ in response to environmental uncertainty. Such questions include 1). If a perturbation affects the entire ecosystem, what are the consequences for a given population of fish? 2). What tactics can a fish employ to increase its chances of leaving offspring? 3). Do fish respond differently to such perturbations depending on the season? 4). How do these changes relate to the overall resilience of the population? The research reported here was designed to address such questions. Mosquito fish (Gambusia affinis) populations in thirteen experimental ponds at Oak Ridge Nat'l Laboratory were sampled ten times between June 1988, and July 1989 in response to a series of chemical disturbances. During each sampling period the population size and total biomass of Gambusia in each pond was estimated using photographs and a length weight regression. Size-frequency histograms were used to examine seasonal and dose-related changes in population structure. Lipid content and reproductive allotment were measured for a series of fish from each pond on all dates to explore the energy allocation patterns at the individual level.

Fish populations declined significantly in each dosed pond. The intensity of the response was related to season. A dose administered on October 29,

1988 eliminated all fish from five of the experimental ponds. In the fall fish from the treated ponds retained a higher rate of reproduction which resulted in pulses of recruitment in the experimental ponds that did not occur in the reference ponds. Fish in the dosed ponds entered the winter with lower lipid levels, however over winter these populations lost less biomass, entering the spring in better condition which resulted in higher growth rates, and greater fecundity. In the spring the phenolic treatment group was the only group that showed recruitment. I attributed the results to reduced intraspecific competition: the dosed ponds had lower population densities of _Gambusia_. Although _Gambusia_ populations appear very resilient to disturbances the effects of the fall treatment were still evident in July 1989, nearly eight months after the last dosing had been administered.

I. INTRODUCTION

1. Objectives:

Seasonal fluctuations in resources and environmental conditions can affect the rate at which fish populations grow. Further these populations can persist in environments that appear unpredictable. The theoretical basis for the evolution of adaptive strategies has been well explored by a number of investigators, Fretwell (1972), Law (1979), Levins (1968), Lynch and Gabriel (1987), Lomnicki (1988), Murphy (1968), Pianka (1976), Schaffer (1974), Stearns (1976, 1977). Experimental studies have also been used to determine how strategies, or tactical variations within these strategies allow organisms to withstand environmental fluctuations (Adams 1982, Bagenal 1957, Booth and Keast 1986, Constanz 1979, Tinkle and Hadley 1975).

Many questions about the linkages between life-history attributes and tactics organisms may employ in response to environmental uncertainty remain unanswered. Of particular interest are questions about how linkages between life history attributes may enhance or constrain the response of a population to an unpredictable perturbation. Such questions have been rarely addressed in the context of ecosystems. This study was conducted to provide answers to some of these questions. I examined the effects a seasonally unpredictable perturbation (repeated doses of phenolics, designed to kill or stress a significant portion of the population) had on populations of Gambusia affinis in replicate experimental ponds, and used this data to identify the mechanisms these fish use to persist despite such perturbations.

I addressed four principal questions, and the subsequent predictions that came out of each:

1). If a perturbation affects the entire ecosystem what are the consequences for a population of mosquito fish within that ecosystem? Food needed to sustain the fish is composed largely of species with short generation times that can potentially respond quickly to the perturbation. Thus, density-dependent constraints on fish population growth should decrease following a perturbation that removes some, but not all of the fish from the perturbed system.

2). Following a perturbation what tactics are adopted by the fish, that may result in an increased chance of leaving offspring? With reduced intraspecific competition it is possible that the surviving fish can sustain higher growth rates, and become more fecund than fish in systems that were not perturbed.

3). Do the fish respond differently to chance perturbations depending on the season? If a perturbation occurs early in the season when fish are actively reproducing, the result may be higher rates of recruitment of new individuals into the population. If the perturbation occurs during the non-reproductive season the effects on recruitment may not be evident until the following spring. However, the fish may overwinter in better condition due to reduced competition, and thus produce larger broods the following spring.

4). How do changes in growth, fecundity and recruitment relate to the resilience of the population? Due to reductions in intraspecific competition, increases in growth, fecundity and recruitment rates could increase the resilience of the population.

2. Literature Review

What factor or factors regulate the total number of individuals living in a population? Why are some species more abundant than others in certain areas? Why do some species exhibit cycles in their patterns of abundance? These three questions have been addressed in almost all research exploring the mechanisms behind population dynamics (Andrewartha and Birch 1954, Hairston et al. 1960, Horn 1971, Levins 1968, Lomnicki 1988, MacArthur and Connell 1966, Solomon 1976, Tanner 1966). In answering these questions others emerge: Are the differences the result of just the environment, or do the biotic interactions of the organisms have a role (Tanner 1966, 1971)? Ultimately the abundance of a limiting resource may predict the maximum number of organisms that can exist in a given habitat, but as researchers have noted this maximum population is rarely, or never achieved.

Many factors, biotic and abiotic, may act to keep populations below a theoretical maximum (Andrewartha 1961, Cohen et al. 1980, Slobodkin 1954). Predation, for example, has been shown to shift habitat usage by bluegill sunfish, thus increasing intraspecific competition for resources in a more confined area (Gilliam and Fraser 1987, Werner et al. 1983, Werner and

Gilliam 1984). Predation may also be selective for body size, sex or color (Britton and Moser 1982, Law 1979, Reznick 1982, Reznick and Endler 1982). Similarly nutritional deficiencies due to abiotic environmental conditions or population density have been shown to limit fecundity in fish and birds (Schoenherr 1977, Wootton 1977, Lack 1966). Responses to seasonally predictable environmental conditions, or physiological inhibition of reproduction by factors such as temperature can affect production (Stearns 1983). Even complex trophic interactions have been implicated in regulating population abundance (May 1973).

Further, species are affected because the resource itself typically varies in abundance and quality both temporally and spatially. Consequently, populations existing in most temperate environments may exhibit cycles in the number of individuals due, at least in part, to systematic decreases and increases in available resources (Cushing 1986, Eisenberg 1966, Fowler 1981, Fretwell 1972, Solomons 1976, Starett 1951). The pattern of population abundance will track the environment depending on the scale of variation an environmental change encompasses, in relation to the length of the organisms life-cycle (Roughgarden 1974). How natural selection acts on the gene frequencies of the population, given design constraints from the organisms evolutionary history, ultimately determines the response to these fluctuations. The establishment of a life-history strategy among members of that population that maximizes reproductive fitness for the individual should be favored (Lynch and Gabriel 1987, Mann et al. 1984, Southwood 1988).

The sensitivity of a population to environmental extremes is both a function of the variance between the individuals in the population, and the possibilities for adaptation by a given individual (Lynch and Gabriel 1987). Because the overall genotypic life-history strategy of an individual consists of many traits, different tactics can develop in response to a varying environment (Harper and Ogden 1970, Mann et al. 1984, Potts and Wootton 1984). The resilience that a population requires to withstand repeated perturbations and environmental heterogeneity depends on the degree of independence between these relationships, because fixed relationships limit both the direction and extent of change a population can undergo (Trendall 1982). Adaptive adjustments in the relationship between life-history traits may result from differences in short-term responses to an environmental perturbation, to seasonal changes, or, in the long run, can lead to evolutionary change (Horn and Rubenstein 1984, Stearns 1983).

Typically life-history traits that have been found to be flexible in response to environmental variability and chance perturbations include (1) the growth rate of individuals; (2) size at reproductive maturity; (3) reproductive life-span; (4) the number and size of young produced; (5) the number of broods and the interval between broods; (6) the fecundity-age relationship; (7) and reproductive allotments (Milton and Arthington 1983, Stearns 1983, Trendall 1982, Wilbur et al. 1974). How might each of these traits vary in the face of environmental change?

Fisher (1930) posed a similar question, albeit perhaps more eloquently. He stated "It would be instructive to know not only by what physiological mechanism a just apportionment is made between nutrient devoted to the gonads and that devoted to the rest of the parental organism, but also what circumstances in the life-history and environment would render profitable the diversion of a greater or lesser share of the available resources towards reproduction.". Fisher's "just apportionment" of resources, better known as resource allocation, can be considered the cummulation of growth, maintenance, and reproduction. More recently, a fourth dimension, that of the storage of energy reserves, has been added to this set (Hahn and Tinkle 1965, Harper and Ogden 1970, Reznick and Braun 1987).

The tradeoff between growth and reproduction is especially important in organisms such as fish and plants which have indeterminate growth (Begon and Mortimer 1981, Harper 1977, Lomnicki 1988). Because growth is a positive function of body size of the individual and fecundity tends to follow growth exponentially, when to produce is a key question. Proximate environmental factors such as temperature affect growth, but so do selective factors that relate to uniform mortality across all age classes, or variations in age and size specific mortality (Constanz 1979, Pianka 1976, Stearns 1977, Werner et al. 1983). One would expect that an increase in resource levels would increase reproductive effort and growth, as well as survival. However reproductive allotment does not always increase even though it may be adaptive; this is because increased costs can be associated with growth or survival (Gadgil and Bossert 1970, Hirschfield 1980, Law 1979, Schaffer 1974, Williams 1966).

Further, how should the reproductive allotment be divided up among the offspring? Is it best to produce a few relatively well endowed offspring, or is it better to produce more, smaller, offspring that may not be as fit (Pianka 1976, Smith and Fretwell 1974)? Implications for differentially devoting investment to one sex or the other should also be considered (Clutton-Brock et al. 1985). These reproductive options occur, because natural selection acts on the combined life-history traits, the phenotype, not individual traits (Dobzhansky 1956). These reproductive options fall under the tenets of alternative evolutionary stable strategies (Smith 1982) and optimal decision theory, or "adaptive coin flipping" (Kaplan and Cooper 1984). In organisms that live in unpredictable environments the adaptive cion flipping principle appears mathematically to be the best strategy. It is advantageous for an organism to produce alternative genotypes so that some will survive. Over the course of many generations this strategy is more successful than the production of offspring with one fixed genotype (Dawkins 1982, Lomnicki 1988).

Age and size at sexual maturity are two of the most critical life-history traits in fish, especially for species that produce multiple broods (Campton and Gall 1988, Wootton 1973). These life-history traits have also been implicated as being very important in amphibians (Howard 1980, 1983). Age and size at sexual maturity determine the fecundity of an individual. Depending on environmental conditions, selection could favor fish that produce offspring early: even though fewer young are produced there may be correspondingly greater survival. The tradeoffs associated with egg production are related to competition for food, predation and demands placed upon the individual by the seasonal climatic cycle (Booth and Keast 1986, Nelson et al. 1987). With these thoughts in mind it is not surprising that studies of life-histories for fishes living in contrasting environments suggest that phenotypic plasticity of life history traits is common (Reznick 1983, Stearns 1983, Wootton 1977).

If brood size and offspring size are a result of natural selection there are three tactics that organisms might adopt: 1) If the environment and offspring survival remain constant, or vary in an unpredictable fashion, one might expect similar amounts of resource to be devoted to each clutch. 2) When the environment is predictable, there should be some period during this time which is best for offspring survival. This is when one would expect the largest clutch sizes. 3) Investment in reproductive tissue should decrease as the reproductive season progresses, because offspring survival is less certain (Bolduc and Fitzgerald 1989, Glebe and Leggett 1981, Harper 1977, Horn and Rubenstein 1984, Schaffer and Rosensweig 1977, Southwood 1988).

This third prediction may serve as an explanation as to how organisms may use a predictable period to enhance offspring survival. In amphibians

(Fitzpatrick 1972), lizards (Hahn and Tinkle 1965), and fish (Reznick and Braun 1987), there are higher than expected levels of offspring production relative to present resource levels. In such cases organisms appear to have gained a degree of independence from the environment (Reznick and Braun 1987). This reproductive adaptation is permitted largely by the storage of lipids which allows energy resources to be stored for the potential production of young, and as a buffer against seasonal fluctuations in food supply (Adams et al. 1982, Derickson 1976, Downer and Matthews 1976, Falk-Petersen et al. 1987, Krapu 1981, Reznick and Braun 1987).

Lipid reserves tend to increase in late summer and early fall when reproduction ceases (Reznick and Braun 1987, Wootton and Mills 1979, Delahunty and deVlaming 1980, Reznick and Braun 1987, Derickson 1976, Hahn and Tinkle 1965). The production of eggs in the spring may then be subsidized by depletion of the stored lipid reserves (Wootton et al. 1978, Wootton and Mills 1979, Wilson and Pitcher 1983).

A change in the allocation of resources from reproduction to the accumulation of lipid reserves in the fall is one possible adaptation for increasing the fitness of the young (Hahn and Tinkle 1965). Such reserves allow for the early production of a brood the following spring, which is presumably the most important brood of the year in many animals (Krumholz 1948). In populations where there is the possibility of producing several generations in a single season, young born at the beginning of the season can mature and reproduce the season of their birth. This provides them a reproductive advantage over those born later in the year (Krumholz 1948, Barney and Anson 1921).

II. MATERIALS AND METHODS

1. Life History Traits of *Gambusia*

The mosquitofish (*Gambusia affinis*) and its close relatives, members of the family Poeciliidae, have long been the subject of studies relating to variation in life history attributes (Barney and Anson 1921, Hughes 1985, Stearns 1983). *Gambusia* typically occurs in highly variable and often ephemeral systems. Through repeated introductions, often for use in mosquito control, it now occurs in many ecological zones throughout the world (Dees 1968). It tolerates wide ranges in temperature, and salinity, reproduces prolifically during the summer, and is ovoviviparous.

A relatively short gut is indicative of *Gambusia*'s predatory nature. Under severe conditions the fish will shift to diets composed mainly of algae or detritus (Harrington and Harrington 1982, Krumholz 1948). The effects that *Gambusia* have on the ecosystems in which they live are well documented and appear to be typical of those caused by fish introductions (Carpenter and Kitchell 1985). *Gambusia* can substantially reduce zooplankton and invertebrate populations (Brooks and Dodson 1965, Goodyear et al. 1972, Hurlbert and Mulla 1982).

Breeding in *Gambusia* tends to be relatively synchronized among the females within a population (Botsford et al 1987). There is no reproduction during the winter (Hughes 1985). In early spring, eggs that were fertilized the previous fall begin to develop. The first broods may be born as early as February; up to nine broods may be produced during the growing season (Milton and Arthington 1983). The earliest broods produced are the largest, and brood size gradually decreases over the course of the season (Barney and Anson 1921). Fecundity also tends to be linearly related to body weight of female fish (Milton and Arthington 1983). The young that are born early in the season can mature and become reproductive adults that season. Female Gambusia are often used for experiments in resource allocation because relative to males of this species they channel a higher proportion of resources into the gonads. This tendency makes reproduction more sensitive to environmental influence (Townshend and Wootton 1984).

Studies of Gambusia have been conducted in areas relating to the link between fecundity and somatic growth (Reznick 1983, Constanz 1979), plasticity for age at maturity (Stearns 1983), the role of lipid storage as a reproductive adaptation (Reznick and Braun 1987), and the role of cannibalism on population development and structure (Meffe and Crump 1987).

2. Study Area

The research and was conducted in 13 ponds, as part of a larger project dealing with resilience of aquatic ecosystems to perturbations. Each pond was lined Hypalon^R and enclosed 10 to 13 m^3 of water maintained at a depth of about 80 cm, adding dechlorinated tap water. In extremely wet periods, water was pumped from the ponds to maintain the desired level and to prevent the possible transfer of Gambusia between adjacent ponds due to flooding. The electric pump used for this purpose did not appear to disturb the Gambusia or suck any individuals into the intake. Each pond contained a layer of loose sediment 15-20 cm in depth as substratum. The sides of the pond were colonized by periphyton.

As reported by Giddings et al. (1984), submersed macrophytes (Elodea canadensis and Potamogeton sp.) were the dominant plants in earlier studies. When pond dynamics are controlled by dense beds of macrophytes, it can be hard to detect some of the more subtle changes in trophic interactions occurring between the planktonic community and Gambusia populations. Accordingly a 20-25 cm Grass carp (Ctenopharyngodon idellus) was added to each pond to prevent the accumulation of excessive quantities of macrophytes.

The ponds were not manipulated after the start of the experiment other than periodic seining for Gambusia and for the collection of samples for water chemistry analysis and enumeration of plankton and zooplankton. During the first six months of the experiment, weekly samples were collected for zooplankton, phytoplankton, periphyton, conductivity, alkalinity, pH and nutrients. After this time sampling frequency was reduced to once a month.

Replicate ponds were dosed with a mixture of phenols (2-4 dinitrophenol, m-creosol, o-creosol, and phenol) once during the summer (June 10, 1988), once in early fall (August 15, 1988), and again early in winter (October 29, 1988). Each dose, computed nominally based on water volume, was large enough (16 mg/L, measured as total phenols) to kill 50 percent of the Gambusia in a pond within 48 hr, based on the results of laboratory acute toxicity tests conducted at 25°C. Similar phenolic concentrations were achieved in each of the 13 ponds, and the chemicals remained in the ponds for about the same length of time in all cases; phenols after dosing were present in detectable quantities for about two weeks.

Gambusia were first added to the ponds in March, 1988. These Gambusia were obtained from a slough adjacent to Poplar Creek, about 1 km upstream of the Oak Ridge Gaseous Diffusion Plant. Approximately 1300 Gambusia were collected and allowed to acclimate in the lab for one week before being stocked into the ponds. All Gambusia were collected from the same slough to reduce the possibility of inter-population differences among ponds. Ninety-eight Gambusia randomly selected from the collection were added to each pond. Gambusia populations were first sampled on June 4, 1988, just before the first dose was administered. A seine, 3.8 m x 1.3 m with 1.5-mm mesh, was used to collect Gambusia from each pond. All ponds were seined again on June 29, about two weeks after the initial dose had been added. Similar collections were made about a week before and about two weeks after the dosings on August 15, and October 29 : Collections were made on June 4, June 29, August 4, August 23, September 15, October 20, November 17, March 27, 1989, June 7, 1989, and July 11, 1989.

The Gambusia populations in each pond were sampled with 4 passes of the seine, moving in the same direction each time. Because the ponds lacked refugea few Gambusia escaped seining. By the fourth haul, few or no Gambusia were captured. From each collection 10-15 reproducing female Gambusia of a similar size were immediately placed on ice and frozen for future analyses. The number of Gambusia selected was based on the density of Gambusia in the pond. Ponds with fewer Gambusia had correspondingly fewer individuals removed.

The Gambusia seined from each pond were placed in a 50 x 70 cm white plastic tray containing about 2 cm of water. A plastic ruler 30 cm in length and a tag indicating the pond I.D. number and the sampling date were also placed in the tray. The Gambusia in the tray were then photographed for later enumeration of the population. Gambusia to be returned to the pond were generally returned less than 10 minutes after being captured;, this minimized inadvertent mortality.

The photographs were prepared as 5 cm x 5 cm slides and the images were then projected onto a screen. The standard length (SL) of each Gambusia was then measured using the ruler in the picture for calibration. The size estimate of the Gambusia were made to the nearest 0.5 mm (SL). The total number of Gambusia in each pond was also counted from the slides.

The total biomass of Gambusia in each pond was estimated using a length-weight regression for Gambusia (Figures 36, 37, and 38). The regression was obtained from 100 randomly selected Gambusia from 4 ponds. Each Gambusia was measured to the nearest 0.5 mm (SL), sexed, and weighed: Dry weight of these individuals was measured to the nearest 0.1 mg. Two regressions, one for Gambusia in the winter when no reproduction occurred and one for Gambusia during the summer when reproduction was high were used to provide the total biomass in each pond. The winter regression used Gambusia taken from ponds B, J, F, H (collected on October 20, and November 17, 1988). The regression for Gambusia in the summer used Gambusia collected from ponds B, J, F, H (June 1989). A single regression line using Ln-transformed data (Figure 36) during the winter months was sufficient to cover all size classes. For months when reproduction occurred two regression lines were used to provide the best estimate of total biomass (Figures 37 and 38). The weight of each Gambusia that was <= 27 mm (SL) was estimated with one regression, and the weight of each Gambusia > 27 mm (SL) was estimated using another regression. As shown by other authors, 26-

28 mm appears to be a point below which most <u>Gambusia</u> are either males or non-reproducing females (Reznick and Braun 1987). <u>Gambusia</u> larger than 27 mm (SL), in contrast, are almost exclusively reproductive females.

3. Laboratory Processing of Gambusia

Lipid extractions of the Gambusia followed the methods of Reznick and Braun (1987). Only reproducing female Gambusia were included in this analysis. The Gambusia to be analyzed for lipids were first measured to the nearest 0.1 mm using calipers. Using a pair of dissecting scissors, a cut was then made around the body cavity, starting at the vent and circling up around the top of the body cavity, terminating at the gill plate. This exposed the ovaries and gastrointestinal tract. The gastrointestinal tract and ovaries of each Gambusia were then removed and preserved separately in formalin.

The dissected Gambusia were placed in individually labeled, pre-weighed glass scintillation vials and dried at 60^0 C for 24 h. Care was taken to make sure the abdominal cavity was spread open to maximize the surface area for extraction and to reduce drying time. Gambusia and vials were allowed to cool to room temperature for 1-2 h before being weighed. About 20 mL of anhydrous diethyl ether was to added each vial to cover the carcass, and the vial was tightly capped. The lipids in the Gambusia were allowed to extract for at least 24 h in the ether. The ether was then decanted off, and the Gambusia were again dried overnight before being re-weighed. The percent dry weight as ether-soluble lipids was then calculated as a percentage of the carcass weight:

(dry weight before extracting)/((dry weight before extracting)-
(dry weight after extracting))

Tests with fish pieces similar in size to _Gambusia_ and containing similar or greater amounts of lipids, were extracted in differing volumes of ether for different lengths of time to verify that the single extraction in 20 mL of ether was sufficient. Above 20 mL I found no significant increase in the amount of lipid extracted. Thus single _Gambusia_ do not contain enough lipid to saturate more than 20 mL of ether.

Eggs obtained from the dissected _Gambusia_ were inspected microscopically. They were counted and graded according to six maturational stages (Schoenherr 1977). The ovaries and eggs of each _Gambusia_ were then dried separately at 60^0 C for 24 hours before being weighed. The reproductive allotment for individual _Gambusia_ was then calculated as the fraction of the _Gambusia_'s total dry weight that was composed of reproductive tissues.

(dry weight of reproductive tissue)/((dry weight of _Gambusia_) + (dry weight of reproductive tissue))

4. Statistical analyses.

The dependent variables used for most of the analysis were: lipid content (expressed as % dry body weight), reproductive allotment (expressed as the fraction of total dry body weight composed of reproductive tissue), and Gambusia length. Dose, biomass, population size, and time of the year were considered as independent variables. Lipid, and reproductive allotment, were also considered as independent variables in tests determining the associations between them.

All analysis were done using SAS GLM or SAS NPAR1WAY (SAS Institute version. 6.03). Levels of significance were based on Type III sums of squares. Other than for the analysis of seasonal cycles in lipid content and reproductive allotment, criteria for the analysis of variance were assumed to be satisfied. These assumptions were satisfied visually; in general, the small sample sizes made it difficult to interpret statistical tests. A dose affect was incorporated in all models. When testing for dose effects, the mean of the dependent variable for each pond was used. Sample sizes varied greatly among ponds (dosed vs. reference) as well as seasonally; these differences were due primarily to differences in total abundance of the Gambusia.

Analysis of variance tests for the affect of dose on lipid content, reproductive allotment and maximum length of reproducing female Gambusia were used in a nested design, with dose as a main effect and ponds nested within dose. I considered ponds a fixed effect, but classification as a random effect may be more appropriate depending upon how one wishes to classify the division of ponds.

Gambusia length, lipid content, and reproductive allotment can all potentially covary with each other. I found no predictable relationship between these parameters within the range of Gambusia sampled within a pond however, and thus felt justified in excluding them for the purposes of this study. Tests of the relationship between lipid content, reproductive allotment and length were done according to the strength of the correlation coefficient between the pond means for these variables and other potential covariates (Figure 7). The procedure was used in an effort to reduce the number of statistical tests, because a strong correlation is generally presumed to indicate a significant relationship between any two variables.

III. RESULTS

1. Population Development: Numbers and Biomass

Population development was initially very rapid for all ponds. Numbers increased in reference ponds until late summer, then started a gradual decline in early fall. This decline continued throughout the winter (Figures 9-14). By August 4, the seven ponds used as reference systems for the first dosing (B, C, F, G, H, L, and Q) had peaked with respect to numbers of Gambusia; the Gambusia populations decreased thereafter. The general pattern of increase was similar across all ponds before the first dosing.

Before dosing, populations were dominated by larger size classes of fish (Figures 23-35). The first pulse in recruitment occurred near the beginning of June, as evidenced by changes in the size-frequency histograms for Gambusia in the ponds on June 4 (Figures 23-35). In the reference ponds this pulse was evident through time in the histograms showing Gambusia size. As the season progressed little new recruitment occurred in the reference populations.

Population biomass was calculated by summing the weights of all individual Gambusia, using separate length-weight regressions for males plus immature females and mature females (Figures 36, 37, and 38). Changes in Gambusia biomass were parallel to those of the population increases, but lagged this parameter by several weeks. Biomass reached a maximum by August 23, 1988. The lag in biomass relative to numbers resulted from growth of individual Gambusia (Figures 9-15), as the population began to decrease. Total biomass in reference ponds was at its highest on August 23, 1988, after the second dosing had been administered. Then, the reference ponds had between about 4.5 and 5 g (dry weight) of Gambusia/m^3 (Figures 9-15).

The pattern of population development in reference ponds was different in the early summer of 1989 when compared to the same period of 1988. The number of Gambusia in each pond was lower in 1989 than in 1988. This difference was due to lower recruitment earlier in the year. Relative to the 1988 populations, the 1989 populations had more Gambusia in the larger size classes. Because of this difference, biomass in reference ponds in 1989 increased more rapidly, in proportion to the population size, than it did in 1988.

When a pulse of recruitment entering a population moves through the various size-classes, both the amplitude and period of the pulse changes. A decrease in the size of the pulse is indicative of mortality. The pulse also tends to flatten out, and may become somewhat bimodal. The smaller size classes are probably comprised of males which rarely exceed 30 mm in standard length (SL), and immature females. The largest male found when doing the length-weight regressions for biomass estimates was 28.7 mm SL. Thus, all size classes >28 mm SL consist exclusively of female Gambusia.

Dosed ponds initially showed the same recruitment patterns as reference ponds, with a single large initial pulse appearing in late spring. Six ponds (A, D, E, J, M, and P) were dosed on June 14. On June 29, significant differences in biomass and numbers were found between dosed and reference ponds: the total number of Gambusia was much lower in the dosed ponds than in the reference ponds. This reduction however, was not significant (p= 0.113). Biomass differences between dosed and reference ponds were significant (p= 0.034, Table 8, Figure 8). The lack of a significant dose effect on population size was attributed to a pulse of recruitment that occurred in several of the dosed ponds between the dosing and sampling dates. This pulse did not significantly affect biomass because the small Gambusia contributed little to the total biomass.

In late fall, a different pattern of population development occurred. Gambusia densities were high in all reference ponds, and recruitment was low. Recruitment rates in the dosed ponds were higher than in the reference ponds. Recruitment, expressed as the proportion of individuals in the population under 20 mm SL, was significantly higher during the November 17, sampling period (p= 0.032, Table 5) and probably on October 20, as well (p= 0.10).

On October 29, 1988, ponds C, E, G, L, M, and P were dosed. This dosing destroyed Gambusia populations in each of the dosed ponds except for C, where about 62 Gambusia remained. Total phenol concentrations were similar to the previous two dosing periods, and persisted in the ponds for about as long as in the previous dosings. In the summer of 1989, pulses of recruitment occurred in the four remaining dosed ponds (A, C, D, and J); these pulses were significantly greater than those that occurred in the reference ponds ($p=.030$, Table 5) during July of 1989. The pulses in recruitment were similar to those following the initial stocking in 1988.

Once dosed, the number and biomass of Gambusia in dosed ponds tended to differ significantly from Gambusia number and biomass in reference ponds (in terms of a dose effect) throughout most of the remaining experimental period (Table 8, Figure 8). During the summer and fall of 1988, the dosed populations tended to be lower than those in reference ponds; during the winter months, populations in dosed and reference ponds were similar; and during the spring and summer of 1989, populations in dosed ponds were larger than those in reference ponds due the greater pulse in recruitment in spring. Biomass followed the same pattern, but I found no significant dose effects during the July 11, 1989 sampling. The lack of a significant difference between population size of the dosed and reference ponds at this time was attributed to differences in recruitment: Additions of younger, smaller, Gambusia to the populations increased population size but added little to the biomass.

2. Lipid Content and Reproductive Allotment.

Within given time periods, Gambusia were pooled (by pond) for analysis of lipid contents and reproductive allotments. This was done in an attempt to simplify the final analysis. Pooling in this manner could possibly affect some interpretations because at times significant correlations were found between reproductive allotments, lipids, and size of Gambusia within ponds. Significant correlations did not occur in all ponds at the same time, or even between the same variables among ponds, but their general patterns of occurrence seemed to be consistent from pond to pond. To minimize within-pond effects, these analyses included only reproducing female Gambusia from the larger size classes within each pond.

I found an inverse relationship between percent lipid content (expressed as a percentage of dry body weight) and reproductive allotment (Figure 16-22). During the periods when Gambusia were reproducing, lipid content was low (typically between 4% and 10%); the lipid content increased in late summer as reproductive allotment decreased. During the autumn and early winter, reproductive allotment declined to about zero; lipid content, however, increased dramatically and peaked in late October. The magnitude of the peak in lipid content differed somewhat from pond to pond (Figure 16-22). All ponds showed cycles, both in reproductive allotment and lipid contents of the

Gambusia (Table 7).

The phenolic doses appeared to affect both the reproductive allotment and the lipid content of the Gambusia. However, these parameters were not necessarily affected at the same time or in a similar manner (Table 1, Figure 1). After the first dosing, dose-related differences were found both in reproductive allotment and lipid content of the Gambusia; these two parameters were lower in Gambusia from the dosed ponds than they were in Gambusia from the reference ponds. A significant dose effect on lipids was not evident again until October 20, 1988; then, lipid content of the Gambusia was again lower in dosed ponds than it was for Gambusia from the reference ponds. A significant dose response was also found on November 17, 1988, March 27, 1989 and June 7, 1989. After June 7, 1989, pond-to-pond variation swamped any residual effects of dose. In November, lipid content of the Gambusia in reference ponds was higher than that of Gambusia in dosed ponds, but by March 1989, this trend had reversed. Thus over-wintering Gambusia from reference ponds lost lipids more rapidly than Gambusia in the dosed ponds (Figure 1). During the late spring and early summer, Gambusia in dosed ponds appear to utilize lipids more rapidly than Gambusia in the reference ponds.

Pond-to-pond variation in reproductive allotment was quite high, but this parameter also responded to dosing. For Gambusia collected on June 29, 1988, the effect was very strong (p= 0.004). However, by the beginning of

August no dose effect could be detected. On August 23, 1988, and on September 15, significant dose effects were found (Table 1). On both dates Gambusia, in dosed ponds had a higher reproductive allotment ($p< 0.001$, $p< 0.001$) than did Gambusia from the reference ponds. During the winter, no significant dose effect or pond difference in reproductive allotment was detected, partly because the Gambusia were no longer reproducing. On June 7, 1989, Gambusia in the dosed ponds again had reproductive allotments that were higher than those of Gambusia in the reference ponds ($p< 0.001$). By July, both the dose effect and the pond effect were no longer significant ($p= 0.09$ and $p=0.22$, respectively).

The rate at which reproductive allotments of the Gambusia decreased in the fall varied among the ponds. The total number of Gambusia per pond was a good predictor of reproductive allotment for Gambusia within ponds on August 4, 1988, and remained so through September 15, 1988. For August 4, only a Gambusia effect and a prior dose effect were evident (Table 4, Figure 4). Thus, the general result of the dose effect was to lower Gambusia populations in the ponds that had been dosed, but to increase reproductive allotments of the Gambusia that survived. On August 4, 1988 lipid content was also significant in relation to population size ($p= 0.009$) but there was no significant effect of dose ($p= 0.115$).

On August 23 and on September 15, 1989, there no longer appeared to be a dose effect on the reproductive allotments in relation to population size

(Table 4, Figures 5 and 6). Population size alone though was a good predictor of reproductive allotment on August 23 ($p= 0.048$), and on September 15 ($p= 0.04$) (Figures 5 and 6). On both dates reproductive allotment was negatively correlated with population size. A dose effect was probably not detected due to the high variability in population sizes between the ponds.

3. Patterns of Covariation

For lipid content and reproductive allotments, the nested-effects analysis of dose revealed significant differences across dates (Table 1). However, many of these differences could have been due more to a covariation with some aspect of population development or life-history attribute of Gambusia than to a direct result of the dosing (Figure 7). Length of the reproducing female Gambusia showed a strong dose effect on June 29, August 4, and August 23, 1988, and again in March and June of 1989. During the summer and autumn of 1988, and during the spring of 1989, reproducing Gambusia from the dosed ponds were significantly smaller than the reproducing Gambusia in reference ponds (Table 1, Figure 1). In June, 1989, however, reproducing Gambusia from the dosed ponds were significantly larger than those from the reference ponds.

In March and June, 1989, Gambusia length and lipid content were strongly linked (p= 0.044 and p= 0.058 respectively) even with the dose term included in the model. The dose effect for was not significant (p= 0.6958) in March, or June (p= 0.630, Table 9). Although lipid content of the Gambusia was significant in relation to Gambusia length for both dates, the correlation between lipid content and length of reproductive Gambusia switches from negative to positive (Figure 7). This indicates that in the autumn, Gambusia in ponds that contained larger Gambusia also had lower lipid content; this pattern was reversed in the spring.

The switch in correlation between March 27, and June 7, 1989 was due to a significant dose effect ($p < 0.001$). Relative to Gambusia in reference ponds Gambusia, in the dosed ponds lost a smaller percentage of their lipids over the winter. This reduction in lipid content also appeared to be related to the increase in the length of the reproducing Gambusia. The size of reproducing Gambusia increased faster in ponds in which Gambusia had high lipid content in the spring than in ponds containing Gambusia with lower lipid content ($p= 0.0011$, Table 12). No residual dose effects were apparent.

Ponds that contained Gambusia with high lipid content after winter also tended to have Gambusia that had higher reproductive allotments in early summer. In June 1989, the lipid content of Gambusia correlated strongly with reproductive allotment (Table 11). However this correlation may have been a relict of dosing ($p= 0.034$), because lipid in this model was only marginally significant ($p=.083$). When both lipid and dose were combined in the model, neither parameter was a significant predictor of reproductive allotment.

Before the first dose was administered, length of Gambusia was a good predictor of reproductive allotment ($p= 0.007$) using a linear regression. On June 4, 1988, lipid content was also a good predictor of reproductive allotment in all ponds ($p< 0.001$). On August 4, 1988, just before the second dose, lipid content of the Gambusia was a good predictor of reproductive allotment in all ponds (p= 0.005); the dose effect, in contrast, was not significant (p= 0.91).

When length-reproductive allotment correlations were high (Figure 7), the effect of dose was not a significant contributing factor (Table 10). The only other time that Gambusia length (across ponds) showed a significant relationship with reproductive allotment was in June, 1989 (p= 0.065). I found no length-dose interaction, and dose had a significant relationship to reproductive allotment (p= 0.079). This fact combined with the previous results, suggested that Gambusia in dosed ponds over-wintered better and were better able to increase the following spring. Over the winter lipid contents of Gambusia in dosed ponds also declined less than lipid contents of Gambusia in reference ponds (p= 0.028; Table 3, Figure 3). Overall, the trend was for dosed ponds to contain larger reproducing Gambusia that had a higher reproductive allotment. Lipid content of the Gambusia during autumn was also a good predictor of the loss of population biomass over the winter (p= 0.002). Finally, I found a strong effect of dose on biomass loss (p= 0.002). Dosed ponds lost proportionately less biomass over the winter (Figure 2).

IV. DISCUSSION

The overall patterns of growth and reproduction of Gambusia in the ponds used in this study were similar to those noted for this fish by others (Hughes 1985, Krumholz 1948). The reproductive season was between early March and the end of September, and the timing of the onset of reproduction was similar across both years of this study. Reproductive allotment was high early in the season, but decreased to near zero during the winter (Barney and Anson 1921, Reznick and Braun 1987). The average size of reproducing females collected also decreased from early summer to mid-November, due to recruitment of young into the reproductive size classes and mortality among large, over-wintering females from the previous season (Figure 1). Finally reproductive allotment and lipid content cycled inversely to each other, which is similar to the cycles observed for this and other species (Reznick and Braun 1987, Wootton and Mills 1979) (Figures 16-22). Collectively these observations indicate that the growth and reproductive patterns noted for Gambusia in this experiment are not atypical for this fish.

The objectives of this study were to 1) illustrate, and quantify differences between the ponds in the observed patterns of population development that emerged as a result of adding phenolics to the system, and 2) examine the data with respect to resilience of the Gambusia populations and the tactical life-history changes that may have affected this resiliency. Gambusia are known to produce their largest clutches early in the season with subsequent clutches decreasing in size as the season progresses until cessation of reproduction in the fall (Barney and Anson 1921, Reznick and Braun 1987). The Gambusia populations in this study showed this trend, as well. However, I found significant differences between ponds in the rate at which Gambusia reproductive allotment declined as the season progressed (Figures 16-22). Reproductive allotment (the proportion of total body weight devoted to reproductive tissue) was inversely related to the total number of fish in the ponds; fish in the more densely populated ponds had reproductive allotments lower than those of fish in ponds with fewer fish (Table 4). This observation raises several questions. Were these differences as a result of intraspecific interactions or were the fish responding to external environmental ques?

The seasonal cycle of reproduction of many fishes appears principally to be controlled by photoperiod and/or temperature, with photoperiod appearing to control reproduction in Gambusia (Milton and Arthington 1983, Brown and Fox 1966). Although temperature may also be involved in some aspect of reproductive timing, some species of Gambusia living under conditions of constant thermal regimes may still reproduce on a seasonal cycle, whereas other species exposed to constant thermal regimes reproduce continually throughout the winter months (Davis 1978). Thus at least some species of Gambusia appear to have a seasonally entrained physiological cycle that operates independently of biotic interactions.

Several theoretical models have been devised to explain the tendency for broods of Gambusia to become smaller as the season progresses. The most widely accepted of these is referred to as "bet-hedging" (Stearns 1976, Murphy 1968, Nussbaum 1981). Clutch size becomes smaller in the autumn because the chance that offspring will survive is lower then: dwindling resources and harsher conditions associated with this season make survival less likely. If offspring have a lower probability of survival in the autumn there should be selection pressure for females to not invest large amounts of energy into reproduction at this time. Similarly, larger investments into earlier clutches, would be advantageous and thus selected for, because of the higher probability of offspring survival, when resources are more abundant (Hahn and Tinkle 1965). In organisms that produce several generations per year, the potential fitness of young produced at the beginning of the year may be greater because the first brood offspring mature and reproduce the same season, whereas offspring produced later in the year mature the following spring and thus may not survive long enough to produce as many offspring (Tinkle 1967).

Why did populations of Gambusia in the dosed ponds continue to produce young during the autumn when Gambusia populations in the reference ponds were shifting reserves away from reproduction? Caswell (1982) suggested a modification of r-K theory for species exhibiting seasonal cycles of population abundance might be needed. During the spring when populations are small, r-selected traits, such as larger brood size, might be favored, whereas in autumn when resources available to fish are more likely to be limiting, more K-selected traits could be favored. Theory suggests that selection pressure would favor fish with a high degree of phenotypic plasticity (Stearns 1976).

The dose, reduced the size of the Gambusia populations. It is then possible that the surviving fish which have a high degree of plasticity were then in effect "reset" to a more r-selected state by the lower population density: relatively high levels of resources can be invoked as a mechanism to explain this. If r-K theory is correct, one would also expect that offspring in "early" and "late" broods would differ with respect to maturation times. Early in the season, selection would favor individuals that matured rapidly so that reproductive output would be maximized. Later in the season, however, selection should favor individuals that delay maturity until the following season. My study did not address the age at maturity in Gambusia in the ponds, so I was not able to assess this possibility. I also did not examine egg size, which, based on r-k and "bet-hedging" theories would be expected to shift from smaller eggs in the spring to larger, energetically better endowed eggs later in the season (Nussbaum 1981).

Dahlgreen (1979) has shown that for another poeciliidae, the guppy Poecilia reticulata, reproduction depends on fish density. Even when fish in populations of different densities were given proportionately similar amounts of food, fish in lower-density populations consistently had higher reproductive output. In another experiment, food was fed adlibitum to Poecilia living at different densities. In this study there were no detectable differences among fish in reproductive output (Dahlgreen 1980). The two experiments suggest that at higher densities, fish need more energy per capita. This requirement could be due in part to the increased number of intraspecific, aggressive interactions that take place at higher densities. Similar observations were made by Warren (1973a, 1973b): increased aggression and reduced courtship were found for Poecilia at higher densities. Although these observations were all made on laboratory population, there is no reason to suspect that they would not be important in natural situations as well, especially for fish populations that are confined in systems such as small ponds.

Many species have been shown to vary clutch size and/or offspring size under environmental conditions that limit the supply rates of resources independently of social interactions. In sticklebacks, for example, egg production is a function of food availability (Wootton 1977). The number of spawns produced was positively correlated to food ration, whereas the interval between spawning events was negatively correlated to ration. Similar findings have been reported for convict cichlids (Townshend and Wootton 1984). In Tilipia, Oreochromis mossambicus, a restricted food supply limits growth and reduces the number of eggs per spawn, but increases the number of spawns and allows a significantly larger portion of energy to be allocated to egg production (Mironova 1977).

If the increase in reproduction is related to population density why wasn't a similar increase observed in the study populations in the summertime: after the dosing fish populations were low? During the summer, Gambusia in the ponds used in this study were presumably producing at as high a rate as possible. Thus even though competition could have been reduced due to reductions in the number of fish per pond, individual fish may have been physiologically constrained so that they were unable to take advantage of the higher level of resources that were present. Constanz (1979) compared population dynamics of a top minnow (Poeciliopsis occidentalis) living in a stable spring system to a population of the same species living in an unstable desert wash system. He noted that the size of the body cavity appeared to limit egg production: There was an upper limit beyond which the fish could not increase brood size. Fish from the stable system did not appear to limited in this regard, indicating that they used a different strategy in responding to the environment they typically encountered. Gambusia also appear to be able to cope well with an unpredictable environment, as might be expected from the typically harsh, ephemeral habitats in which they are often found. During the autumn primary productivity decreases. Then the differences in reproduction in Gambusia between ponds of different fish densities are apparent. With fish in the ponds of highest density suffering the ramifications of increased competition while the fish from the dosed ponds were still able to exploit the relatively more abundant resources by sustaining higher rates of reproduction.

For the strategy of continued reproduction to be effective, the costs of the "last brood" must be lower than those of the perceived growth or reproductive benefits that could otherwise have been achieved by the adults the following spring. I showed that fish in the dosed ponds had a pulse of recruitment in the late fall that was not present in the reference ponds which indicates maintaining reproductive effort for a longer period of time increased recruitment to the population (Table 5). Fish populations can suffer large over-wintering losses due to harsh environmental conditions. Such losses can be important in controlling population dynamics. Many populations are temporally variable, with periods of high mortality being associated with various events (Schoenherr 1977, Hughes 1985, Krumholz 1948, Oliver 1979). Few studies have evaluated the survival probability of young in broods produced late in the year. If survival is very low, there would be selection pressure against this form of plasticity. My studies have shown that juvenile Gambusia do overwinter: this was evident from the relatively stable populations of fish in the dosed ponds over the winter (Figure 8).

Although young Gambusia do survive the winter, they appeared to grow little during this period. Wurtsbaugh and Cech (1983) showed that at low temperatures (i.e. those approximating winter conditions), growth rates of Gambusia are nearly zero. If the offspring do not grow much over the winter and many die, would it not be advantageous for the adult fish to delay reproduction until spring, when they could produce a larger brood at a time of the year when offspring survival is more likely? As stated earlier, selection for fish to do this would occur only if future growth and reproductive costs are less than those needed to produce the late autumn brood of young. The most obvious and presumably the largest costs associated with the production of a late autumn brood would be a reduction in the storage of reserves, reduced growth rates, and smaller broods the following spring.

Lipids serve as a buffer against seasonal fluctuations in prey densities (Adams et al. 1982), and are an efficient means of storing large amounts of energy in a relatively small amount of volume (Derickson 1976). Fish in the dosed ponds did not have lipid levels as high as those of fish in the reference ponds during the winter (Figure 1). However, the fish in the dosed ponds used a smaller proportion of the lipids over the winter so that fish from the dosed ponds had higher lipid levels than fish in the reference ponds by spring. The lipid content of a fish is a good predictor of that fishes probability of surviving over the winter. However, fish in dosed ponds lost less biomass, relative to the decrease in lipid levels than did fish in the reference ponds (Figure 2, Table 2). Thus, although resources may have been limiting to fish in both the dosed and the reference ponds based on overwinter reductions in lipids, they may not have been as limiting in the dosed ponds, perhaps due to the lower population densities. Consequently, fish in the dosed ponds over wintered in better condition than those in the reference ponds.

Lipids also are used in part to produce the large broods, that are for

Gambusia and many other fish typically early in spring (Wootton et al. 1978). Wootton and Mills (1979) noted that maturation of the ovaries in the minnow Phoxinus phoxinus coincided with a depletion in body and liver condition; the lipid reserves were then restored after reproduction had occurred. Similarly a decrease in somatic condition associated with increased gonadal growth occurs in the pollan Coregonus autumalis pollan (Wilson and Pitcher 1983). In Gambusia, lipids and reproductive allotment are inversely related to each other indicating a linkage between the two (Reznick and Braun 1987). In the spring, I found that fish were of similar size in both treatment groups and had similar reproductive allotments (Figure 1). Thus the cost of producing the last brood in the fall appears to be minimal. In June, reproductive allotment was significantly greater for fish in the dosed ponds than it was for fish in the refernce ponds (Figure 1, Table 1). I suggest that the higher levels of reproduction were sustained by resources. Although the dosed ponds had a Gambusia population increase in autumn, their populations were still significantly lower than those in the reference ponds.

Although fecund fish were observed in both treatment groups in the spring there was little or no recruitment of new individuals into the populations in the reference ponds but there was significant recruitment of young into the Gambusia populations in the dosed ponds (Table 5). This indicates survival of juvenile Gambusia in the reference ponds was low during in the spring. Juvenile fish typically are the first to starve in times of food scarcity. Based on the condition of the adult fish there was no evidence for food scarcity. Predation seems a supposed explanation for the difference in recruitment rates between the dosed and reference ponds. In the reference ponds many adult Gambusia contained juveniles in their stomachs (M. Horn personal observation) indicating cannibalism could be important. Cannibalism can provide a quick source of high quality energy, and may be of a reproductive benefit because conditions would not favor juvenile survival (Meffe and Crump 1987). Although disadvantageous from an evolutionary perspective it may be a by-product of Gambusia's aggressive non-specific predatory behavior. Cannibalism would be lower in dosed ponds simply because these ponds contained fewer fish, reducing the probability that an adult would encounter a juvenile.

V. CONCLUSIONS

The overall effect of adding phenols to the ponds was to reduce Gambusia populations to levels well below those in the reference ponds (Figure 8). The reductions occurred quickly indicating the concentrations of phenols administered were acutely toxic to the fish. The high degree of phenotypic plasticity in reproduction noted for Gambusia allowed individuals that survived the acute exposure to make the best of the situation at a time of the year when other populations were curtailing reproduction. The costs of producing a late autumn brood, were apparently low relative to cost of forgoing reproduction until the spring. Young Gambusia produced in late autumn in the dosed ponds survived the winter and the adults in these ponds, although not having lipid stores as high as Gambusia in the reference ponds overwintered in better condition. Furthermore the reproductive allotments of Gambusia in the dosed ponds were just as large as those of Gambusia in the reference ponds the following spring (Figure 1). Thus the phenols did not damage the long-term reproductive output of the fish.

These results indicate that Gambusia populations inherently have a high degree of resiliency, perhaps because these fish are often found in what appear to be very unpredictable environments. Although they exhibit a growth and reproductive cycle based on the predictable cycle of the seasons, they are evolutionarily equipped to deal with unpredictability in the environment (Stearns 1983). The dosing regime of phenols used in this study resulted in a large fish kill and no lingering, secondary effects: this is probably analogous to a flood, or drying of a habitat, to which the fish respond similarly.

In using results such as these to assess possible impacts of disturbance on the fish populations, and their subsequent recovery, it should be noted that my observations are likely to apply only to species that have life-histories similar to those of <u>Gambusia</u>. The results may pertain to an organism that can produce several generations per year and that is very opportunistic in its reproductive behavior. For fish species having evolved in more predictable habitats, disturbances are likely to have very different effects on population recovery.

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APPENDIX

ORNL-DWG 90-13120

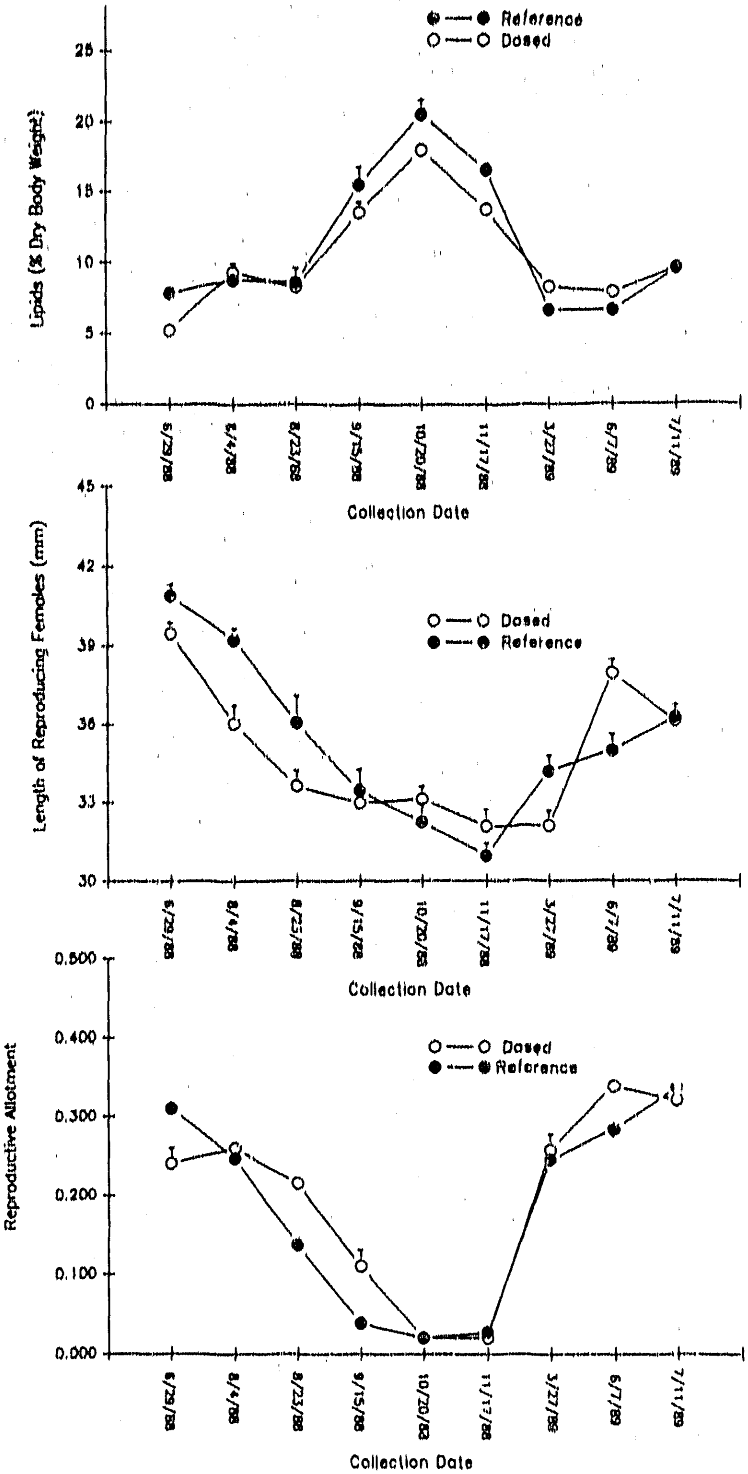


Figure 1. Seasonal comparisons between dosed and reference ponds. a. Lipid content; b. Length of reproducing fish; c. Reproductive allotment: Vertical lines show one standard error of the mean.

ORNL-DWG 90-13121

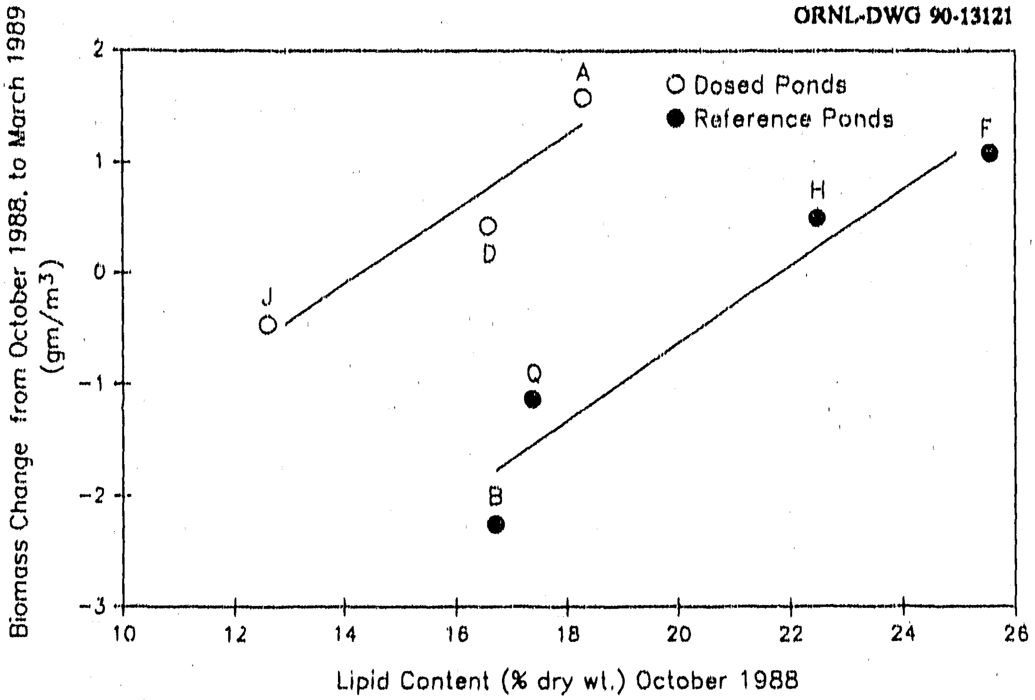


Figure 2. Loss of population biomass from October 1988 through March 1989, in relation to lipid content of fish in October 1988, for dosed and reference ponds

ORNL-DWG 90-13122

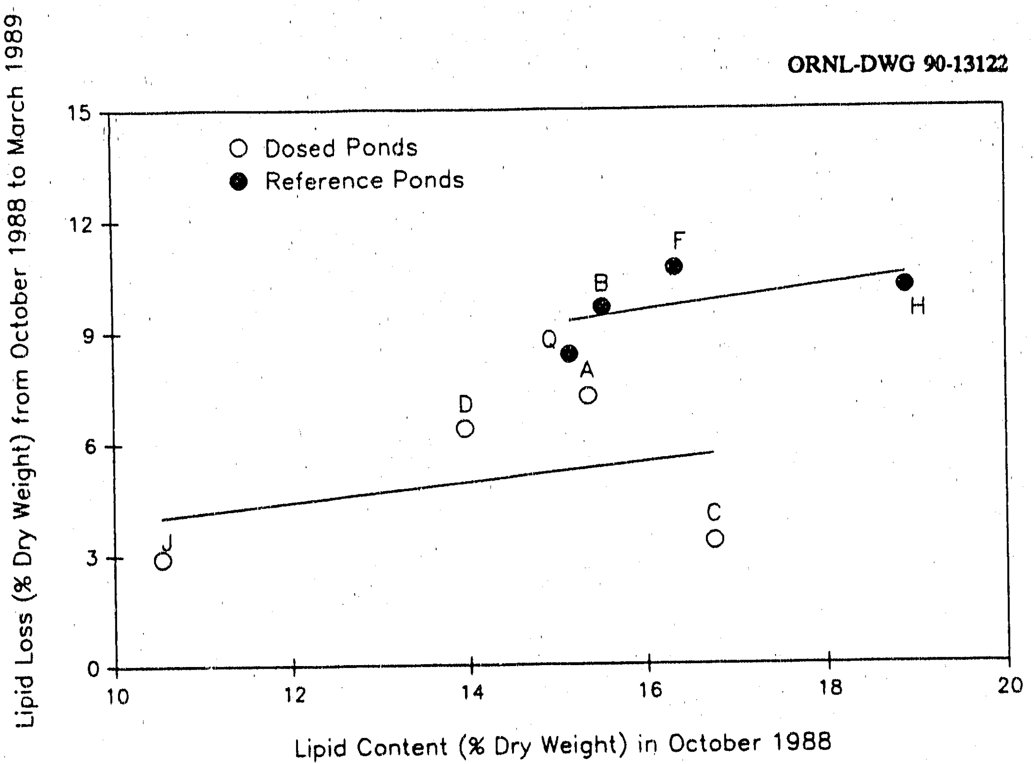


Figure 3. Percent loss of lipids for fish between October 1988 and March 1989 in relation to lipid content in October 1988.

ORNL-DWG 90-13123

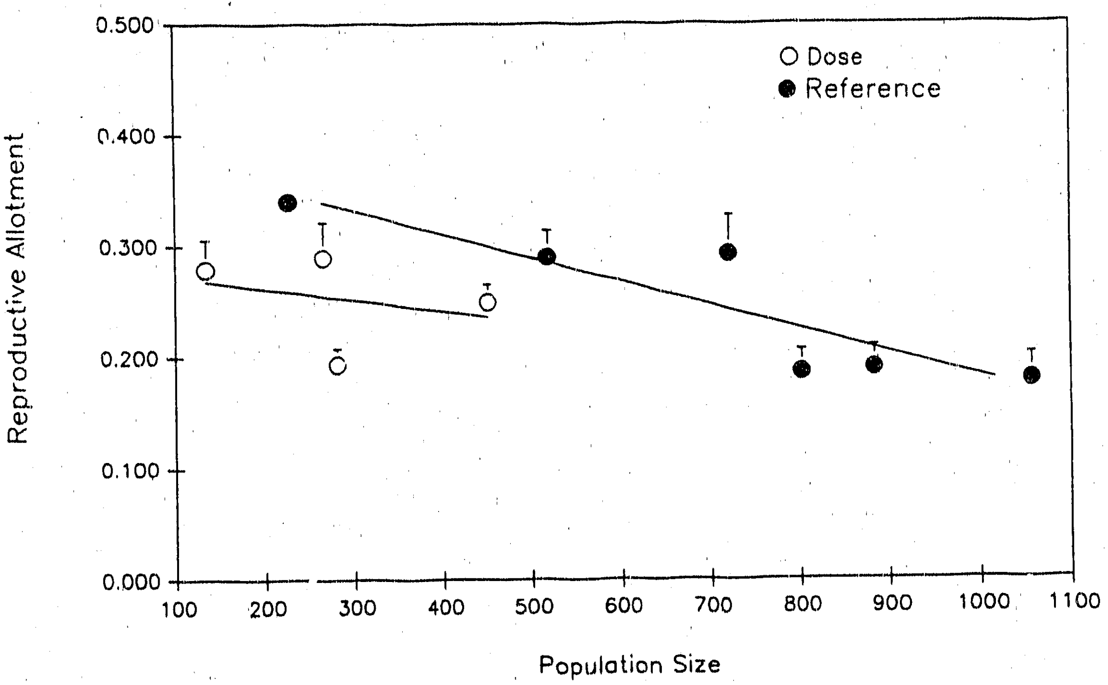


Figure 4. Comparison between dosed and reference ponds, in an analysis of reproductive allotment for fish in relation to the number of fish in ponds on August 23, 1988.

ORNL-DWG 90-13124

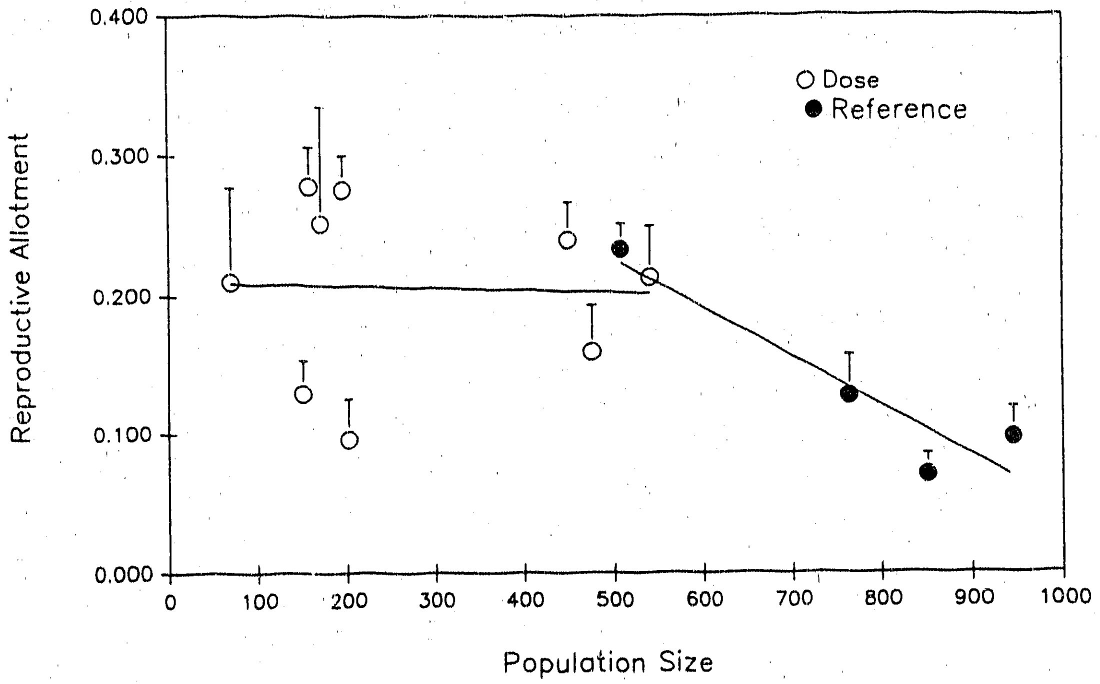


Figure 5. Comparison between dosed and reference ponds, in an analysis of reproductive allotment for fish in relation to the number of fish in ponds on August 23, 1988.

ORNL-DWG 90-13125

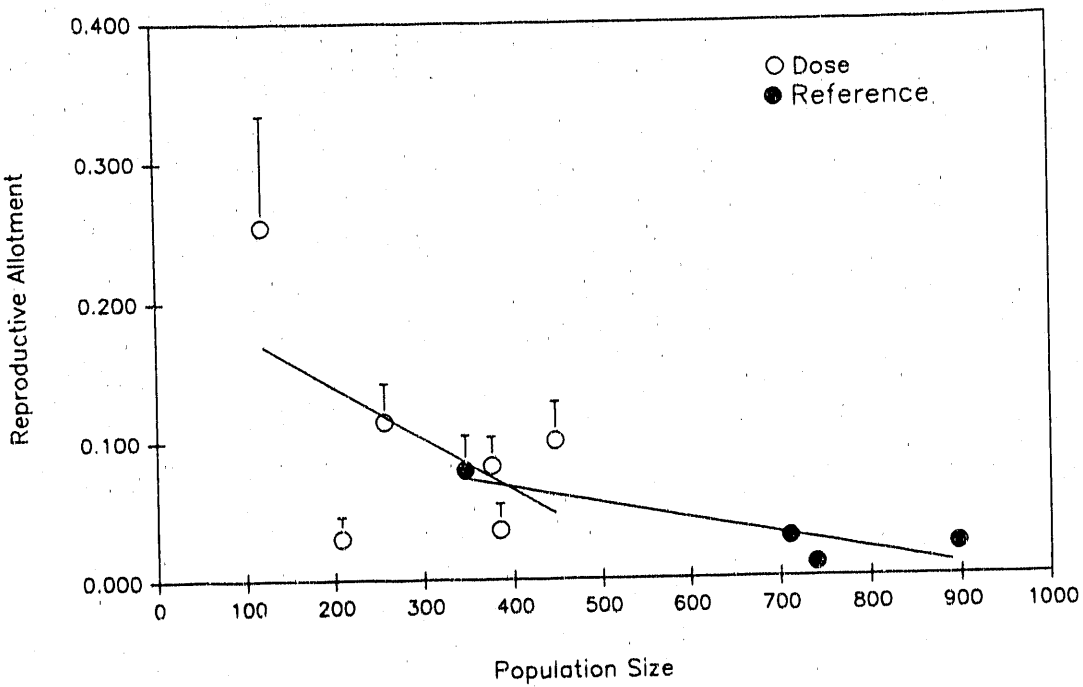


Figure 6. Comparison between dosed and reference ponds, in an analysis of reproductive allotment for fish in relation to the number of fish in ponds on September 15, 1988.

ORNL-DWG 90-13126

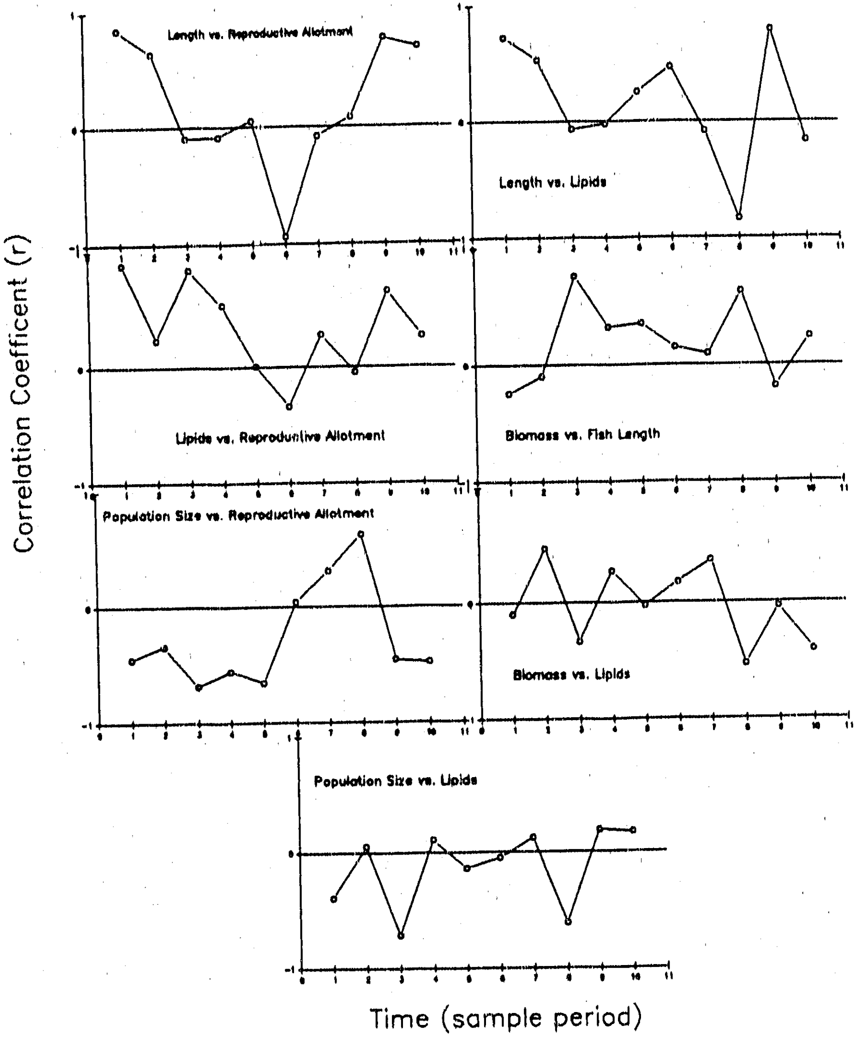


Figure 7. Seasonal trends in the strengths of the correlation coefficients between life history parameters.

ORNL-DWG 90-13127

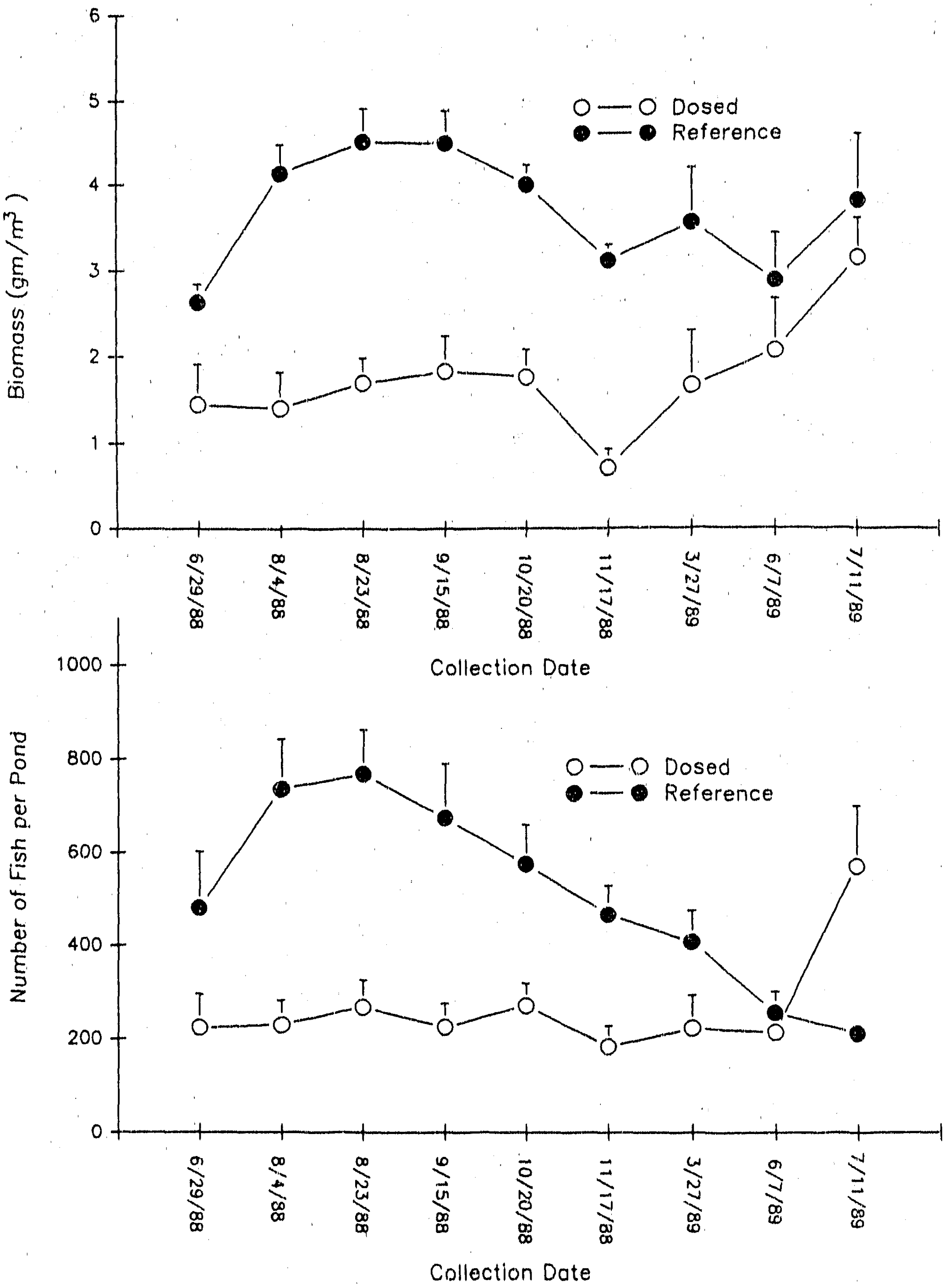


Figure 8. Seasonal comparison of the number of fish and biomass within dosed and reference ponds. Values are the means for each group. Vertical bars show one standard error of the mean.

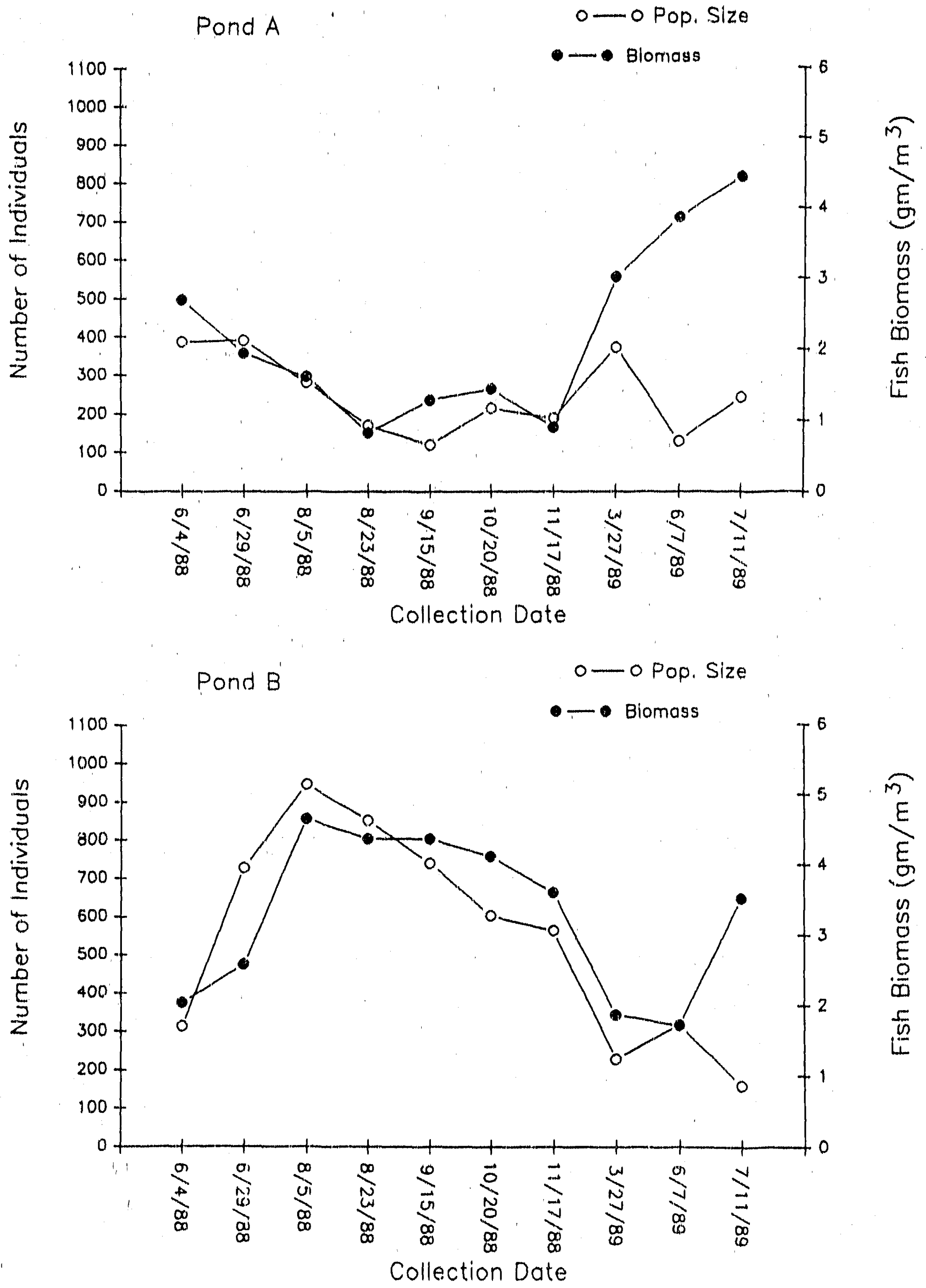
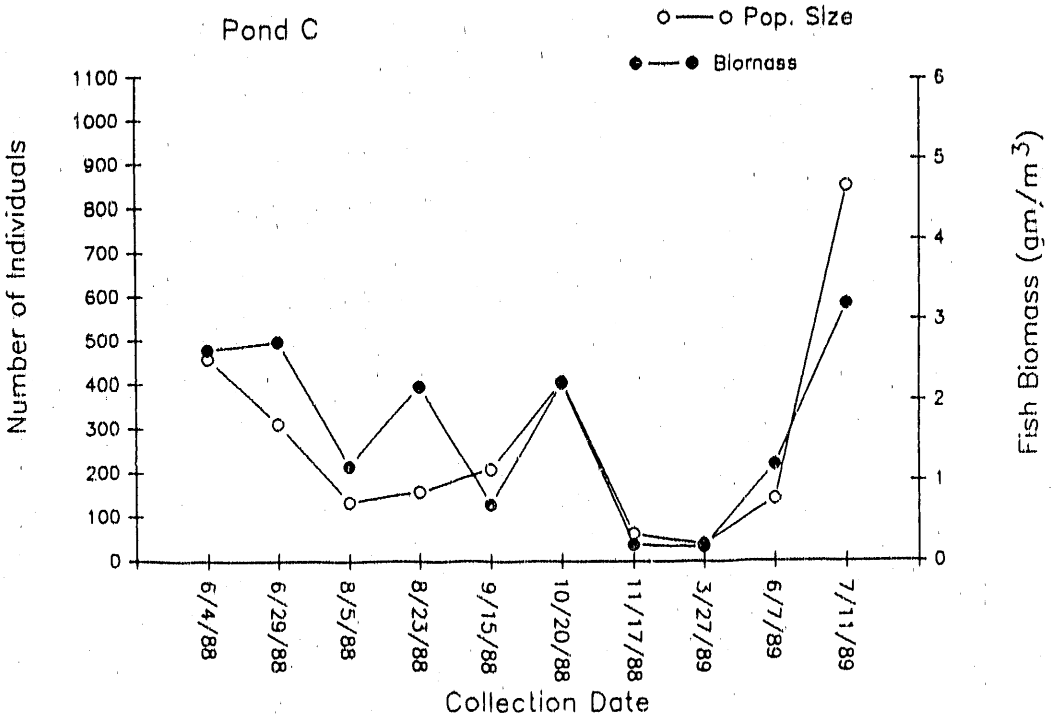


Figure 9. Changes in fish biomass and fish numbers through time for ponds A and B.



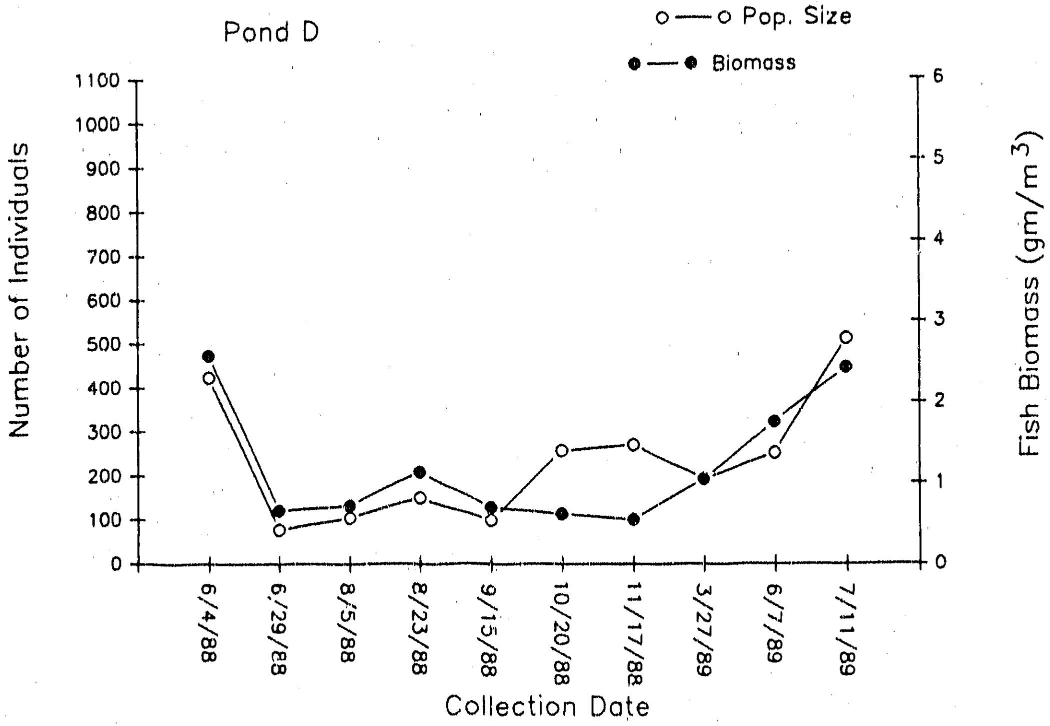


Figure 10. **Changes in fish biomass and fish numbers through time for ponds C and D.**

ORNL-DWG 90-13130

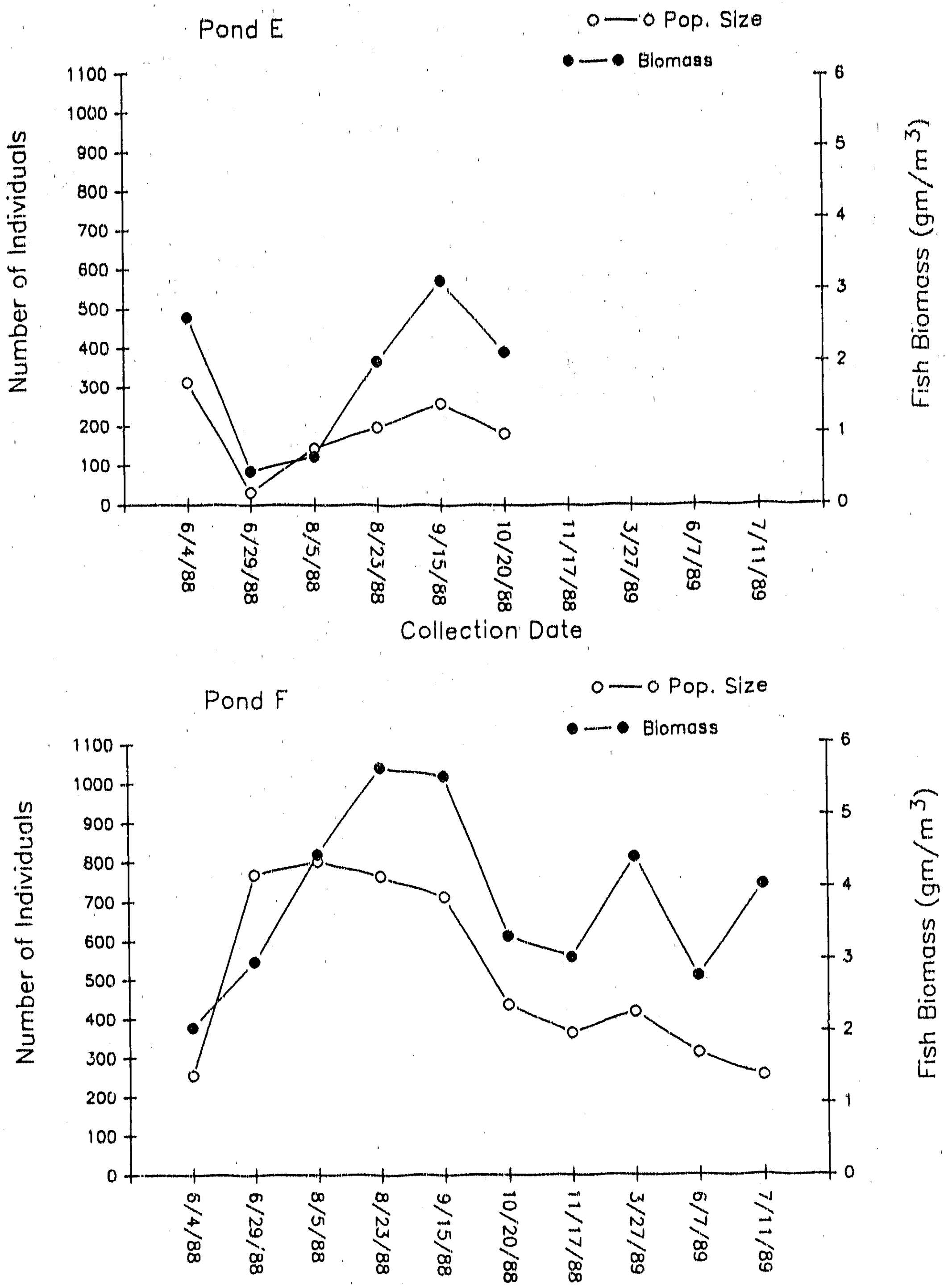


Figure 11. Changes in fish biomass and fish numbers through time for ponds E and F.

ORNL-DWG 90-13131

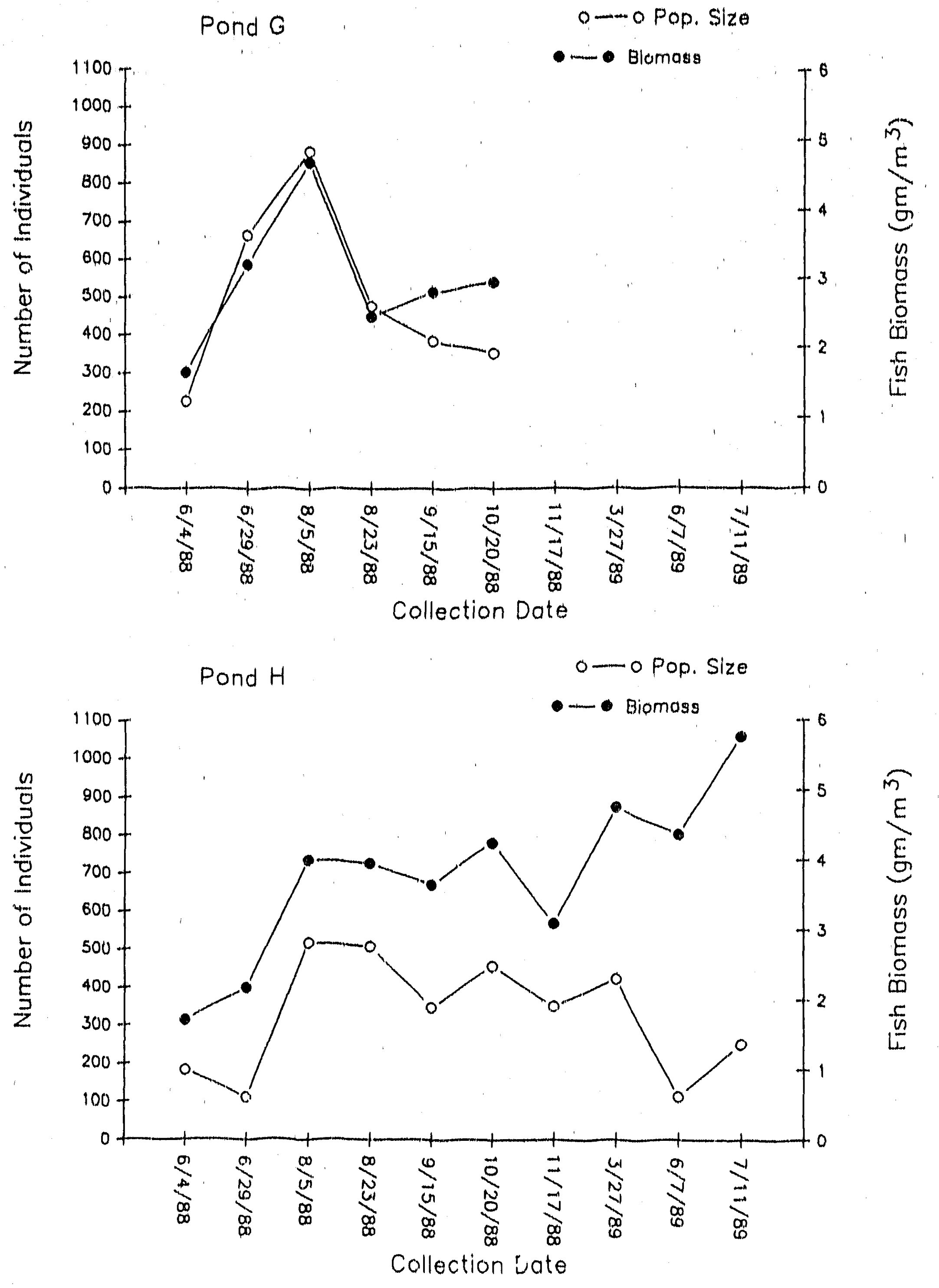
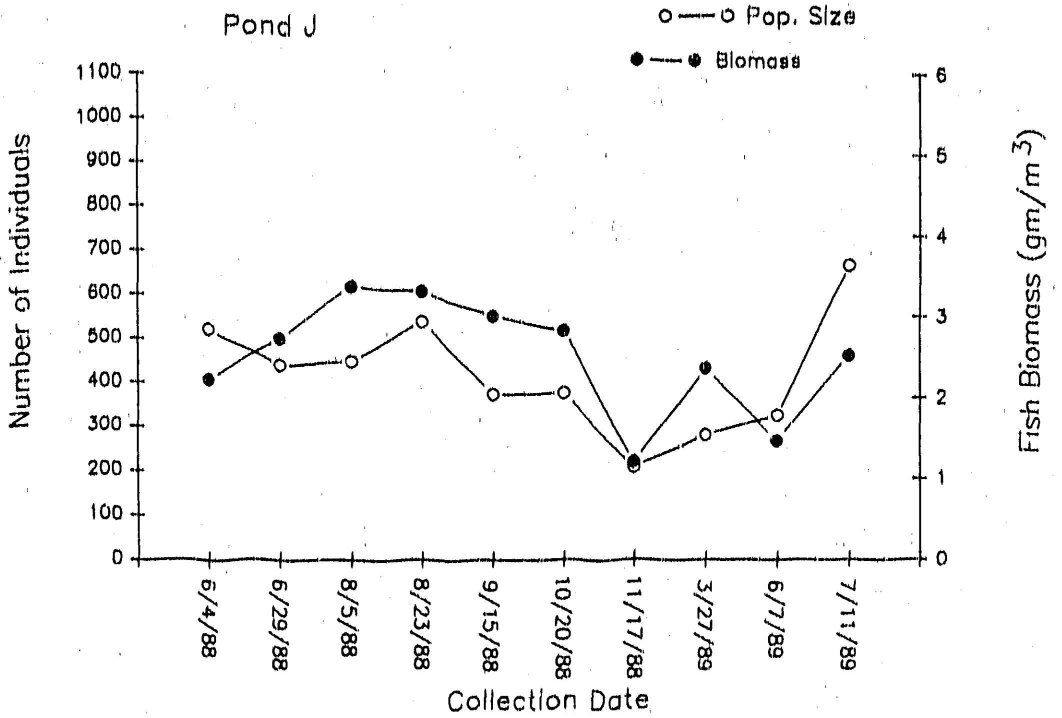


Figure 12. Changes in fish biomass and fish numbers through time for ponds G and H.

ORNL-DWG 90-13132



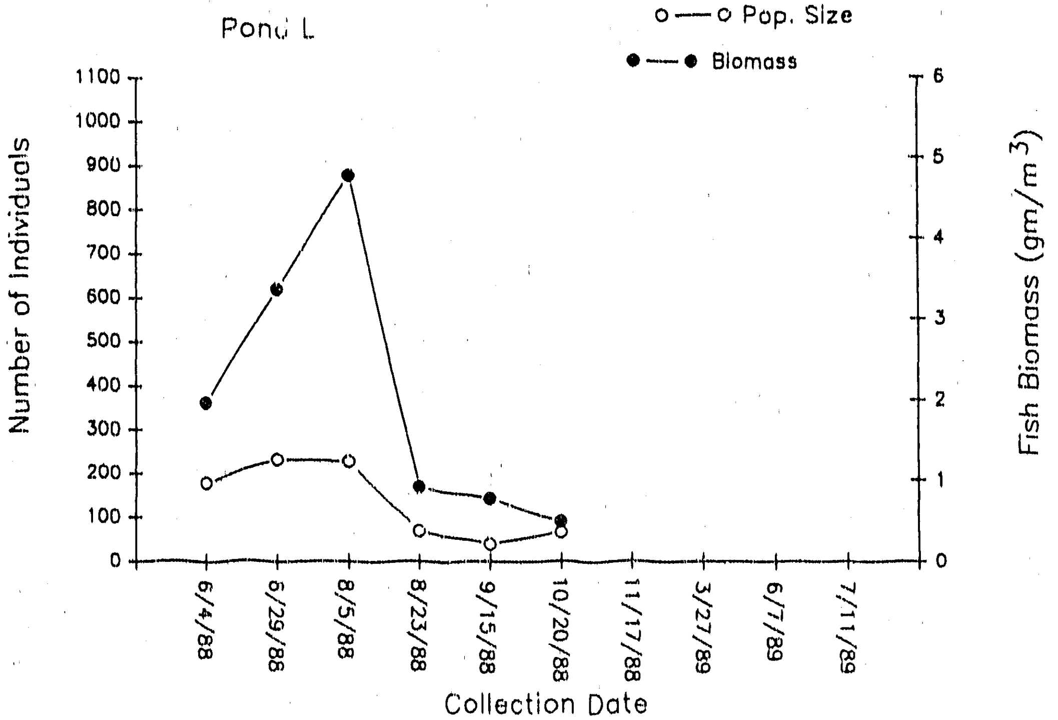


Figure 13. Changes in fish biomass and fish numbers through time for ponds J and L.

ORNL-DWG 90-13133

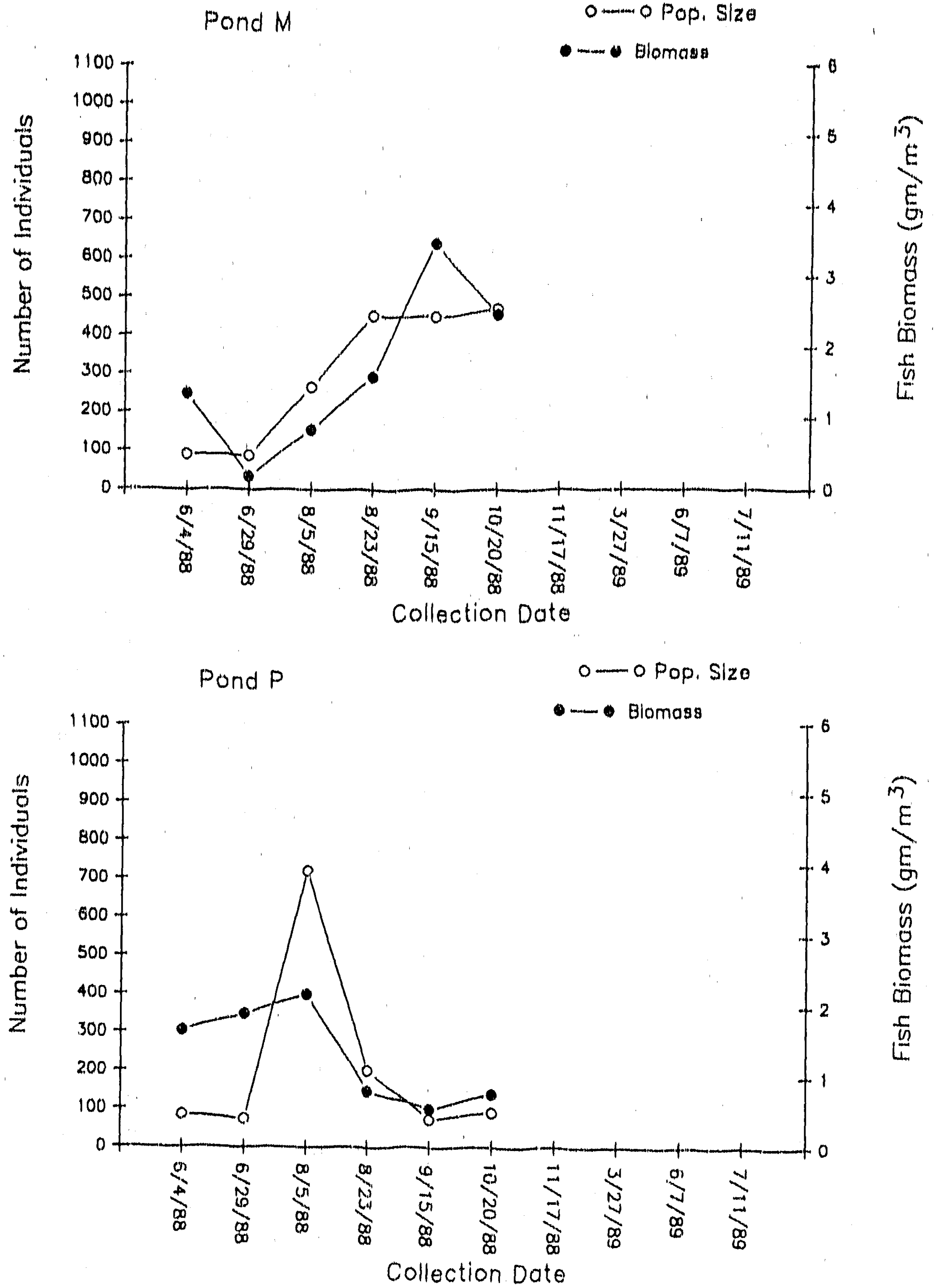


Figure 14. Changes in fish biomass and fish numbers through time for ponds M and P.

ORNL-DWG 90-13134

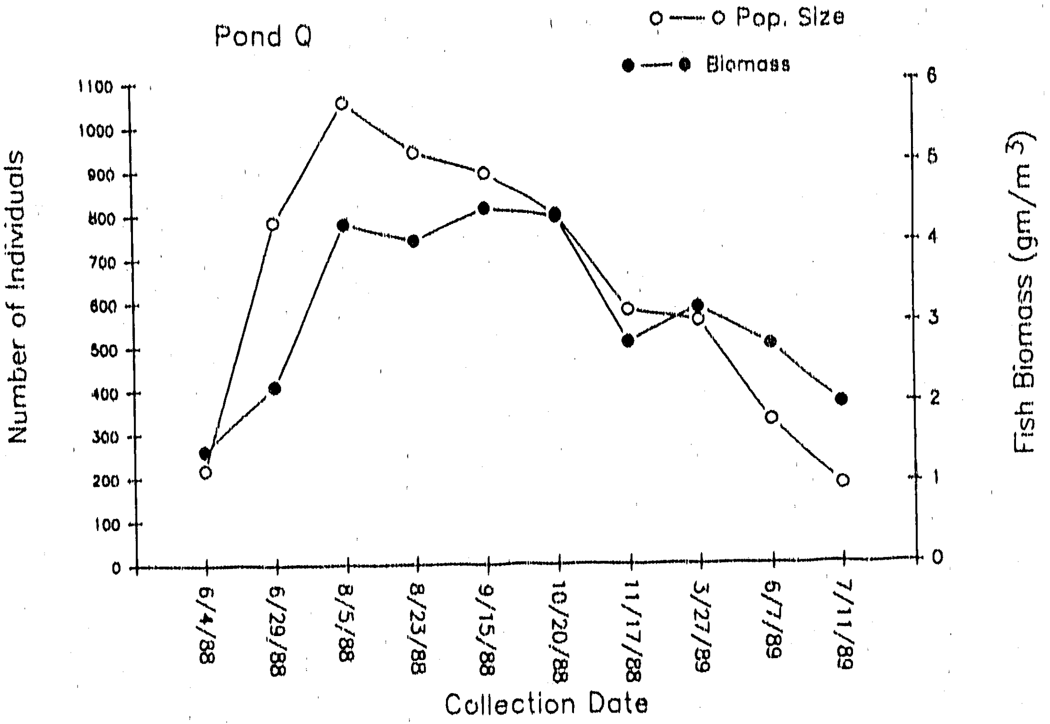


Figure 15. Changes in fish biomass and fish numbers through time for pond Q.

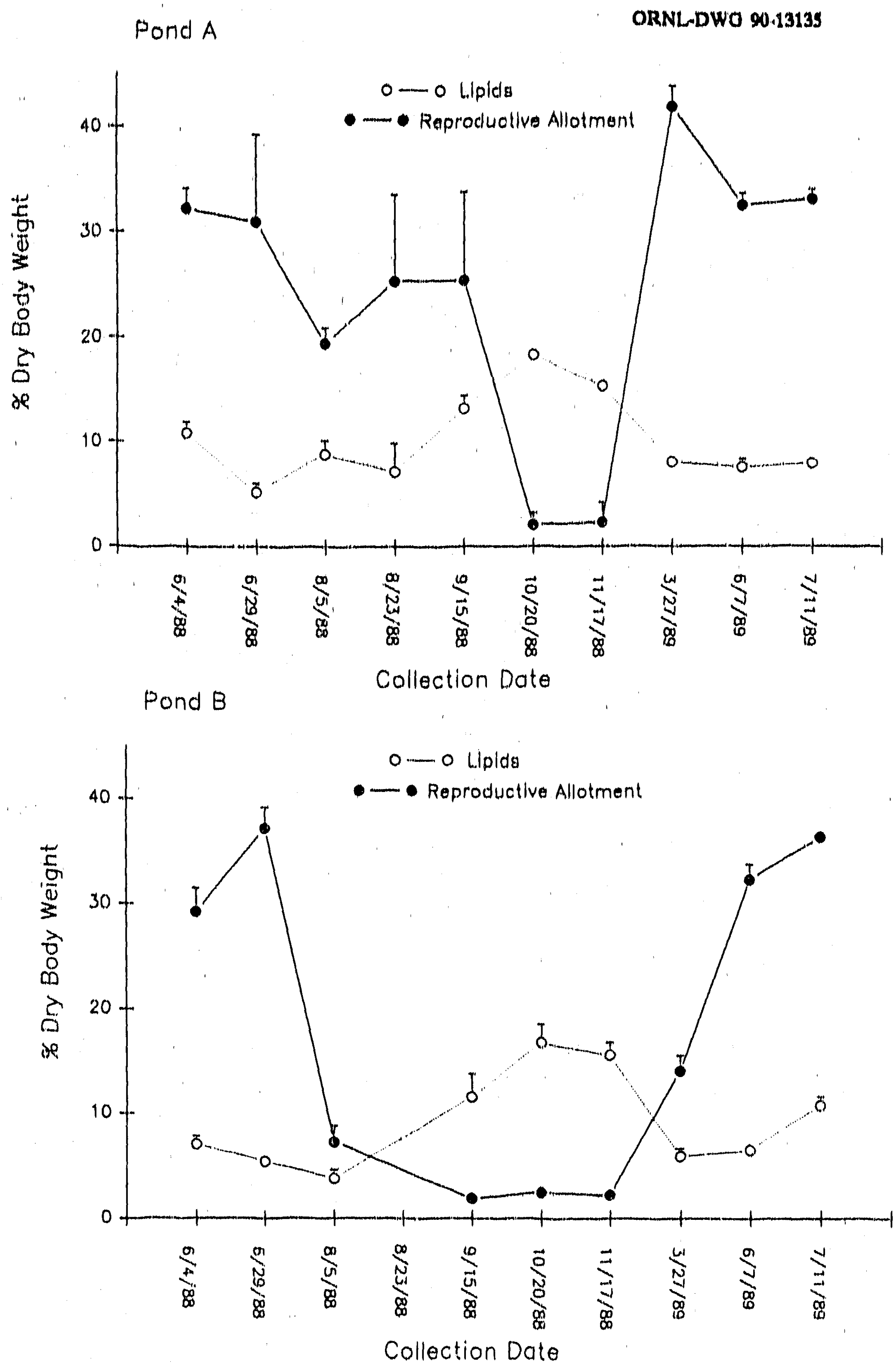


Figure 16. Seasonal pattern of reproductive allotment and lipid content in fish (expressed as a percentage of dry body weight) for ponds A and B.

ORNL-DWG 90-13136

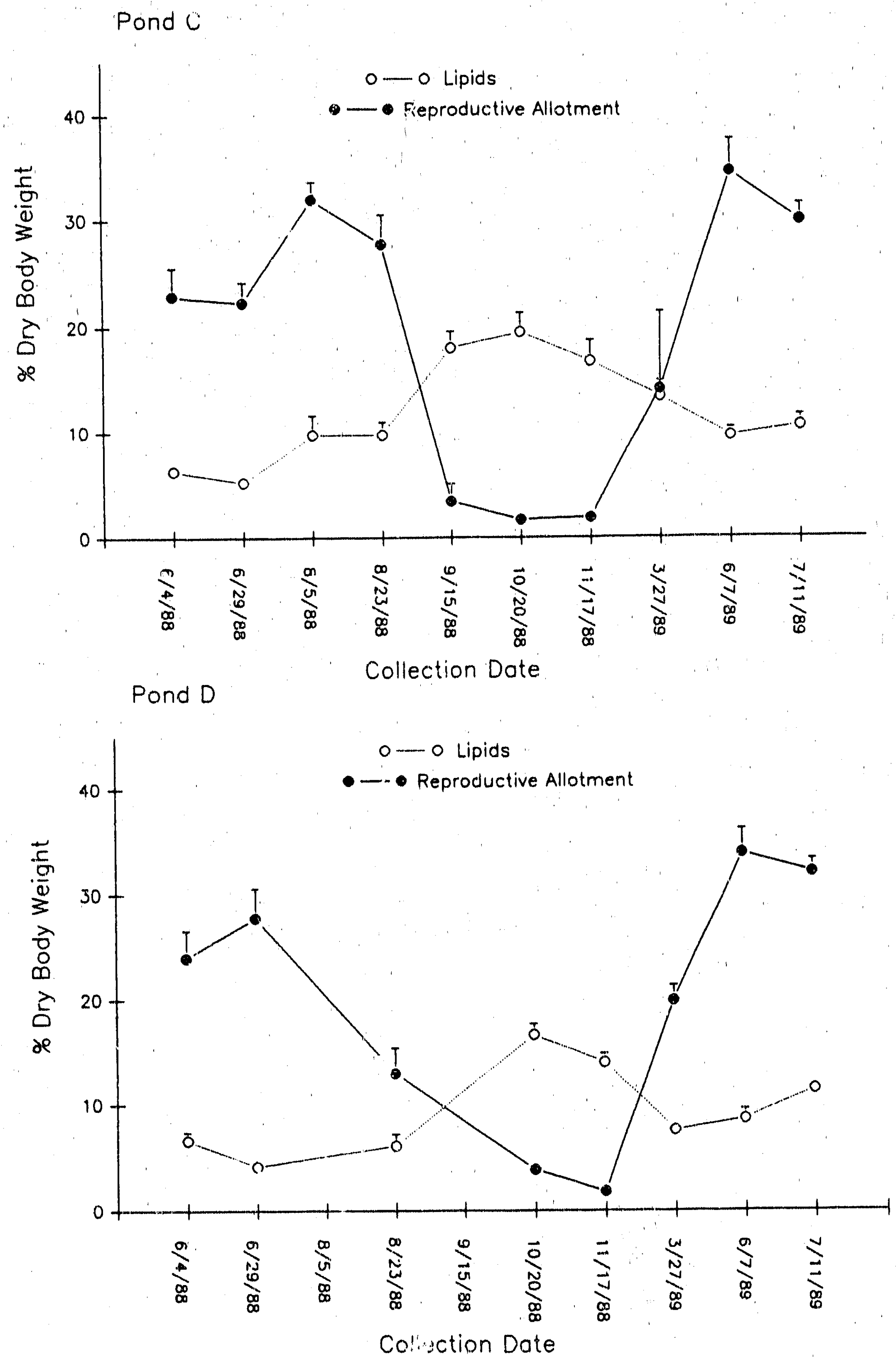
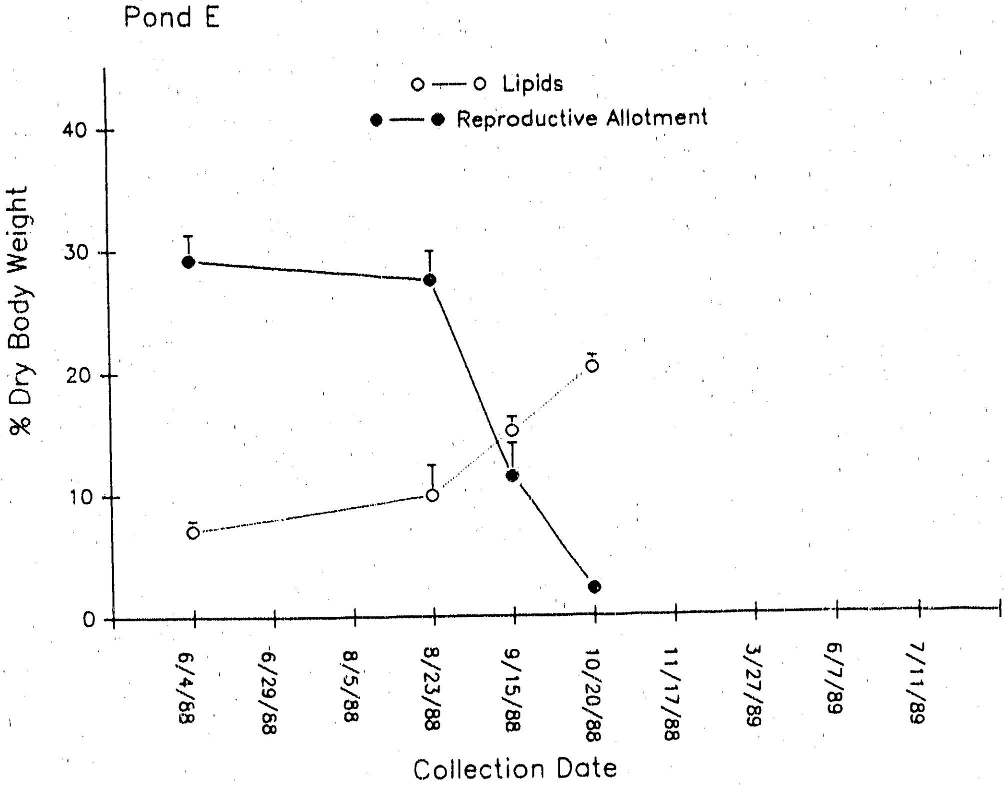


Figure 17. Seasonal pattern of reproductive allotment and lipid content in fish (expressed as a percentage of dry body weight) for ponds C and D.

ORNL-DWG 90-13137



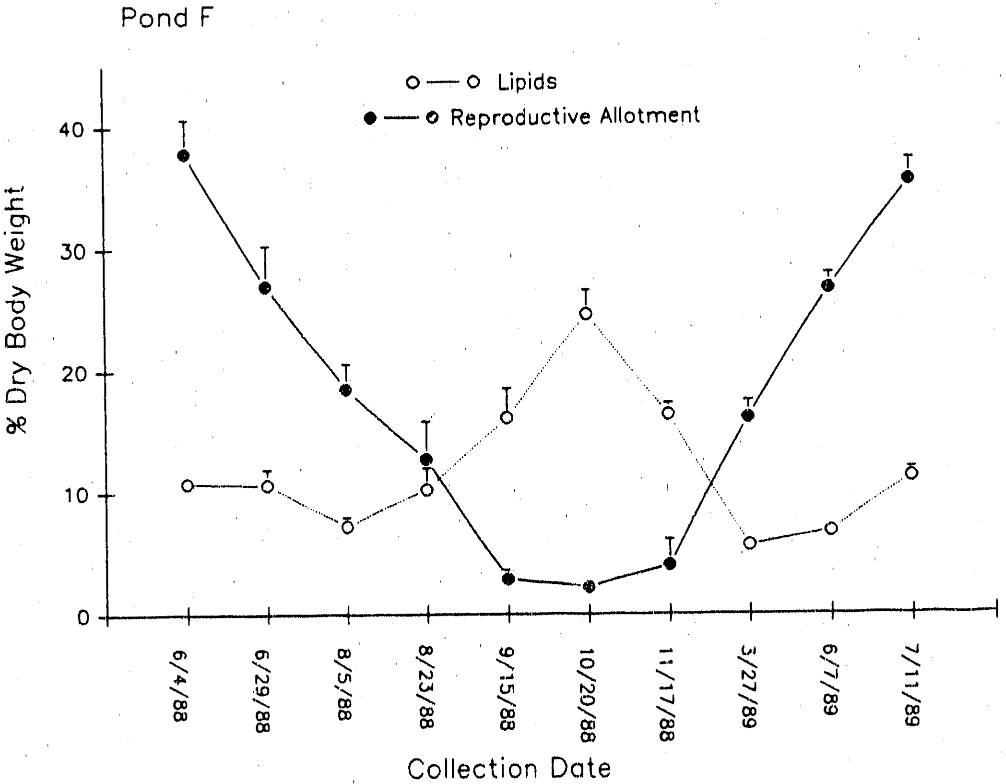


Figure 18. Seasonal pattern of reproductive allotment and lipid content in fish (expressed as a percentage of dry body weight) for ponds E and F.

ORNL-DWG 90-13138

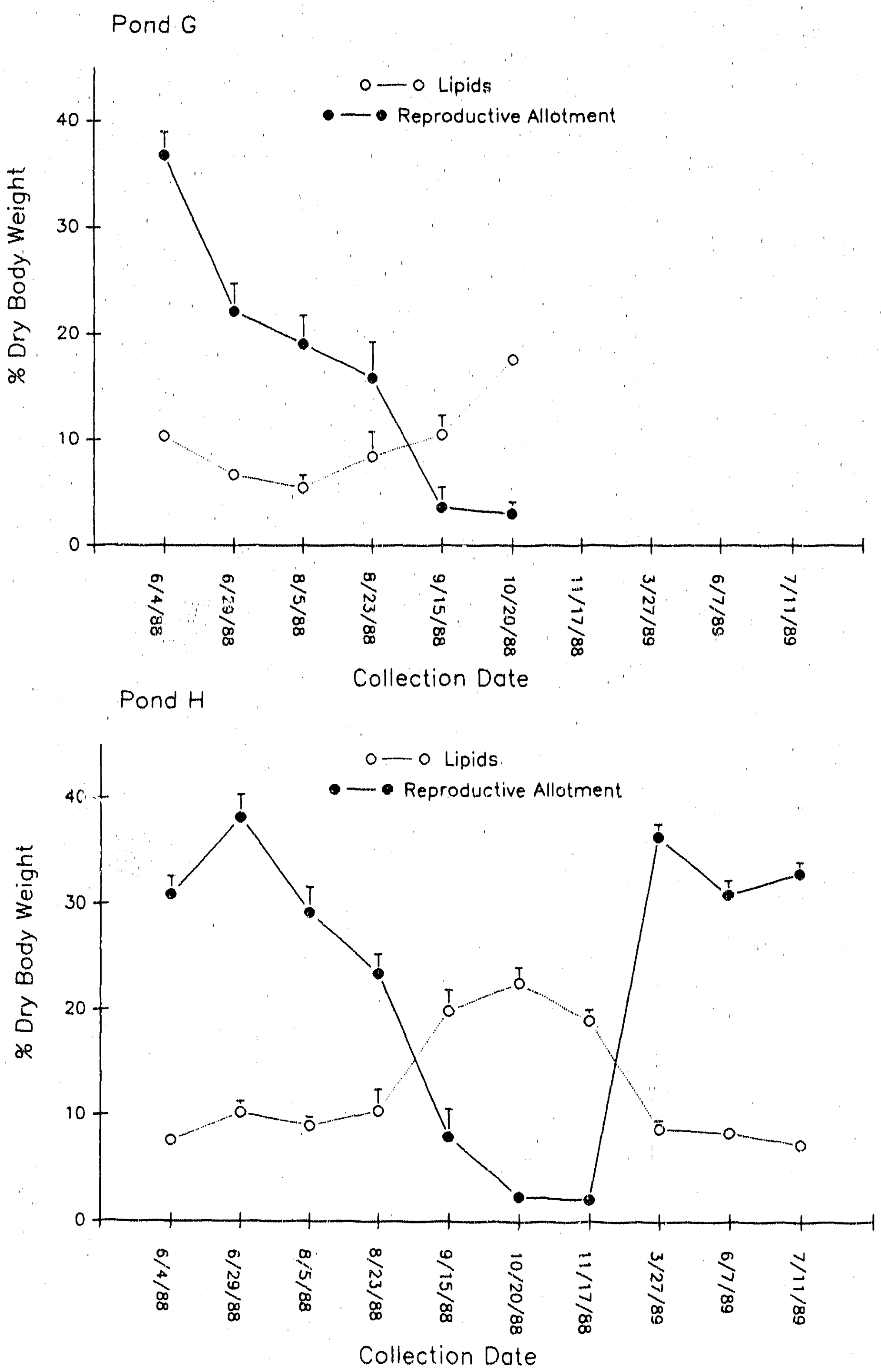


Figure 19. Seasonal pattern of reproductive allotment and lipid content in fish (expressed as a percentage of dry body weight) for ponds G and H.

ORNL-DWG 90-13139

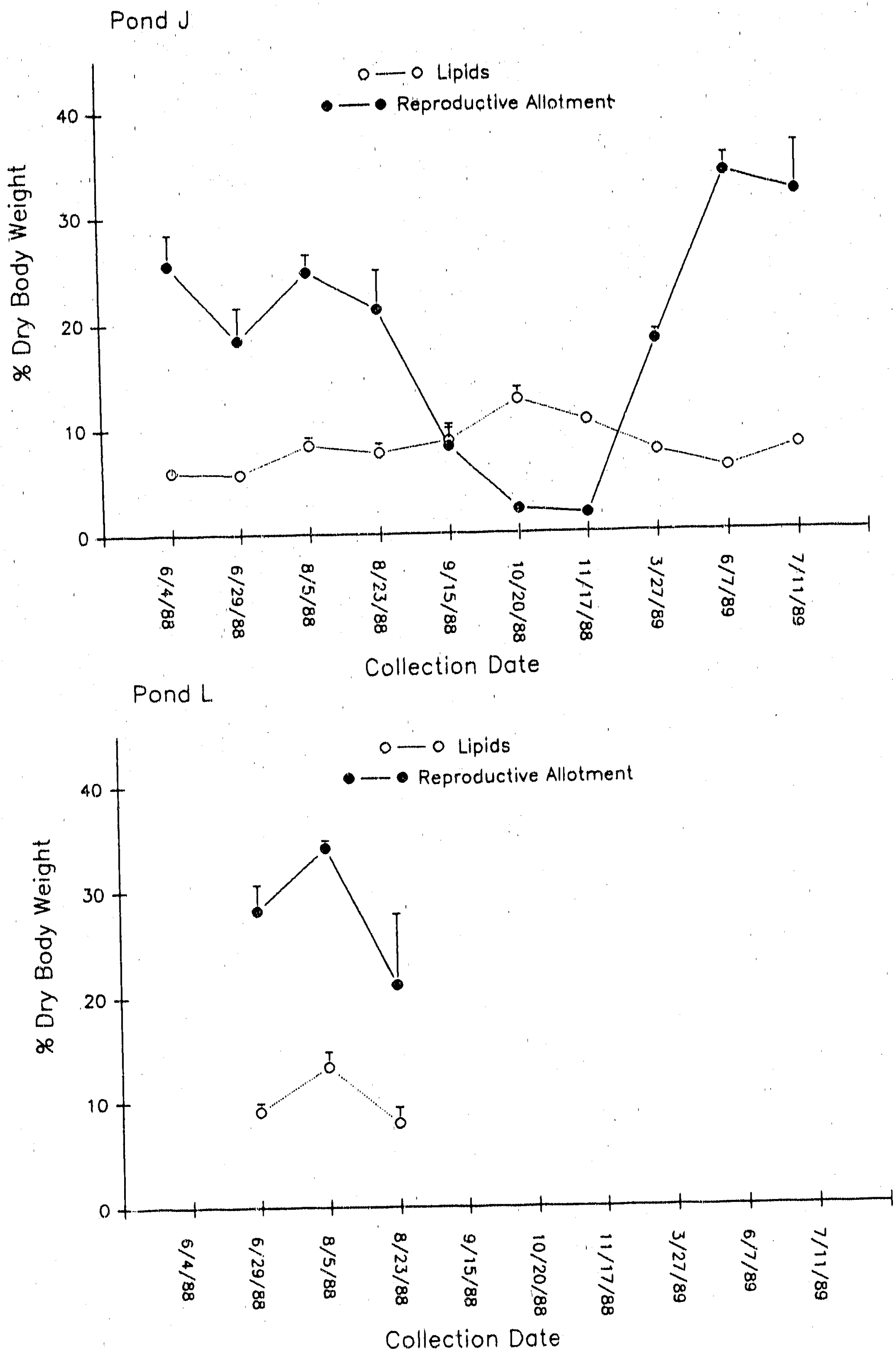
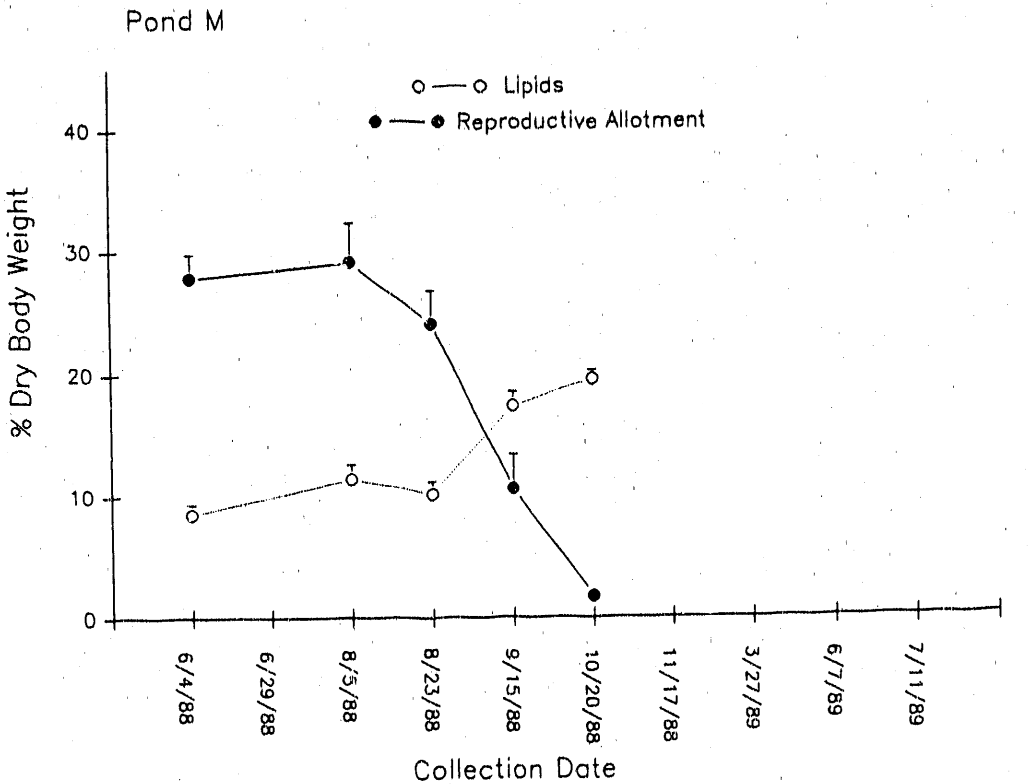


Figure 20. Seasonal pattern of reproductive allotment and lipid content in fish (expressed as a percentage of dry body weight) for ponds J and L.



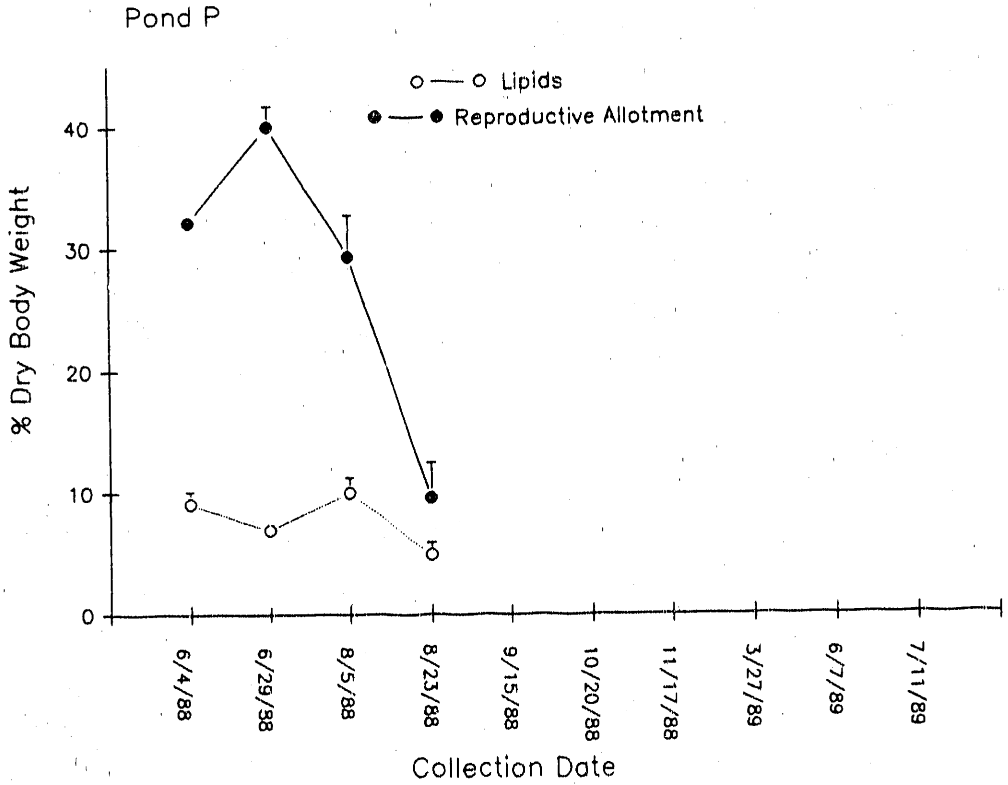


Figure 21. Seasonal pattern of reproductive allotment and lipid content in fish (expressed as a percentage of dry body weight) for ponds M and P.

ORNL-DWG 90-13141

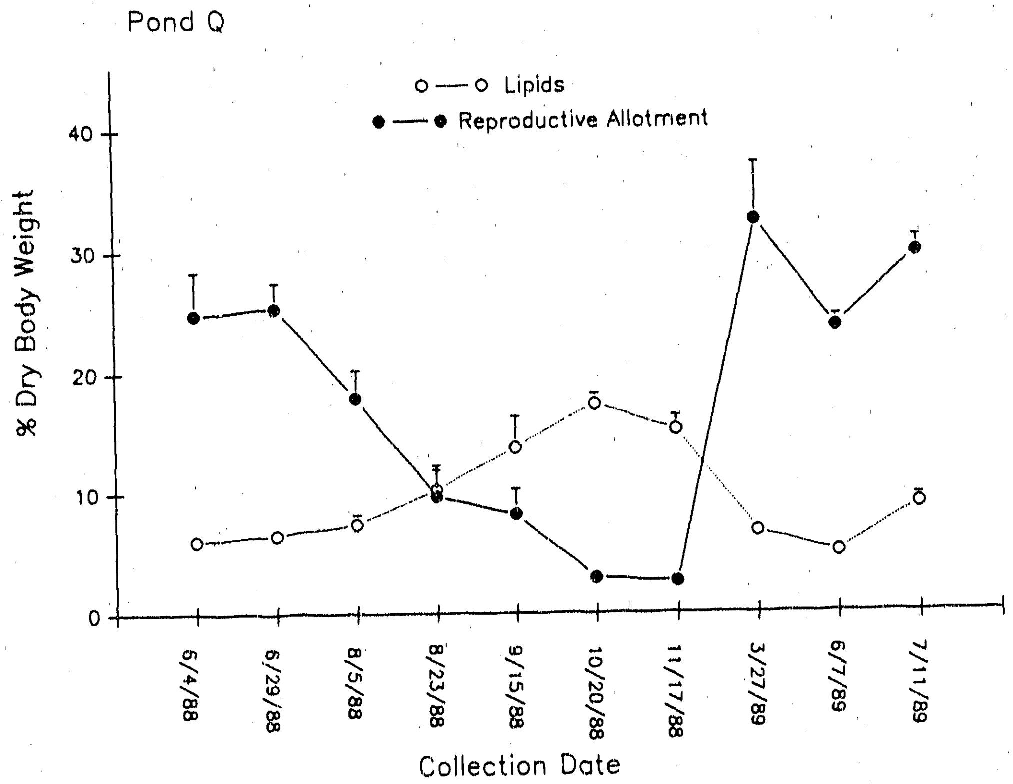


Figure 22. Seasonal pattern of reproductive allotment and lipid content in fish (expressed as a percentage of dry body weight) for pond Q.

ORNL-DWG 90-13142

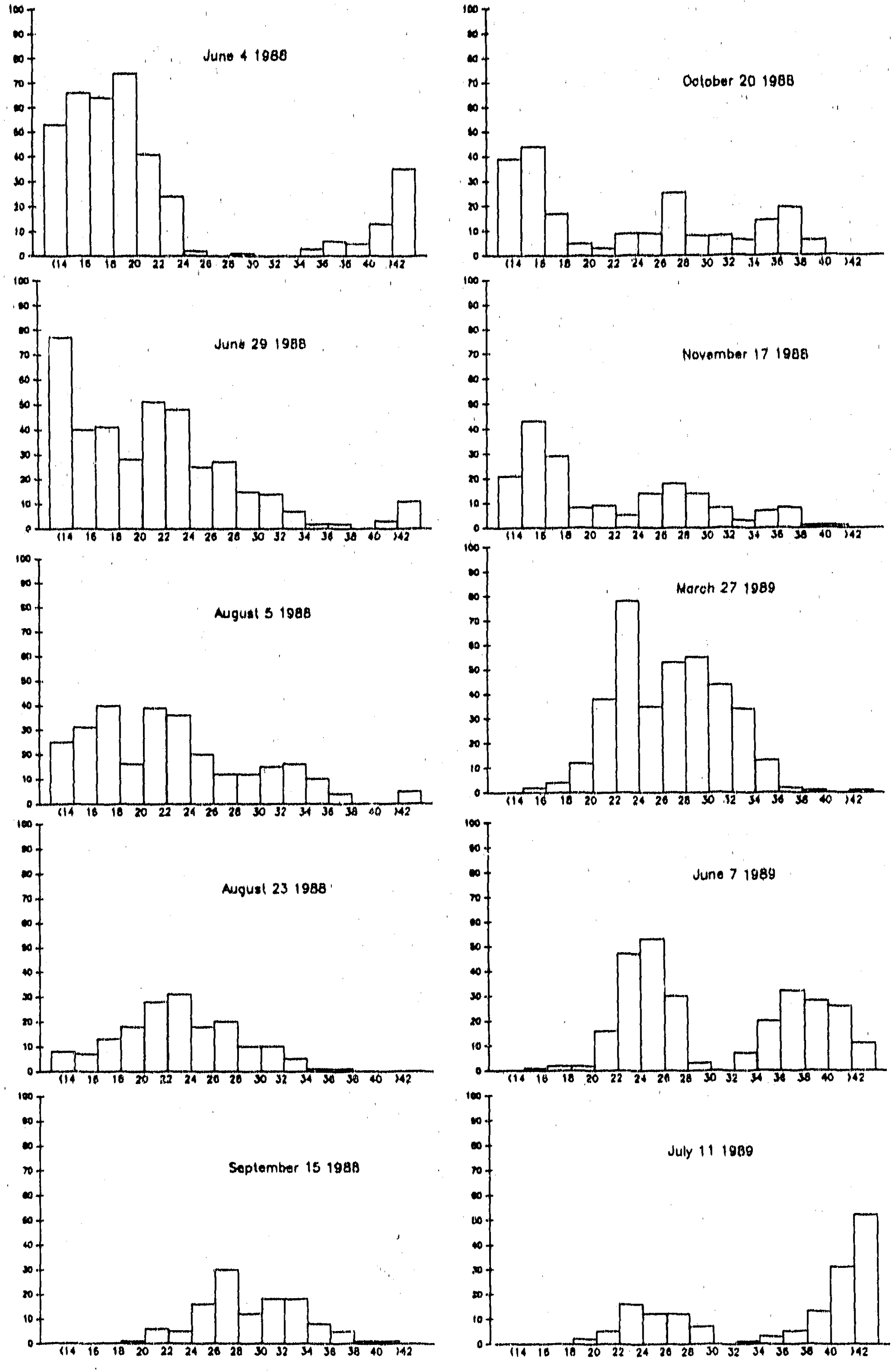


Figure 23. Size frequency histogram showing the seasonal pattern of population development in pond A.

ORNL-DWG 90-13143

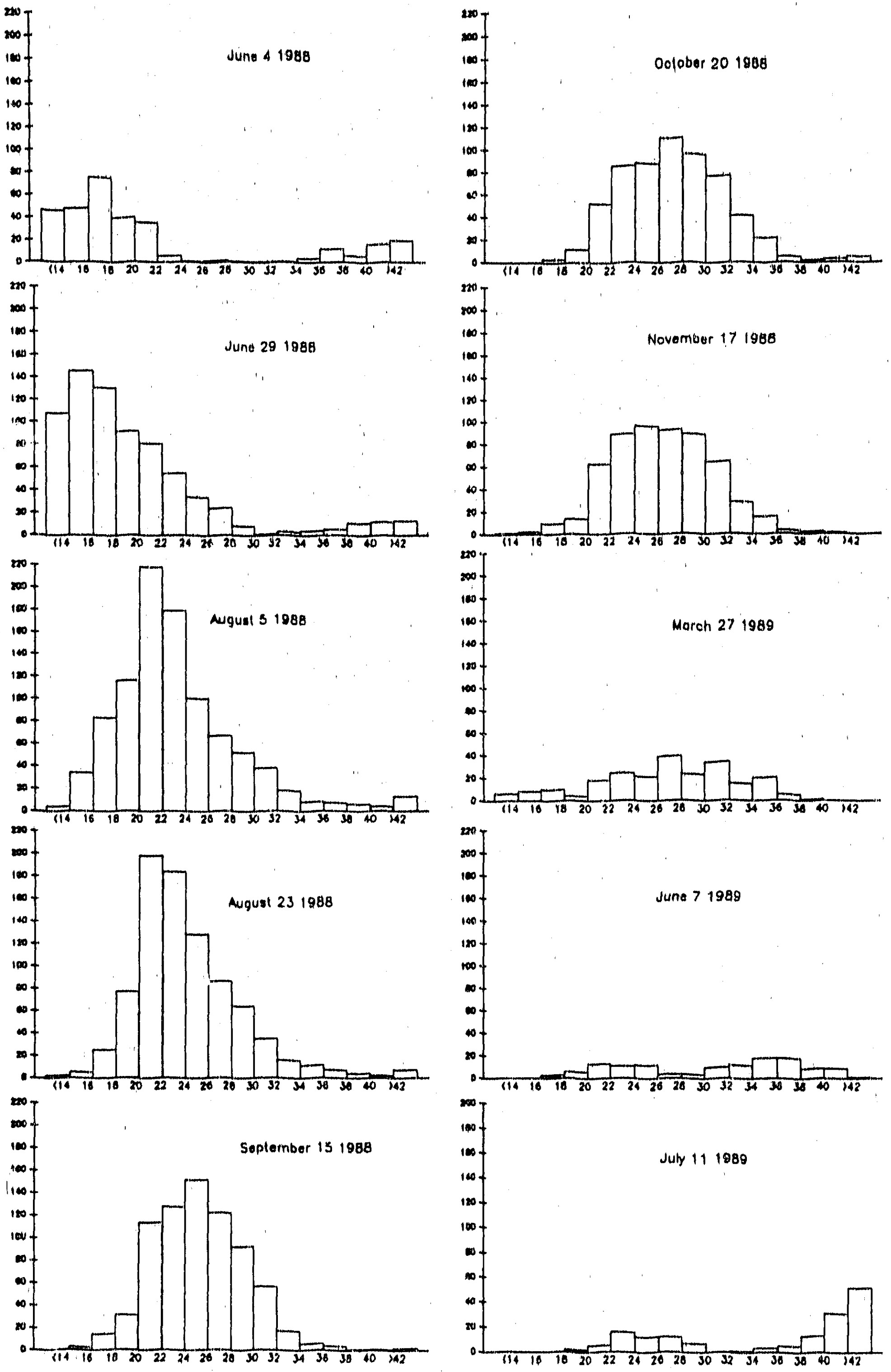


Figure 24. Size frequency histogram showing the seasonal pattern of population development in pond B.

ORNL-DWG 90-13144

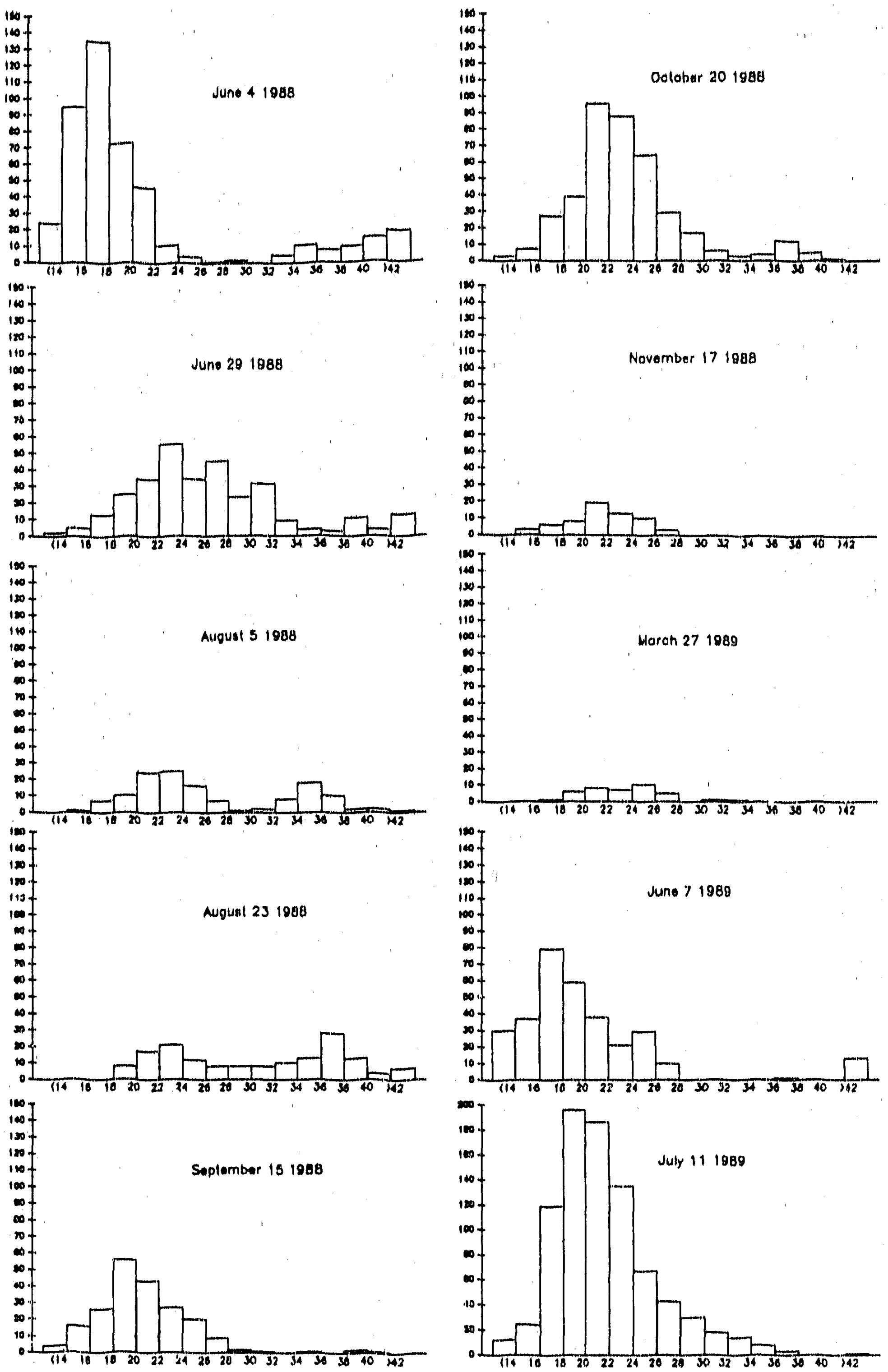


Figure 25. Size frequency histogram showing the seasonal pattern of population development in pond C.

ORNL-DWG 90-13145

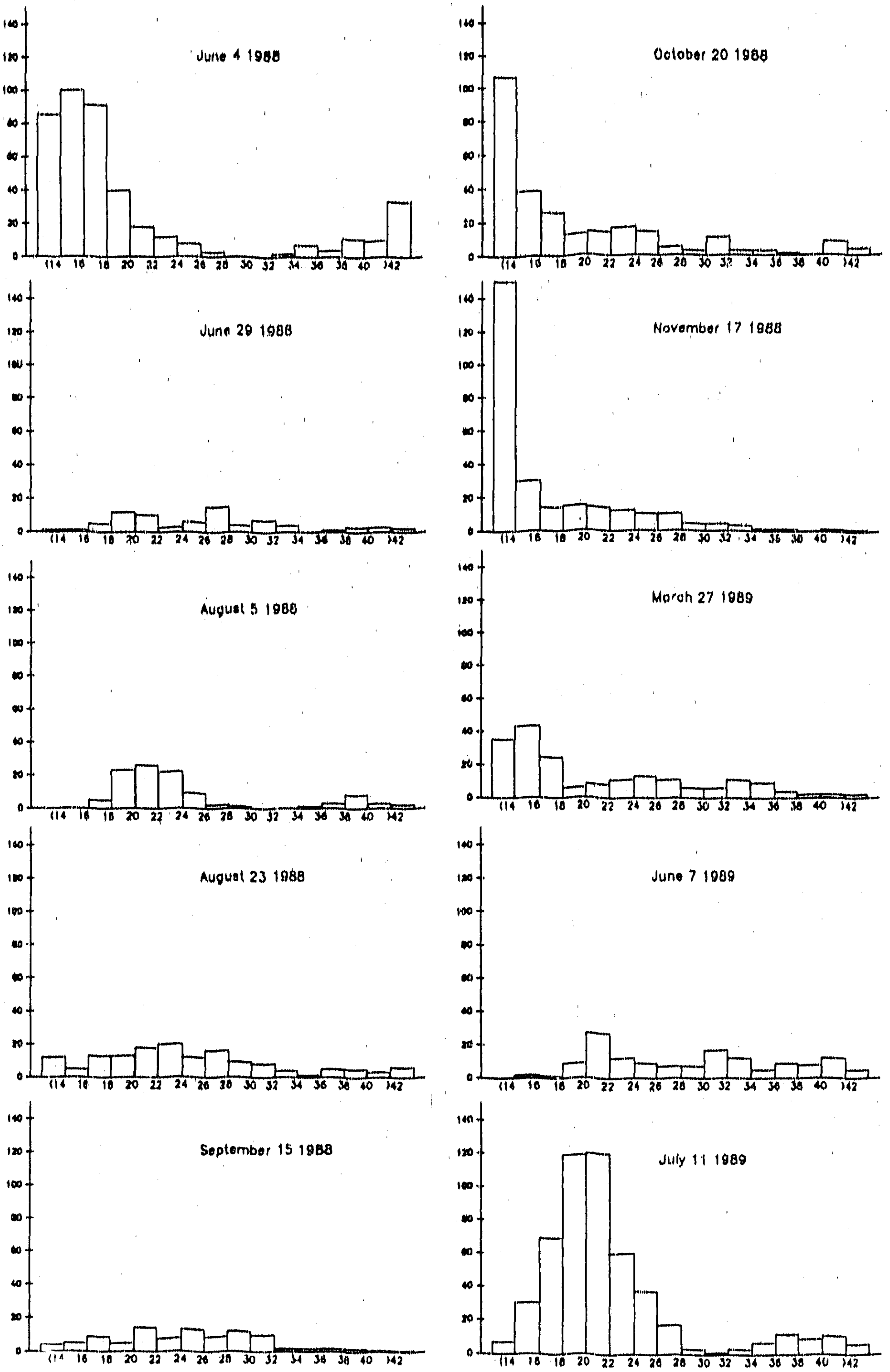


Figure 26. Size frequency histogram showing the seasonal pattern of population development in pond D.

ORNL-DWG 90-13146

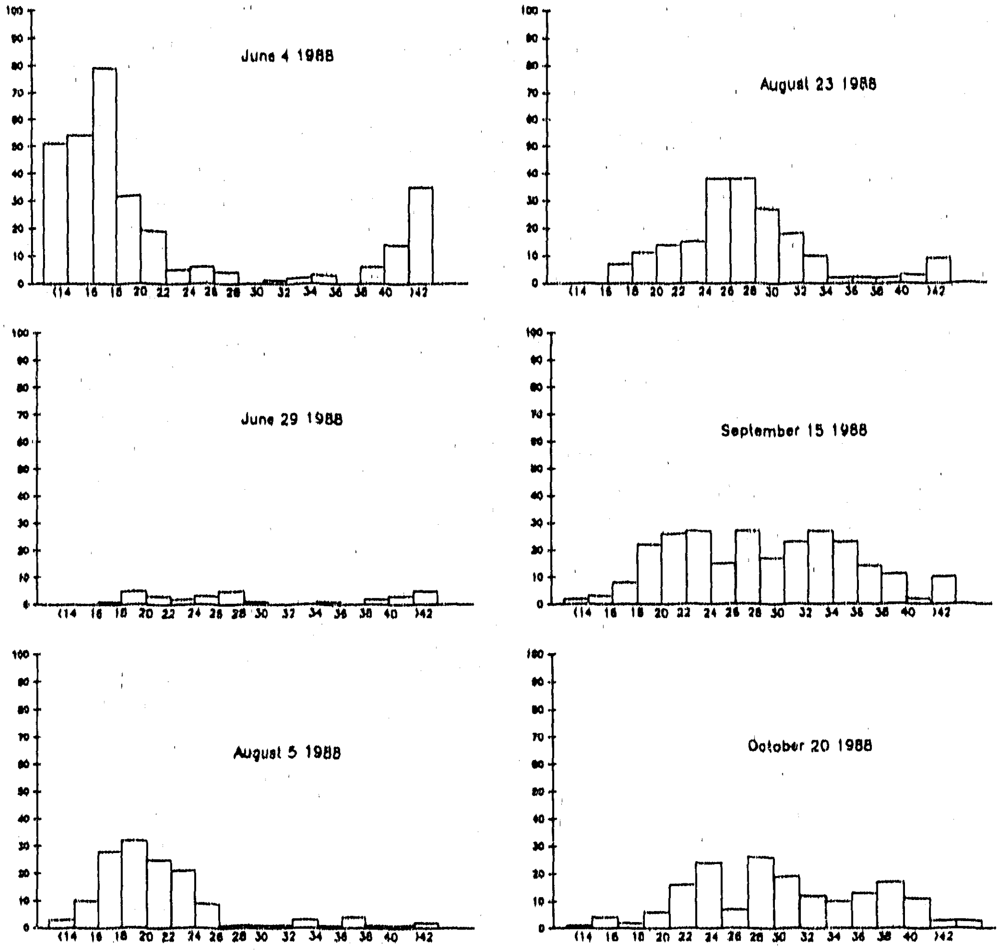


Figure 27. Size frequency histogram showing the seasonal pattern of population development in pond E.

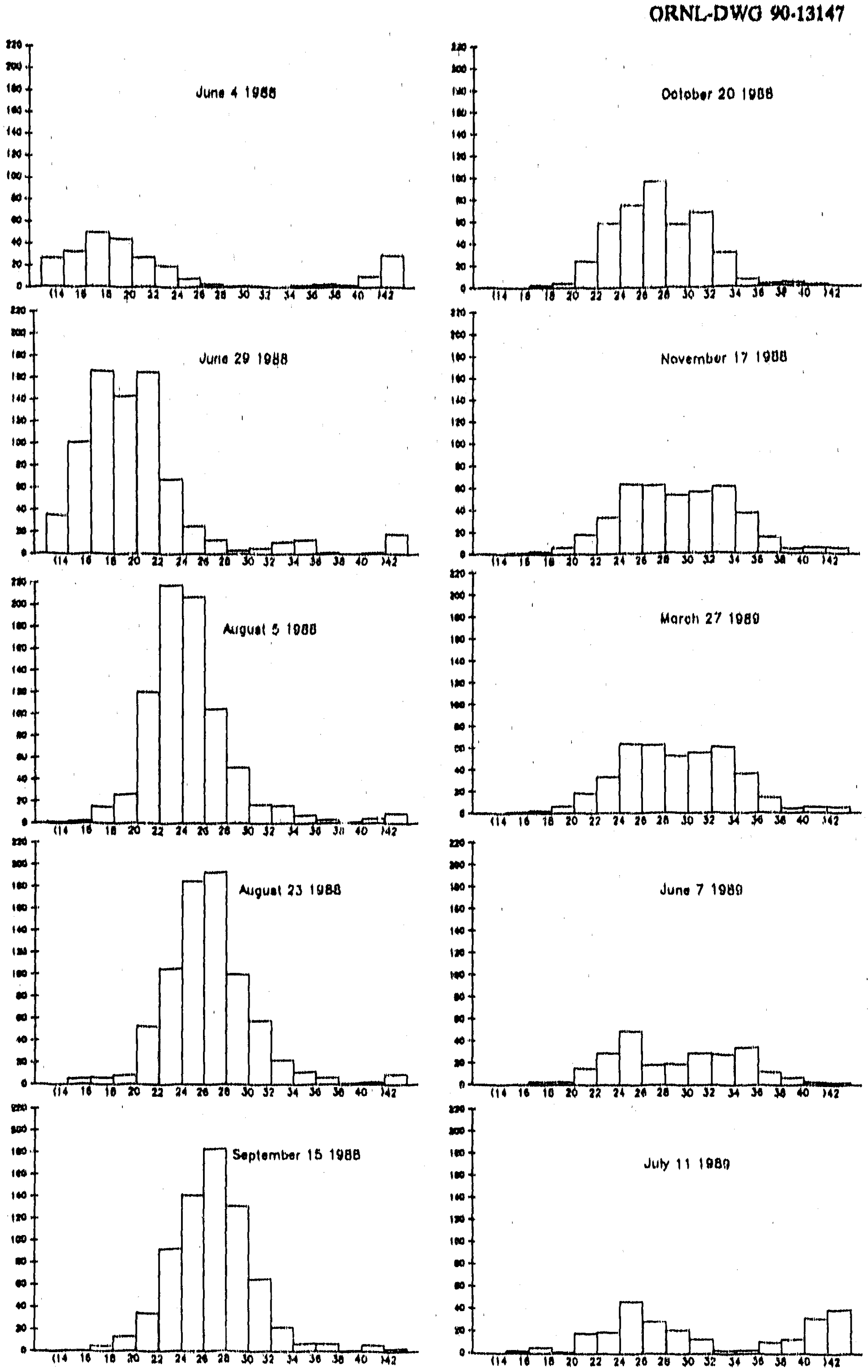


Figure 28. Size frequency histogram showing the seasonal pattern of population development in pond F.

ORNL-DWG 90-13148

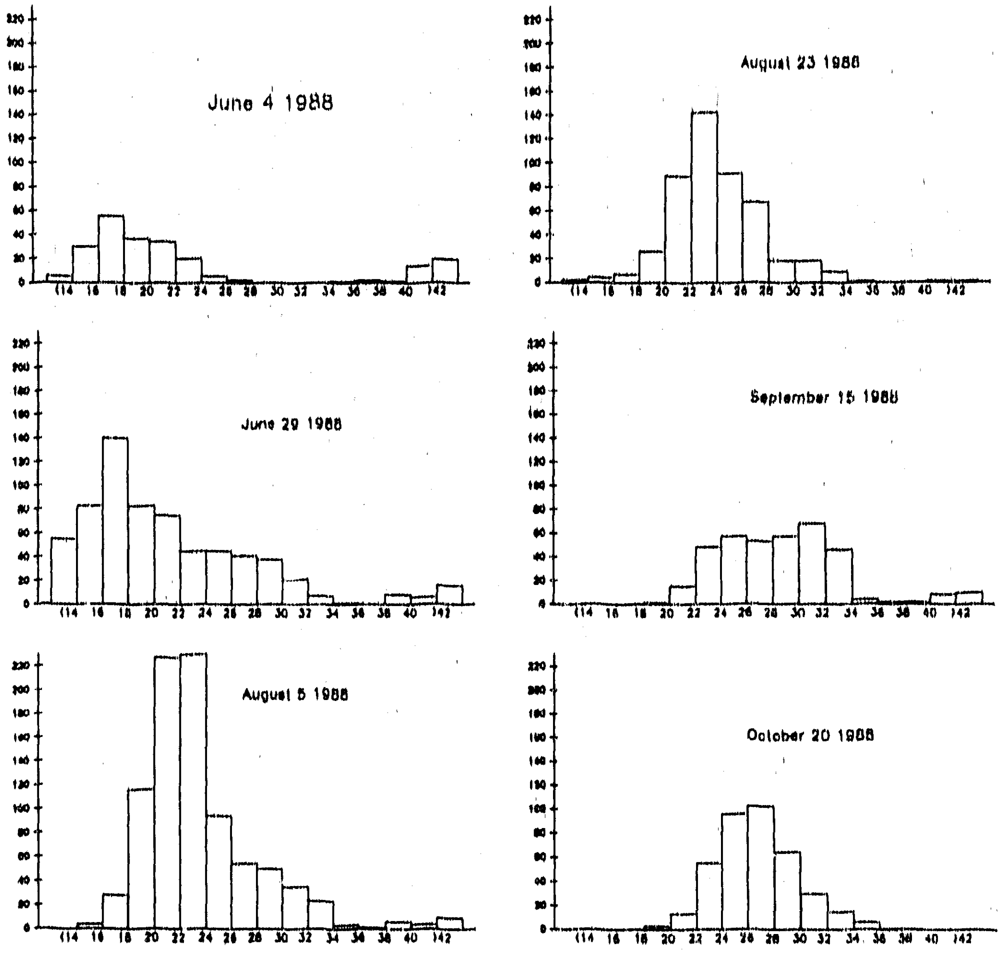


Figure 29. Size frequency histogram showing the seasonal pattern of population development in pond G.

ORNL-DWG 90-13149

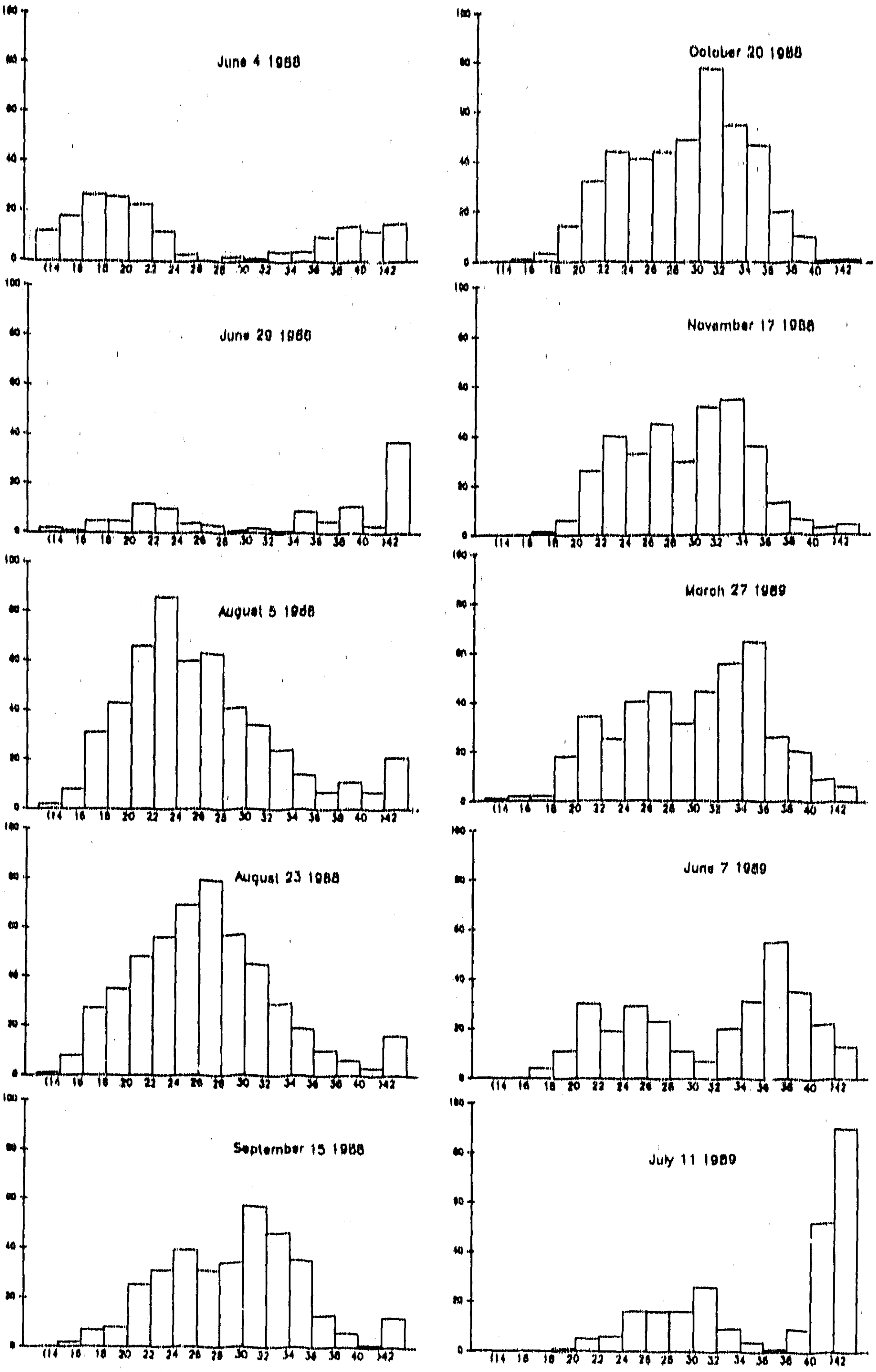


Figure 30. Size frequency histogram showing the seasonal pattern of population development in pond H.

ORNL-DWG 90-13140

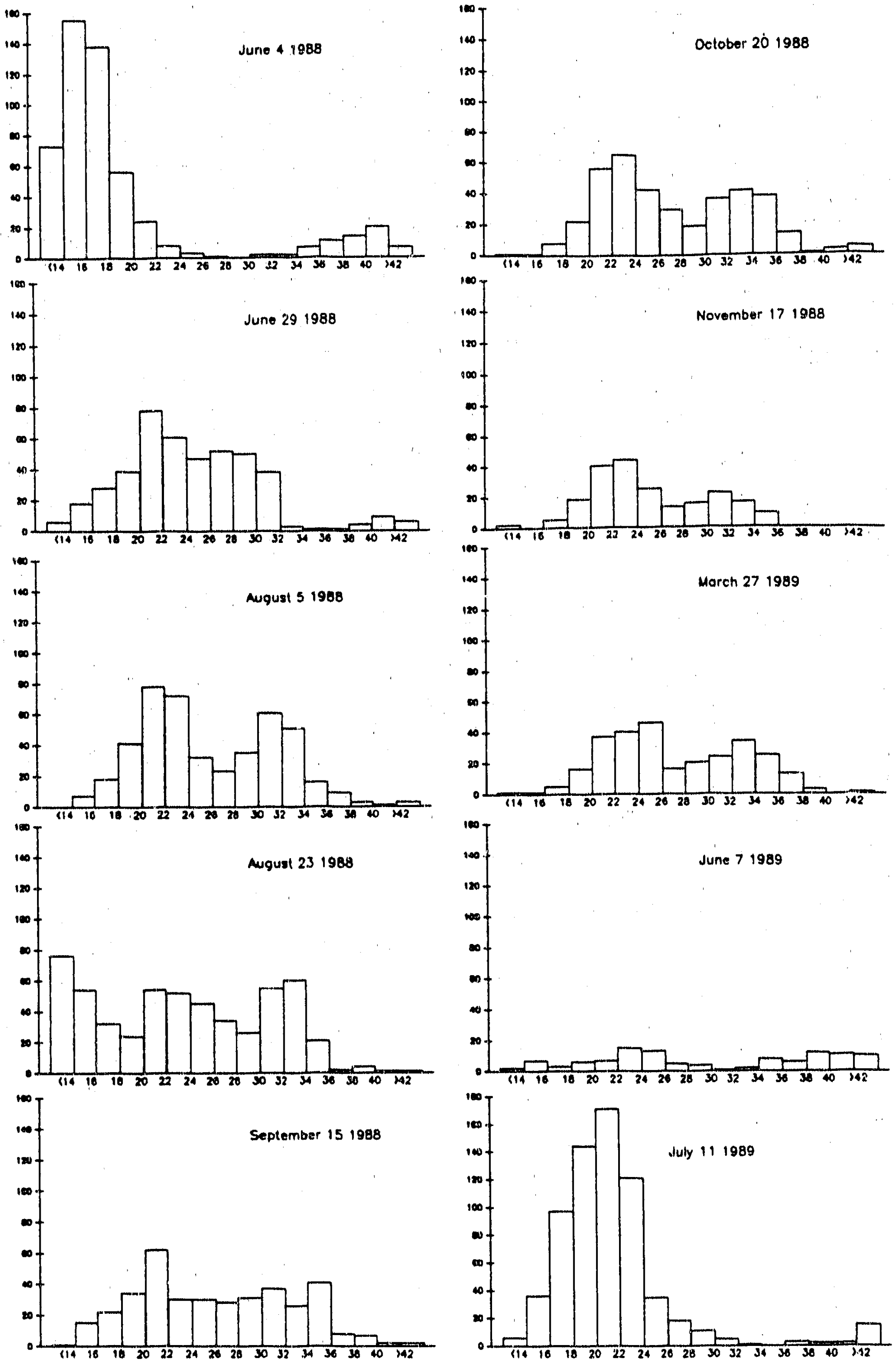


Figure 31. Size frequency histogram showing the seasonal pattern of population development in pond J.

ORNL-DWG 90-13141

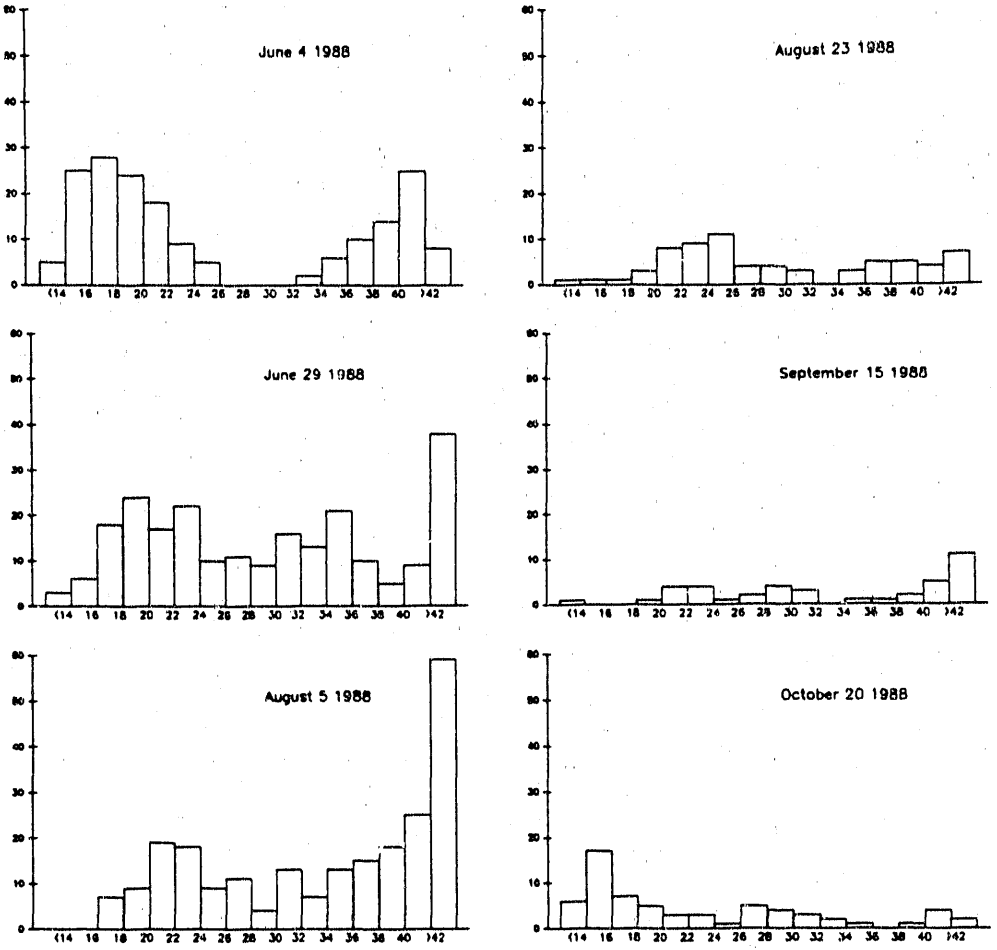


Figure 32. Size frequency histogram showing the seasonal pattern of population development in pond L.

ORNL-DWG 90-13142

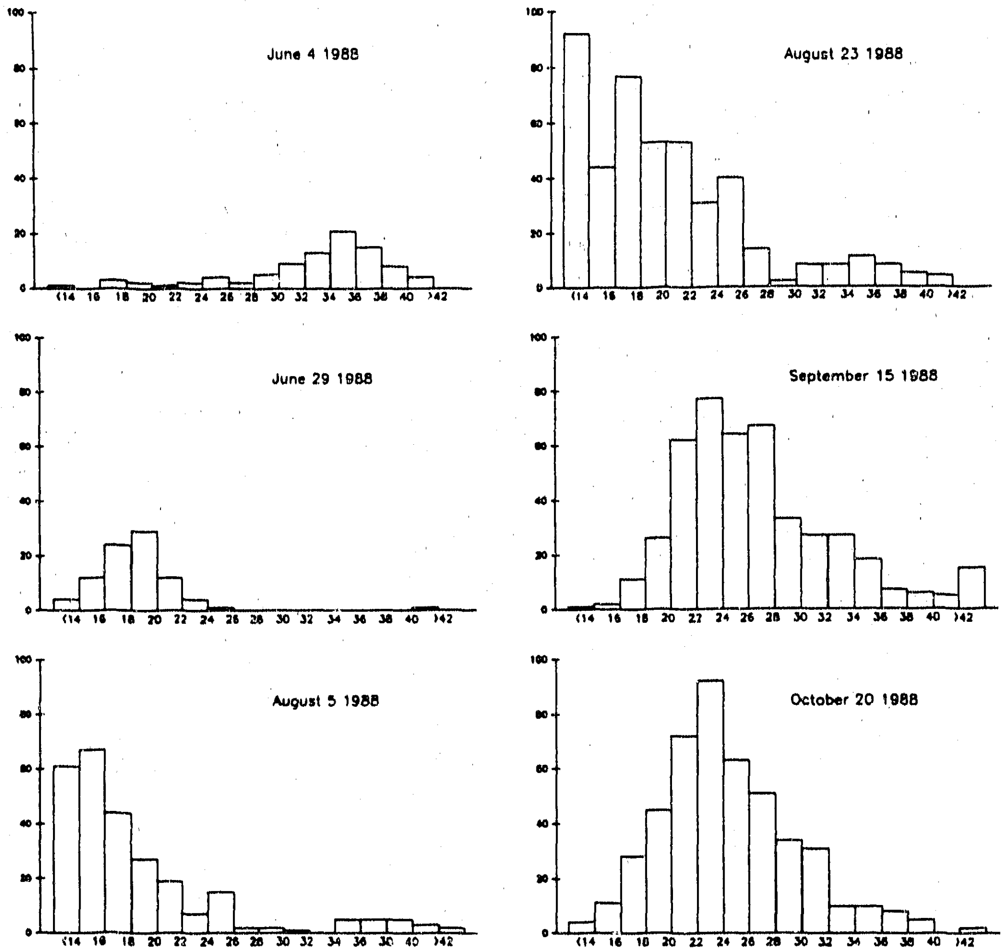


Figure 33. Size frequency histogram showing the seasonal pattern of population development in pond M.

ORNL-DWG 90-13143

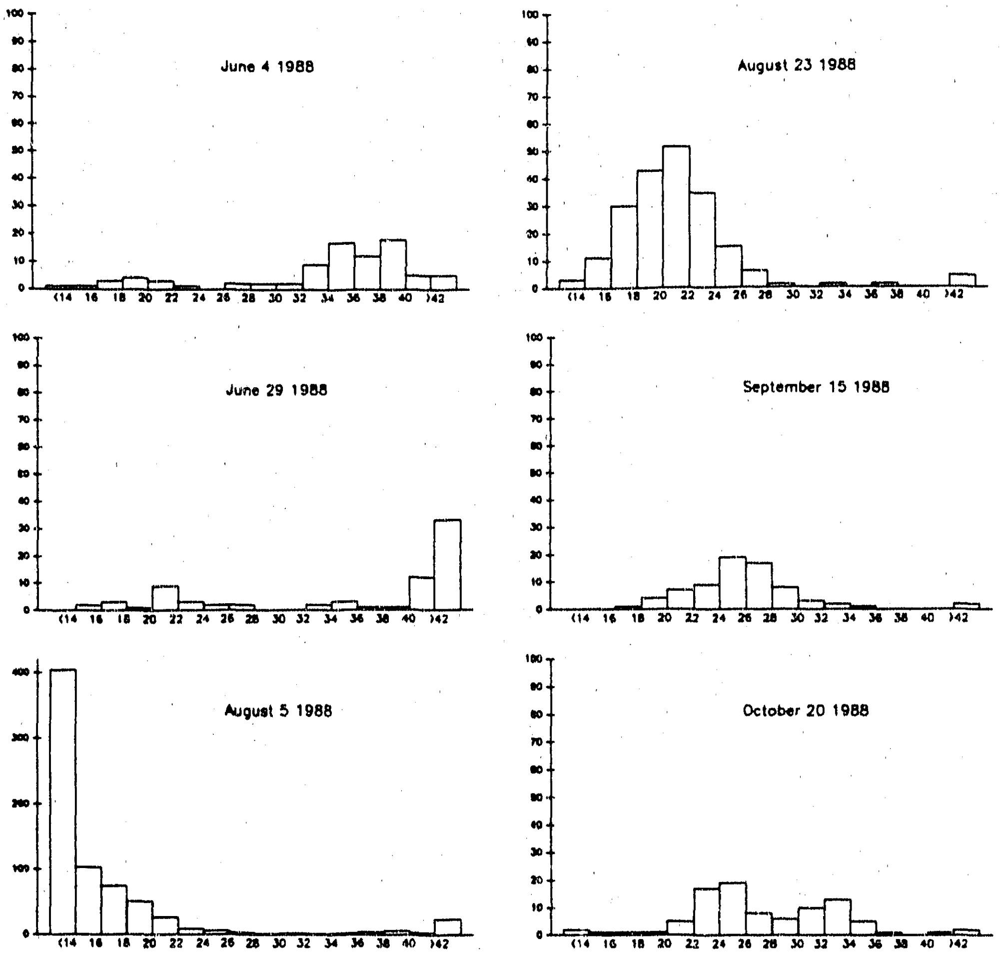


Figure 34. Size frequency histogram showing the seasonal pattern of population development in pond P.

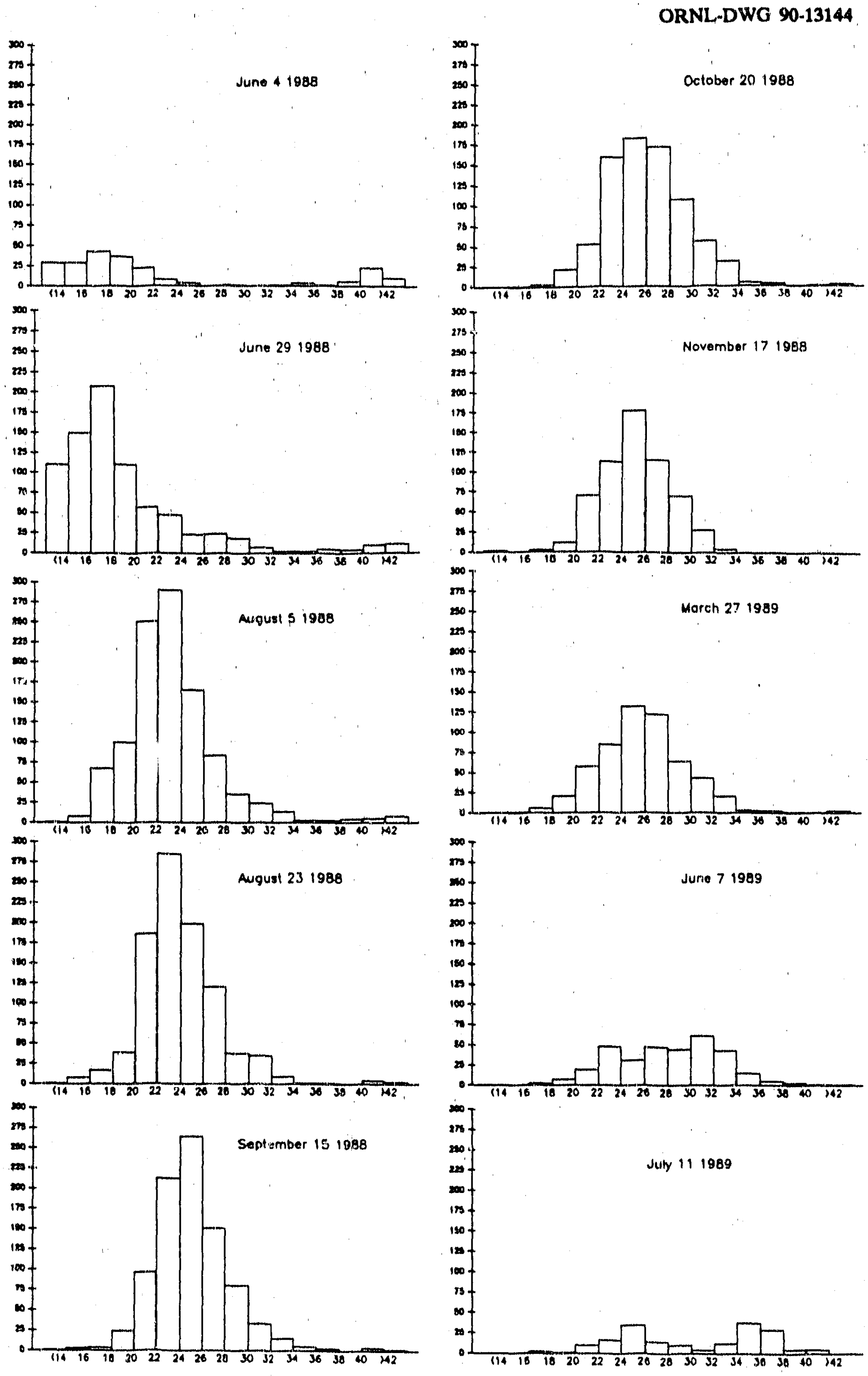


Figure 35. Size frequency histogram showing the seasonal pattern of population development in pond Q.

ORNL-DWG 90-13145

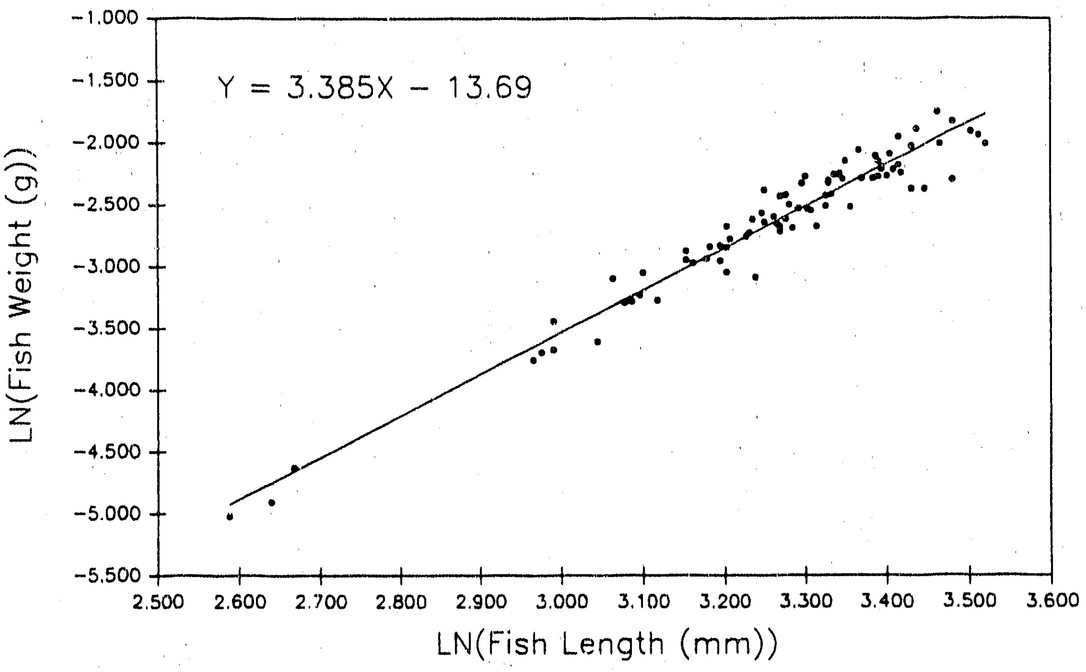


Figure 36. Length-weight regression for fish during October through November when no reproduction occurred. The equation for the regression fitting the data is given in the figure.

ORNL-DWG 90-13146

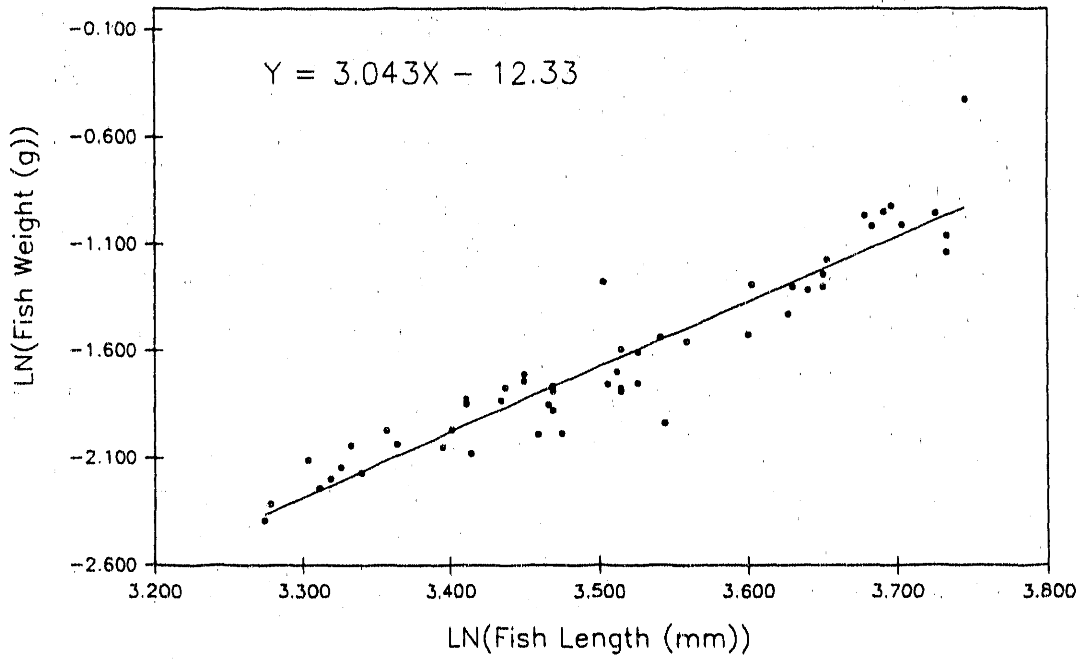


Figure 37. Length-weight regression for reproducing fish during March and September. Reproducing fish were considered to be all fish >= 27 mm standard length.

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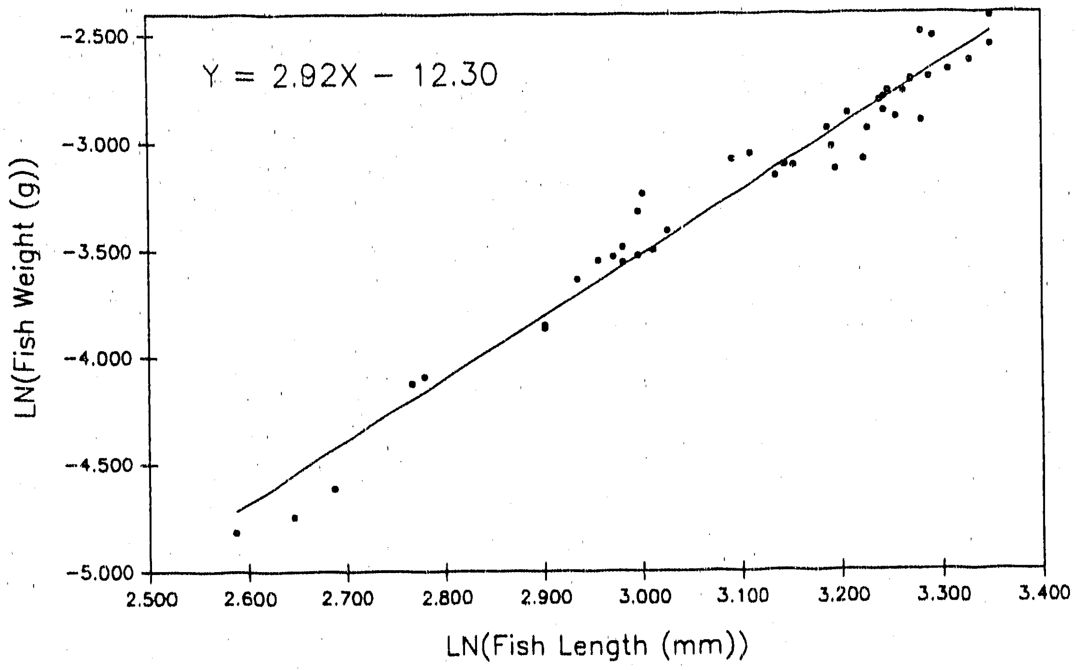

Figure 38. Length-weight regression for male plus immature female fish between March and September. All fish <= 27 mm standard length were either male or immature females.

Table 1. Analysis of the effect of dose on lipids, reproductive allotment and length of reproducing female <u>Gambusia</u> on each sampling date. A nested model was used with ponds nested as fixed effects within the main effect, dose. Degrees of freedom (DF) are the same for all three variables.

	Lipid Content		Rep. Allotment		Length		
	F	P	F	P	F	P	DF
June 29, 1988							
Dose	32.96	< 0.001	8.58	0.004	3.69	0.058	1
Pond(Dose)	5.28	< 0.001	4.38	< 0.001	0.81	0.606	9
August 4, 1988							
Dose	1.17	0.282	1.32	0.255	17.47	< 0.001	1
Pond(Dose)	4.23	< 0.001	9.44	< 0.001	0.72	0.674	8
August 23, 1988							
Dose	0.38	0.540	13.35	< 0.001	3.9	0.049	1
Pond(Dose)	1.63	0.105	3.65	< 0.001	1.6	0.103	11
September 15, 1988							
Dose	1.28	0.262	12.25	< 0.001	1.89	0.173	1
Pond(Dose)	3.24	0.003	3.71	0.001	3.80	<0.001	8
October 20, 1988							
Dose	7.46	0.008	0.05	0.83	0.03	0.862	1
Pond(Dose)	5.61	< 0.001	0.93	0.504	9.61	< 0.001	9
November 17, 1988							
Dose	9.05	0.004	1 .12	0.295	0.11	0.747	1
Pond(Dose)	4.27	0.001	0.57	0.749	11.90	< 0.001	6

Table 1. (Continued)

	Lipid Content		Rep. Allotment		Length		
	F	P	F	P	F	P	DF
March 27, 1989							
Dose	24.63	< 0.001	0.36	0.548	15.61	< 0.001	1
Pond(Dose)	6.52	< 0.001	23.87	< 0.001	4.71	< 0.001	6
June 7, 1989							
Dose	8.07	0.006	21.52	< 0.001	32.43	< 0.001	1
Pond(Dose)	4.56	< 0.001	2.98	0.011	21.12	< 0.001	6
July 11, 1989							
Dose	0.02	0.89	2.94	0.09	0.13	0.719	1
Pond(Dose)	6.28	< 0.001	2.65	0.22	23.21	< 0.001	6

Table 2. Relationship between lipid levels for Gambusia in ponds on October 20, 1988 and the overwintering loss of population biomass loss for ponds from October 20, 1988 through March 27, 1989.

Source	SS	DF	F	P
Lipid	8.497	1	16.46	0.0017
Dose	7.62	1	50.55	0.0021
Error	0.603	4		
	$R^2 = 0.944$			

Table 3. Analysis of lipid loss over the winter for Gambusia in ponds in relation to the lipid content of the fish on October 20, 1988.

Source	SS	DF	F	P
Lipid	5.09	1	9.49	0.0275
Dose	10.97	1	20.46	0.0063
Error	2.68	5		

$R^2 = 0.924$

Table 4. Number of Gambusia in ponds versus reproductive allotment of individuals within ponds. Comparisons between dosed and reference ponds are given for August 4, August 23, and September 15, 1988. F-test values for dosed and reference ponds indicate the fit of a regression line in each case. On August 23 and September 15, the result of an analysis of variance model with fish abundance is also given.

	F	P	R^2
August 4, 1988			
Dosed	9.98	< 0.001	
Reference	39.55	< 0.001	
Fish	14.01	0.007	0.673
Dose	4.32	0.076	
August 23, 1988			
Dosed	29.07	0.611	
Reference	0.26	0.001	
Fish	1.12	0.315	0.312
Dose	0.04	0.846	
Fish	4.93	0.048	0.309
September 15, 1988			
Dosed	5.39	0.025	
Reference	10.49	0.003	
Fish	2.4	0.165	0.429
Dose	0.01	0.928	
Fish	6.00	0.040	0.428

Table 5. Effect of dosing regime on the recruitment of young <u>Gambusia</u> into the populations in ponds. New recruits are considered to be all fish <= 20mm standard length.

	SS	DF	F	P
June 29, 1988				
Dose	10990.03	1	1.82	0.205
Error	6595.72	11		
	$R^2 = 0.14$			
August 4, 1988				
Dose	1231.37	1	1.96	0.189
Error	6897.58	11		
	$R^2 = 0.15$			
August 23, 1988				
Dose	690.50	1	2.64	0.132
Error	2876.31	11		
	$R^2 = 0.19$			
September 15, 1988				
Dose	263.34	1	1.56	0.237
Error	1853.83	11		
	$R^2 = 0.12$			
October 20, 1988				
Dose	1472.49	1	3.11	0.106
Error	5209.82	11		
	$R^2 = 0.22$			

Table 5. (continued)

	SS	DF	F	P
November 17, 1988				
Dose	3233.25	1	7.74	0.032
Error	2505.92	6		
	$R^2 = 0.56$			
March 27, 1989				
Dose	482.79	1	1.69	0.241
Error	1711.10	6		
	$R^2 = 0.22$			
June 7, 1989				
Dose	686.07	1	1.67	0.243
Error	1711.10	6		
	$R^2 = 0.22$			
July 11, 1989				
Dose	1828.85	1	8.05	0.030
Error	1362.57	6		
	$R^2 = 0.57$			

Table 6. Analysis of variance for average length of reproducing female Gambusia with population biomass on August 4, 1989.

Source	SS	DF	F	P
Biomass	0.832	1	1.17	0.321
Dose	8.66	1	12.18	0.013
Biomass*Dose	3.509	1	4.93	0.068
Error	4.27	6		
	$R^2 = 0.882$			

Table 7. Results of Kruskal-Wallis, non-parametric analysis of variance test of lipid content and proportion of total body weight devoted to reproductive allotment for female Gambusia in experimental ponds.

	Lipid Content		Reproductive Allotment	
Pond	chisq	DF	chisq	DF
A	50.24 p=.0001	9	58.92 p=.0001	9
B	55.58 p=.0001	8	73.88 p=.0001	8
C	49.98 p=.0001	9	47.29 p=.0001	9
D	37.91 p=.0001	7	44.97 p=.0001	7
E	23.69 p=.0001	3	27.21 p=.0001	3
F	58.19 p=.0001	9	78.17 p=.0001	9
G	28.29 p=.0001	5	38.18 p=.0001	5
H	58.53 p=.0001	9	74.95 p=.0001	9
J	42.26 p=.0001	9	70.86 p=.0001	9
L	5.90 p=.0523	2	5.45 p=.0657	2

Table 7. (Continued)

M	27.92 p=.0001	4	28.16 p=.0001	4
P	8.91 p=.0305	3	15.30 p=.0016	3
Q	46.18 p=.0001	9	70.57 p=.0001	9

Table 8. Effect of dose (by date) on biomass and number of <u>Gambusia</u> in dosed and reference ponds. F-test values are for one model and six error degrees of freedom in each case.

	Fish Biomass per Pond		Number of Fish per Pond	
Date	F	P	F	P
<u>Dosing #1</u>				
June 29, 1988	5.84	0.034	2.96	0.113
Aug. 4, 1988	26.03	< 0.001	16.14	0.002
<u>Dosing #2</u>				
Aug. 23, 1988	30.92	< 0.001	22.21	< 0.001
Sept. 15, 1988	15.42	0.002	17.9	0.001
Oct. 20, 1988	18.9	0.001	11.37	0.006
<u>Dosing #3</u>				
Nov. 17, 1988	74.18	< 0.001	13.60	0.010
March 27,1989	4.32	0.083	3.53	0.110
June 7, 1989	1.01	0.356	0.4	0.553
July 11, 1989	0.55	0.485	7.41	0.035

Table 9. Analysis of lipid content of female Gambusia in dosed and reference ponds in relation to length. On June 4, 1988, results of an analysis of variance are given for lipids only, because this date was before the first dose was administered. A dose effect is incorporated into the model for March 27, 1989 and June 7, 1989.

	SS	DF	F	P
June 4, 1988				
Length	19.96	1	35.96	0.007
Error	17.44	10		
	R^2 = 0.534			
March 27, 1989				
Length	17.70	1	7.16	0.044
Dose	0.42	1	0.17	0.696
Error	12.36	5		
	R^2 = 0.706			
June 7, 1989				
Length	7.05	1	6.02	0.058
Dose	0.30	1	0.26	0.630
Error	5.86	5		
	R^2 = 0.648			

Table 10. Analysis of reproductive allotment based on average female Gambusia length in ponds on five dates (June 4, 1988, June 29, 1988, October 20, 1988, June 7, 1989, and July 11, 1989). On June 4, only a regression was fit because the ponds had not yet been dosed.

	SS	DF	F	P
June 4, 1988				
Length	0.018	1	22.19	0.001
Error	0.025	9		
R^2 =	0.712			
June 29, 1988				
Length	0.0003	1	63.90	< 0.001
Dose	0.000005	1	1.09	0.328
Error	0.00004	8		
R^2 =	0.890			
October 20, 1988				
Length	0.00033	1	60.45	< 0.001
Dose	0.00000081	1	0.15	0.707
Error	0.000044	8		
R^2 =	0.884			
June 7, 1989				
Length	0.0025	1	5.54	0.065
Dose	0.0022	1	4.80	0.079
Error	0.0023	5		
R^2 =	0.788			

Table 10. (Continued)

	SS	DF	F	P
July 11, 1989				
Length	0.0022	1	31.67	0.005
Dose	0.00085	1	12.08	0.025
Length*Dose	0.00094	1	13.38	0.022
Error	0.00026	4		
R^2 =	0.911			

Table 11. Analysis of reproductive allotment for female <u>Gambusia</u> in relation to lipid content. Collections from June 4, 1988 have only a regression. June 7 has separate analysis of variance models for dose and fish.

	SS	DF	F	P
June 4, 1988				
Lipid	0.0199	1	35.96	< 0.001
Error	0.0056			
	R^2 = 0.782			
August 4, 1988				
Lipid	0.023	1	16.02	0.005
Dose	0.00001	1	0.01	0.910
Error	0.010	7		
	R^2 = 0.702			
June 7, 1989				
Lipid	0.001	1	1.66	0.254
Dose	0.0027	1	3.68	0.113
Error	0.0037	5		
	R^2 = 0.665			
Dose	0.006	1	7.46	0.034
Error	0.004	6		
Lipid	0.0046	1	4.32	0.083
Error	0.0064	6		

Table 12. Analysis of variance of length increase for reproducing female Gambusia on March 27, 1989 to June 7, 1989 as related to lipid content on March 27, 1989.

Source	SS	DF	F	P
Lipid	146.57	1	46.10	0.001
Dose	0.75	1	0.24	0.647
Error	15.89	5		
	$R^2 = 0.933$			

Table 13. Analysis of variance of the dose effect on the mean reproductive allotment for female Gambusia on June 7, 1989.

Source	SS	DF	F	P
Dose	0.0060	1	7.49	0.0339
Error	0.0049	6		

$R^2 = 0.555$

Table 14. Overall model of recruitment for dosed and reference ponds across the sampling season. The model includes the effects of dose, date, and the interaction between these two factors on recruitment.

Source	SS	DF	F	P
Dose	5356.39	1	12.76	< 0.001
Date	21236.339	9	5.62	< 0.001
Dose*Date	6533.423	9	1.73	0.094
Error	37769.883	90		
	$R^2 = 0.455$			

Table 15. Analysis of variance of seasonal trends in Gambusia population size and biomass. The significant interaction term indicates the relationship between populations size and biomass is changing through the season.

	SS	DF	F	P
Biomass				
Dose	82.756	1	89.11	< 0.001
Date	20.448	8	2.59	0.015
Dose*Date	13.908	8	1.76	0.098
Error	13.909	79		
	R^2 = .608			
Fish				
Dose	1269616.122	1	36.54	< 0.001
Date	632696.732	8	2.28	0.030
Dose*Date	1330929.497	8	4.79	< 0.001
Error	6247267.443	79		
	R^2 = .561			

Table 16. Analysis of lipid content of reproducing female Gambusia with total population size in each pond on August 4, 1988.

Source	SS	DF	F	P
Population	27.79	1	12.96	0.009
Dose	6.96	1	5.96	0.115
Error	15.01	7		
	$R^2 = 0.663$			

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