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MORPHOLOGICAL, HISTOLOGICAL, AND PHYSIOLOGICAL ASPECTS OF ASSIMILATION IN LARVAL SPOT, LEIOSTOMUS XANTHURUS LACEPEDE

The College of William and Mary in Virginia

PH.D. 1980

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MORPHOLOGICAL, HISTOLOGICAL, AND PHYSIOLOGICAL ASPECTS OF ASSIMILATION IN LARVAL SPOT, *LEIOSTOMUS XANTHURUS* LACEPEDE

A Dissertation

Presented to

The Faculty of the School of Marine Science

The College of William and Mary in Virginia

In Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

---

by

John Jeffrey Govoni

1980

APPROVAL SHEET

This dissertation is submitted in partial fulfillment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

John Jeffrey Govoni

Approved, April 1980

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ACKNOWLEDGMENTS

I take pleasure in acknowledging Dr. John V. Merriner who in large part afforded me the opportunity of further graduate study and who supported me throughout. I thank Drs. J. A. Musick, D. S. Peters, C. R. Smith, and R. L. Wetzel for their suggestions, criticisms, and review of the manuscript. Additional graditude is due Drs. Peters and Wetzel who endured many long hours of discussion concerning animal energetics and radiotracer methodology and who, along with Dr. P. L. Zubkoff, provided necessary laboratory space and equipment. Conversations with Drs. J. H. S. Blaxter, G. H. Boehlert, E. D. Houde, R. Lasker, G. C. Laurence, and C. P. O'Connel were also most helpful.

This work would have been impossible without the support of the Southeast Fishery Center, NOAA-NMFS, Beaufort, North Carolina. I thank the entire staff for their cordial help and assistance. Special thanks are due D. S. Peters who sponsored my work and A. B. Powell whose amiable cooperation was invaluable.

A multitude of people contributed much to the material presented herein. Among them I acknowledge Dr. C. L. Ruddell for histological instruction and assistance; J. C. Hoagman and M. H. Peoples for drafting; R. T. Doyle for photomicroscopy; Dr. J. L. Dupuy, N. T. Windsor, and A. T. Leggett for <u>Chlorella</u> and <u>Brachionus plicatilis</u>

cultures; J. E. Warriner for technical assistance and handling of isotopes; J. Colvocoresses for computer programing; and A.D. Estes and H. Powles for field specimens.

I wish to thank Dr. R.M. Ibara for his friendship and continual interest in my progress as a student. I thank my fellow students, especially J. Colvocoresses, B. Kilch, D. Markle, L. Mercer, J. Olney, B. Raschi, J. Ross, G. Sedberry, J. Smith and C. and B. Wenner for their sympathetic ears.

Finally, and certainly most, I am indebted to Maryjane Govoni for her loyalty, sacrifice, and enduring support - both material and morale.

PROLOGUE

"It may be worthy mentioning here that the original meaning of the word "organ" is "working tool." This etymology symbolizes the fact that describing an organ and studying it by the techniques of all science cannot so far provide a complete understanding of it. The organ acquires its full significance only when it functions and when its performance is integrated in the life of the organism."

from "Illusions of Understanding"
_In_ Rene Dubos, _The Dreams of Reason: Science and Utopias_. 1961.

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ABSTRACT

In view of questions concerning the digestive and assimilative abilities of larval marine fishes, the changes in these processes with development, and their relationship with starvation-induced mortality; this study has three objectives: (1) to examine the morphological and histological development of the alimentary canal and associated structures of larval spot, Leiostomus xanthurus (Pisces: Sciaenidae); (2) to assess short-term carbon assimilation in discrete age cohorts of larval spot; and (3) to relate any changes in assimilation to development. The alimentary canal and associated structures of larval spot from hatching through transformation (metamorphosis) were examined using light microscopy. These findings were related to results of experimental-radiotracer studies of carbon assimilation in discrete age cohorts of larval spot.

Cytological evidence suggested that the alimentary canal and associated structures were functional in first-feeding larvae after the completion of yolk-sac and oil-globule absorption, and changed little prior to transformation. There is no elaboration of tissues during the larval phase. Major developmental changes associated with transformation were accompanied by changes in habitat and feeding regime.

The behavior of carbon-14 indicated that food was quickly digested and carbon assimilated following the ingestion of a uniformly labeled ration. A percentage of newly assimilated carbon entered rapidly into short-term metabolic processes and exited the larva via respiration beginning as early as 1 h after ingestion. Gut evacuation was complete within 6 or 7 h.

Uptake, short-term retention, and loss of ^{14}C were used to compute two parameters, carbon retention and carbon absorption, that were taken as relative indices of the coefficient of utilization and absorption efficiency. The relationships between these two indices of carbon assimilation and measures of development indicated that assimilative abilities did not improve with larval development. Because there were no major changes in alimentary canal tissues, this might be expected.

The indices of assimilation were negatively related to larval condition factor and this relationship has important implications to larval growth and survival. Condition factor is an indicator of larval growth and robustness. Larvae that have been feeding well enough to survive, but less than optimally for maximum growth, apparently assimilate more carbon from a ration than do larvae with a better growth history.

These results are plausible in evolutionary terms. Larvae may mitigate the risk of a patchy food distribution by fully utilizing available resources. Such compensatory mechanisms may be of great adaptive significance to pelagic marine larvae.

MORPHOLOGICAL, HISTOLOGICAL, AND PHYSIOLOGICAL ASPECTS OF ASSIMILATION IN LARVAL SPOT, *LEIOSTOMUS XANTHURUS* LACEPEDE

INTRODUCTION

Research presented here is intended as one part of the manifold approach necessary to understand the relationship between stock and recruitment of marine fishes with pelagic eggs and larvae. Because of the high annual variability in recruitment, due in part to differential starvation-induced mortality, this research examines the ability of a larva to assimilate available food resources.

The survival rate of many marine fishes through their early life history is extremely low and may be the major determinant of numerical year-class strength (Beverton, 1962; Gulland, 1965; Cushing, 1969). Mortality is often near 99.9%, especially among highly fecund fishes with pelagic eggs and larvae (see review in Dahlberg, 1979). To explain survival in terms of any single factor is impossible for many synergistic and species-specific factors influence mortality. Yet, a general partitioning of active mechanisms has enhanced an understanding of the problem. Egg and larvae mortality can be attributed to adverse environmental conditions (e.g., Bonnet, 1939; Lillelund, 1965; Blaxter, 1969; Dickson, et al., 1974; Lett and Kohler, 1976; Rogers, 1976; Rosenthal and Alderice, 1976; Forrester, 1977), genetic defects (Vladimirov, 1970), and predation (Lebour, 1925; Murphy, 1961; Fraser, 1969; Hempel, 1965; Dekhnik, et al., 1970; Lillelund and Lasker, 1971; Theilacker and Lasker, 1974; Westernhagen and Rosenthal, 1976; Kuhlmann, 1977; Laurence, et al., 1979). Fish larvae experience additional mortality attributable to starvation. As the major causes of larval mortality (Hempel, 1974; Cushing, 1976; Hunter, 1976), starvation and predation probably interact with synergistic effects; the larva that feeds poorly grows poorly and this abates feeding success and predator avoidance (Cushing, 1976).

Starvation induced mortality was recognized as early as 1897 (Fabre-Domergue and Bietrix) and related research has progressed along several lines. The "critical period concept", hypothesizing that year class strength is determined by the availability of planktonic food immediately after yolk-sac absorption, was first proposed by Hjort (1914; 1926). Research designed to test this hypothesis included the analysis of survival curves for natural populations (Marr, 1955), and the correlation of larval gut contents and microzooplankton abundance in the sea with larval condition and abundance (see reviews by May, 1974). Though not all agree that critical periods exist or that food limitation is significant in nature (Dekhnik et al., 1970; Dekhnik and Singukova, 1976), catastrophic mortalities apparently associated with inadequate feeding conditions in the sea occur during the larval stage (e.g., Wiborg, 1976). Inasmuch as starvation mortality has never been proven categorically, more evidence is needed using diagnostic histological, morphological, and biochemical criteria (Ehrlich, 1974a, 1974b; Umeda and Ochiai, 1975; Ehrlich et al., 1976; O'Connell, 1976; Theilacker, 1978), and experimental bioassay techniques (Lasker, 1975).

Experimental research has accelerated with the ability to successfully rear larval marine fishes in the laboratory. Experiments have assessed the sensitivity of larvae to delayed first-feeding, the effects of food density and small scale distribution on larval survival and growth, and larval searching ability and feeding efficiency (see reviews in May, 1974; Ahlstrom and Moser, 1976; Vlymen, 1977; Houde, 1978). The results of these studies suggest that the integrated concentration of food organisms in the ocean (e.g., Beers and Stewart, 1967, 1971; Arthur, 1977) may be insufficient to support larval fish survival; though concentrations in bays and estuaries may be adequate (see review in Houde, 1978). The contagious small-scale distribution of food organisms in the open sea may also be critical (Lasker, 1975; Vlymen, 1977; Houde and Schekter, 1978), and the interaction of food availability, growth, and predation remain obscure.

Most recently, physiological energetic studies have enhanced our understanding of starvation, growth, and survival of marine fish larvae. Researchers have attempted to establish the amount of food necessary to support larval fish metabolism and growth by estimating the terms of energy budget equations (Ivlev, 1939; Winberg, 1956; Paloheimo and Dickie, 1966a, 1966b; Warren and Davis, 1967). Their results indicate that energy reserves upon yolk-sac absorption are inadequate to support metabolism and growth without feeding (Lasker, 1964; Blaxter, 1969) and that natural densities of food organisms are synotically inadequate to support survival (see review in Laurence, 1977, Houde, 1978). Vlymen's (1977) model, based in part on swimming energetics, predicts that the contagious microdistribution of food organisms is actually necessary for larval survival.

Most physiological energetic studies on fish larvae have estimated equation terms as instantaneous rates at a single developmental interval and have not attempted to resolve the changes in matter-energy utilization through entire larval life. Larval fishes are, however, developing animals; matter-energy utilization involves dynamic processes influenced possibly by age as well as ration size and environmental factors.

The amount of matter-energy available for metabolism and growth is determined, in part, by the ability of the larva to digest and assimilate nutrients (Phillips, 1969). Some have speculated that assimilation is initially low but improves with larval age as the result of the morphological development of the alimentary canal (Rosenthal and Hempel, 1970; Nishikawa, 1975). Besides Laurence (1971,1977) there is little quantitative evidence to support this.

This dissertation has three objectives: (1) to examine the morphological and histological development of the alimentary canal and associated structures of larval spot, *Leiostomus xanthurus*; (2) to assess short-term carbon assimilation in discrete age cohorts of larval spot; and (3) to relate any changes in assimilation to development. It is hoped that the information herein contributes to the understanding of the broader question of physiolgical energetics and survival of larval marine fishes.

THE EARLY LIFE HISTORY OF *LEIOSTOMUS XANTHURUS*:

A BRIEF REVIEW

Spot, Leiostomus xanthurus Lacepede, is a highly fecund, coastal-marine, sciaenid fish ranging from Cape Cod, Massachusetts, to Bay of Campeche, Mexico (Dawson, 1958). Populations fluctuate dramatically, and year-class strength is probably determined offshore during the pelagic egg and larval phases (Joseph, 1972).

Spot spawn over the continental shelf in late fall and winter (see review in Johnson, 1978). Ova counts range from 70,000 to 90,000 per female (Dawson, 1958). Ovarian eggs of several sizes within single females (Hildebrand and Cable, 1930) and repeated spawning in the laboratory suggest multiple spawning in nature. The spawning period may not be protracted, however, as preliminary analyses of daily otolith growth increments indicate a single, rather short, spawning season[1]. Spot begin the offshore spawning migration (Pacheco, 1962) after their second summer (Hildebrand and Cable, 1930; Pearson, 1929). The spherical, pelagic eggs range from 0.72 to 0.87 mm in diameter and have either a single, spherical oil globule or multiple oil globules grouped together within the vitelline membrane (Powell and Gordy, 1980). Oil globules coalesce during development leaving a single globule in the newly hatched yolk-sac larva (Powell

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1 Peters, D. S., J. C. DeVane, M. T. Boyd, L. C. Clements, and A. B. Powell. 1978. Preliminary observations on feeding, growth, and energy budget of larval spot (Leiostomus xanthurus). Ann. Rept. of the Beaufort Laboratory to the U.S. Dept. of Energy. pp. 377-397.

and Gordy, 1980). Hatching occurs in ca. 2 days at 20°C (see Appendix).

Larvae have been described by many authors (see reviews in Fruge and Truesdale, 1978; Johnson, 1978), but their ecology is not well documented. Newly-hatched, yolk-sac larvae range from 1.6 to 2.5 mm notochord length (NL). Yolk-sac and oil-globule absorption are complete in 4 to 7 days at 20°C (see Appendix). The larvae, now 2.0 to 2.4 mm NL, begin feeding as members of the plankton community (Part I). Spot larvae, like most marine fish larvae, are visual feeders with feeding generally restricted to daylight hours (Kjelson et al., 1976; Kjelson and Johnson, 1976). Development to transformation (metamorphosis) takes place in waters overlying the continental shelf (e.g., Powles and Stender, 1976). Preliminary evidence suggests that larval growth is extremely rapid. Transformation begins by ca. 7.0 mm standard length (SL) and is complete by 16.0 to 18.0 mm SL (Powell and Gordy, 1980; also see Appendix). The average growth rate suggests that transforming larvae and early juveniles are not limited by food availability.[1] Food limitation may, however, be an important factor in the survival of first-feeding larvae; a short spawning period would amplify the critical coincidence of zooplankton abundance and first feeding.

Spot, along with many other sciaenids, utilize estuaries as nursery grounds (see review in Chao and Musick, 1977). Transforming larvae enter estuaries during the first half of the year when as small as 10.0 mm SL. Larvae complete transformation within estuaries and coastal bays during spring and summer of their first year. Transforming larvae feed initially on epifaunal invertebrates followed by infaunal invertebrates and vegetable detritus (Part I). Growth is rapid during the first summer, and by December when most have moved offshore to over-winter, they have grown to about 150 mm SL with some as large as 190 mm SL (Chao and Musick, 1977). Some smaller juveniles over-winter in the deeper channels of estuaries (Chao and Musick, 1977). Juveniles may penetrate freshwater as well as hypersaline lagoons and marshes (see review in Johnson, 1978).

The tolerance of eggs, larvae, and juvenile spot to temperature and salinity variation is not well documented. Eggs are not buoyant and hatching success is reduced in water less than 26 ‰. Likewise, hatching success and survival through yolk-sac absorption is reduced below 15°C (A.B. Powell, personal communication). Eggs and larvae generally occur in 18 to 22°C and 30 to 35 ‰ water in the South Atlantic Bight (Powles and Stender, 1976); though larvae and juveniles are taken in temperatures from 6.3 to 32.5°C and salinities from 0 to 34.2 ‰ (see review in Johnson, 1978). Temperatures of 4 to 5°C may be lethal to juveniles (Hildebrand and Cable, 1930; Dawson, 1958). The distribution of larvae and juveniles is apparently limited by temperature rather than salinity (Parker, 1971). Temperature preferences can be inferred from the seasonal movement of larvae and juveniles into and out of estuarine nursery areas. Transforming larvae and early juveniles enter estuaries in spring as the temperature rises above 6°C; juveniles generally depart in fall as the temperature drops below 10°C (Parker, 1971; Chao and Musick, 1977).

ASPECTS OF THE MORPHOLOGICAL, HISTOLOGICAL, AND
FUNCTIONAL DEVELOPMENT OF THE ALIMENTARY CANAL
AND ASSOCIATED STRUCTURES

INTRODUCTION

Starvation-induced mortality of pelagic marine fish larvae as a determinant of fluctuations in numerical year class strength has been the subject of controversy for almost a century (see reviews by Blaxter, 1969; May, 1974). Starvation has received much attention, including extensive field and laboratory study, but the digestive and assimilative abilities of larval fishes remain to be determined (Hunter, 1976). While some have assumed that these abilities are initially very poor but improve with larval age as the result of the development of alimentary canal tissues (Rosenthal and Hempel, 1970; Nishikawa, 1975; Laurence, 1977), little supporting evidence exists. Changes in digestion and assimilation should be concomitant with changes in the alimentary canal and associated structures.

This study was undertaken to describe certain aspects of the functional development of the alimentary canal, liver, pancreas, jaws, and teeth of spot, *Leiostomus xanthurus*. Spot is a highly fecund, sciaenid fish with pelagic eggs and larvae. Year-class size fluctuates drastically, probably due to differential larval mortality (Joseph, 1972); starvation may be a causative factor. Knowledge of the extent and timing of developmental changes in the alimentary canal and associated structures should provide a basis for evaluating changes in the digestive and assimilative abilities of spot during its early life history.

MATERIALS AND METHODS

Specimens for dissection and histological study were obtained from two sources. Eggs were obtained from fish induced to spawn through temperature and photoperiod manipulation and hormone injection[2]. Eggs and larvae were reared at 20 $\pm$ 2^{o}C in 30-35 ^{o}/oo salinity seawater and fed sequentially with the dinoflagellate, Gymnodinium splendens, the rotifer, Brachionus plicatilis, and wild plankton. Specimens were fixed in chilled neutral buffered formalin plus acrolein, dehydrated in methanol, infiltrated with and embedded in glycol methacrylate (2-hydroxy-ethyl methacrylate), and sectioned at 2 and 4 μm according to modifications of Ruddell (1971). For comparison, field collections of the Virginia Institute of Marine Science (VIMS), fixed in buffered formalin in seawater, were dehydrated in isopropanol, infiltrated with and embedded in paraffin, and sectioned at 5 μm. Histological and histochemical stains used for glycol methacrylate sections were acid fuchsin - toluidine blue O, alkaline blue 6B - neutral red, and periodic acid Schiff's reagent (PAS) - toluidine blue O. Paraffin embedded specimens were stained in hematoxylin - astra blue - eosin Y.

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[2] Hettler, W.F., A.B. Powell, and L.C. Clements. 1978. Laboratory induced spawning of spot, Leiostomus xanthurus (Lacepede). Ann. Rept. of the Beaufort Laboratory to the U.S. Department of Energy. pp. 351-356.

The development of jaws and dentition was studied on specimens cleared and stained with alizarin red S - alcian blue by a modification of Dingeraus and Uhler (1977).

Specimens for gut content analysis were obtained from field collections of the South Carolina Wildlife and Marine Resources Department, NOAA-NMFS Sandy Hook Laboratory, and VIMS. Food items were excised from 59 specimens, mounted in CMC-S (Turtox), and identified to the lowest possible taxon.

Lengths reported herein are notochord lengths (NL) measured before the complete formation of hypural elements and standard length (SL) measured thereafter.

RESULTS

Though development is continuous, the alimentary canal is discussed within the framework of three phases that generally correspond with changes in gross morphology: the yolk-sac larva (1.6 - 2.5 mm NL), the larva (2.5 - 7.0 mm NL), and the transforming larva (7.0 -18.0 mm SL). At a rearing temperature of 20°C these phases correspond to 2-7, 7-31, and 31-60 days from hatching. Pertinent details of the early juvenile alimentary canal are also described. While many terminologies of fish development intervals exist (e.g., Hubbs, 1943; Ahlstrom, 1968; Balon, 1975), the present terms afford simplicity and close alignment with alimentary canal development.

Alimentary Canal

The yolk-sac larva. In the yolk-sac larva the alimentary canal is undifferentiated along its length and not functional. The mouth is not open. The bucco-pharynx is lined with squamous epithelium and scattered mucous cells of uniform appearance underlain by fibrous connective tissue. The incipient gut is a straight tube lying in the dorsal visceral cavity above the yolk mass and deflecting ventrally to a closed anus (Figure 1). The narrow lumen is open posteriorly (Figure 7), but is partially occluded in the anterior half by pressure from the oil globule. It is lined with closely packed, columnar epithelial cells, 12-15 μm thick. The nuclei of these cells are located basally and are ca. 3 μm in diameter. The cytoplasm is basophilic and granular. Mitochondria are not visible. Apically, the epithelial cells have a prominent, acidophilic striated border with microvilli 3-4 μm deep (Figure 8). A 5 μm layer of areolar connective tissue with fibroblasts underlies the epithelium. This lining, lacking rugae, constitutes the early mucosa, but a submucosa and muscularis are not apparent. A thin serosa is discernable.

The alimentary canal becomes functional during the completion of yolk-sac and oil-globule absorption. This transition is rapid, taking about 30 h at 20^{o}C. The yolk is absorbed before the oil globule. During its absorption it remains as a cap of yolk granules enclosed by the vitelline syncytium lying over the anterior part of the oil globule (Figure 2). The posterior half of the alimentary canal with a lumen abuts the oil globule whereas the anterior alimentary canal remains partially occluded and lies between the oil globule and the dorsal wall of the visceral cavity (Figure 2). During final yolk and oil-globule absorption, the lumen extends the entire length of the alimentary canal, and the alimentary canal intorts to form a loop midway along its length. Two circular constrictions, the pyloric port and the ileocaecal port, develop further dividing the alimentary canal into four sections: the bucco-pharynx, the foregut, midgut, and hindgut (Figure 3). The mouth and anus are now open, and first feeding may occur. The number of PAS positive mucous cells increases along the single layer of squamous epithelial cells lining the bucco-pharynx.

The larva. The larval bucco-pharynx, fore-, mid-, and hindgut are distinguished by histological differences and remain unchanged until transformation. The alimentary canal length and surface area, however, increase with increased body size.

The walls of the bucco-pharynx are lined with stratified squamous epithelium, 3 to 4 μm deep. Two layers, an inner 7 to 8 μm layer of areolar connective tissue, and an outer 4 m layer of fibrous connective tissue, lie beneath the epithelium. Fibroblasts are seen in these connective tissue layers, and a few uniform PAS positive mucous cells occur in the epithelium. The ventral walls have more numerous and larger PAS positive mucous cells. Taste buds appear in the dorsal and ventral walls; though they lack gustatory pores and are presumably not functional. They are more numerous on folds and ridges of the ventral wall.

The foregut connects the bucco-pharynx with the midgut (Figures 4,9) and differentiates from the anterior closed section of the incipient gut. Its mucosa consists of cuboidal epithelial cells, 10 to 20 μm thick, with basophilic cytoplasm. The basally located nuclei are also basophilic. Nucleoli are not visible. The surface of the mucosa often has jagged projections that are possibly remnants of sloughed cells. Two types of mucous cells, distinguished on the basis of cellular morphology and staining reactions, are tentatively identified in the foregut mucosa (Figure 10). Most numerous (Type 1) are PAS positive mucous cells (8-12 μm in diameter) which resemble typical vertebrate goblet cells and are similar to those of the bucco-pharynx. They stain metachromatically with basic dyes suggesting that they contain acid mucopolysaccharides. The second type (Type 2) are large (20 μm in diamter), basophilic, and PAS negative cells, often aggregated in groups. The foregut mucosa has shallow longitudinal folds. A 30 μm thick lamina propria of fibrous connective tissue with fibroblasts lies subjacent to the epithelium. There is no muscularis mucosa or submucosa. The muscularis consists of 6 to 7 μm of circular smooth muscle fibers, and a very thin serosa is visible.

The midgut, limited by the pyloric and ileocaecal ports, is bulbous with a much wider lumen than either the fore- or hindgut (Figure 9). The mucosa becomes highly convoluted with deep (40-50 μm) rugae, thus increasing surface area. Its epithelium consists of basophilic columnar cells, 25 to 30 μm high, with a 2 to 3 μm striated border of acidophilic microvilli underlain by a terminal web. Nuclei are basophilic with visible nucleoli. Numerous supra- and infranuclear mitochondria are seen within the cytoplasm. The most characteristic feature of these cells is the presence of numerous, supranuclear, vacuolar, intracellular inclusions (Figure 11). The mucosa lacks mucous cells. A thin (8-10 μm) lamina propria of areolar connective tissue with fibroblasts underlies the epithelium. There is no distinct muscularis mucosa or submucosa. The muscularis consists of a 5 μm thick layer of circular smooth muscle fibers. A very thin serosa is visible.

The hindgut begins at the ileocaecal port, ends at the developing anal sphincter (Figure 4, 9), and differs from the midgut mainly in the cytology of its mucosal epithelial cells. The hindgut mucosa has shallower (ca. 25 μm) rugae than those of the midgut. Its. mucosa consists of a layer of uniform columnar epithelial cells, slightly shorter (25 μm) than those of the midgut, with a low (1 or 2 μm) acidophilic striated border. There is no evidence of a terminal web beneath the microvilli. The basal cytoplasm of the epithelial cells is basophilic while the apical cytoplasm is acidophilic. The nucleus is located basally. As in the midgut epithelium, there are numerous supra- and infranuclear mitochondria and supranuclear cytoplasmic inclusions, but these inclusions are granular and acidophilic (Figure 12). The hindgut has a thin (8 - 10 μm) lamina propria subjacent to the epithelium, but no muscularis mucosa or submucosa. The muscularis consists of a 2 μm thick layer of circular smooth muscle cells. There is a thin serosa. Erythrocytes and an assortment of leucocytes are visible within the mid- and hindgut muscularis.

<u>The transforming larva and early juvenile</u>. The major changes in the alimentary canal are associated with transformation (metamorphosis). The alimentary canal completes its development with the formation of the esophagus, stomach, intestine with pyloric caecae, and rectum (Figure 6).

The bucco-pharynx remains unchanged with a few exceptions. A buccal valve develops midway separating the pharynx and the buccal cavities. The taste buds, now open via gustatory pores, become more numerous, and presumably functional.

The esophagus differentiates from the foregut and merges posteriorly with the developing stomach. The esophageal mucosa consists of stratified cuboidal epithelial cells 10 μm thick lacking cilia. The number of Type 1 mucous cells in the mucosa greatly increases (Figure 15), but Type 2 mucous cells were not seen in the esophagus. The lamina propria and submucosa differentiate by ca. 13 mm SL. This distinction is based on the texture of connective tissue for no muscularis mucosa develops in the alimentary canal. The thin (6 μm) lamina propria underlies the epithelium and consists of fibrous connective tissue. The submucosa, comprised of 15-20 μm of areolar connective tissue with a few dispersed longitudinal muscle cells, lies subjacent to the lamina propria. A 10 μm layer of circular, smooth muscle fibers with a few striated muscle cells appear in addition to the inner layer of longitudinal muscle by ca. 13 mm SL. These layers decussate at the esophageal gastric junction.

The stomach differentiates from the posterior section of the foregut. The stomach anlage appears as a blind sac that bulges from the posterior end of the foregut as it meets the midgut (Figure 5). This outpocketing continues to expand and extends as a bulbous sac dorsal to the junction with the midgut. Uniform secretory cells appear in the blind sac by ca. 10 mm SL (Figure 17). These cells increase in number and eventually line distinct gastric glands in the corpus (<u>sensus</u> Barrington, 1957) by 18 mm SL (Figure 17). The gastric mucosa consists of simple columnar epithelial cells without a striated border. The apical cytoplasm is slightly acidophilic, perhaps because of the presence of mucigen, and contrasts with the basophilic basal cytoplasm. Mucous cells, similar to Type 1 cells in form, appear while the mucosa develops deep rugae. A lamina propria underlies the developing gastric epithelium. The stomach has a 15 μm thick submucosa of areolar connective tissue. A layer of a few circular muscle fibers is seen within the mucosa. The gastric muscularis consists of a 40 μm thick inner layer of smooth, circular muscle and a 3 μm outer layer of smooth, longitudinal muscle. The serosa is sometimes visible. The junction of the esophagus and the stomach is marked by an abrupt change in epithelium (Figure 16).

The intestine is separated from the stomach by a developing pyloric sphincter and differentiates from the midgut. It is generally J-shaped (Figure 6) and separated from the straight rectum by the ileocaecal valve. Cytologically its epithelium is similar to the midgut (Figure 18). A few mucous cells, similar to Type 1 cells, appear in the mucosa. A distinction between the lamina propria and submucosa is not apparent as it is in the esophagus and stomach. Instead, a gradual transition from inner fibrous to outer areolar connective tissue develops. The rugae of the midgut deepen in the intestine. The muscularis thickens greatly and an outer layer of longitudinal muscle appears by ca. 13 mm SL. Pyloric caeca develop as blind outgrowths of anterior intestine just posterior to the pyloric sphincter and are histologically similar to the intestine (Figure 20). The first caecum appears at ca. 10 mm SL. The juvenile complement of seven to eight is complete by 18 mm SL and is attendant with the completion of stomach development.

The rectum differentiates from the hindgut but shares a similar epithelium (Figure 19). As in the intestine, mucous cells appear in the mucosa and increase in number near the anal sphincter. The mucosa develops secondary folds. The lamina propria and submucosa become distinguishable by ca. 23 mm SL. The lamina propria consists of aerolar connective tissue whereas the submucosa consists of fibrous connective tissue, a reverse of the esophageal, gastric, and intestinal condition. Within the muscularis, the inner layer of circular muscle expands and a thick outer layer of smooth, longitudinal muscle appears by ca. 13 mm SL. The differentially staining cytoplasmic inclusions, so conspicuous in the larval mid- and hindgut, are also obvious in transforming larvae and early juveniles (Figures 18, 19).

Associated Structures

The liver. The liver is differentiated at hatching and lies dorsal to the yolk mass (Figure 7). Hepatocytes are compact, granular, basophilic, and lack of vacuoles in yolk-sac larvae. In larvae the liver lies beneath and partially surrounding the alimentary canal (Figure 9). It increases in size and becomes bilobed. Large (12 μm in diameter) hepatocytes surround distinct canaliculi. These cells contain voluminous (4 μm in diameter) PAS positive vacuoles (Figure 13) that are presumably the sites of glycogen storage (no diastase controls were used). The gall bladder appears on the right lateral lobe of the larval liver by ca. 3.0 mm NL. The liver in early juveniles occupies more space in the visceral cavity than any other period in the spot's early life history. Its two lobes extend around the posterior loop of the intestine.

The pancreas. The exocrine pancreas, an insular organ in adult spot, is differentiated at hatching with well developed basophilic acinar cells. Pancreatic ducts and zymogen granules are not yet apparent. The larval pancreas lies primarily along the right side of the midgut but sends disseminated branches into the liver. Pancreatic ducts appear soon after yolk absorption, and conspicuous acidophilic zymogen granules appear within the pancreatic acini (Figure 14). The pancreas remains unchanged during transformation.

Jaws. At hatching anlagen of the upper and lower jaws are formed as procartilage with basophilic chondrocytes lying within a thin matrix of chondromucen. At first feeding (ca. 2.0 mm NL) the upper jaw is comprised of paired premaxillae with the premaxilla completely excluding the maxilla from the gape; the lower jaw is comprised of paired Meckel's cartilages. Dentaries and articulars with angular processes are discernable as separate lower-jaw elements by ca. 4.0 mm NL. Calcification of the jaw elements begins by ca. 8.5 mm SL in the anterior shafts and ascending processess of the premaxillae, the anterior shafts of the maxillae, and the posterior flanges of the articulars. The anterior tip of the dentaries begins to calcify by 12 mm SL. Calcification proceeds posteriad along the axes of the premaxillae, maxillae, and dentaries and spreads into the lateral flanges of these elements by 16 mm SL. Meckel's cartilages persist in juveniles as cartilagenous shafts enclosed within each articular.

Caution must be exercised in the interpretation of staining reactions. Premaxillae, maxillae, dentaries, and articulars are dermal bones not preformed as cartilage, yet they often stain with alcian blue. Alcian blue reacts with many acid mucopolysaccharides including chondromucin (Pearse, 1968). Histologically, hyaline cartilage with chondrocytes completely filling lacunae embedded within a thick chondromucin matrix, predominates in endochondral elements of larvae and early juveniles. Perichondral bone appears on the periphery of some elements by ca. 23 mm SL; though the alizarin reaction is visible in elements of much smaller specimens. Alizarin red S chelates Ca ions (Pearse, 1968) and thus may indicate the presences of calcified cartilage as well as true ossified bone.

The gape, terminal in larvae and transforming larvae, becomes progressively inferior in position during the early juvenile stage.

<u>Dentition</u>. Developing teeth are first seen in the areolar connective tissue underlying the bucco-pharyngeal epithelium of the larvae. Retrorse conical teeth erupt on the premaxillae at ca. 4.0 mm NL and on the dentaries by 5.2 mm NL (Table 1). At ca. 16 mm a second horizontal row of recurved villiform teeth erupts linquad on the premaxillae and dentaries. This row of villiform teeth replaces larval teeth as additional rows of recurved villiform teeth erupt linquad forming tooth rows. At complete transformation, the polyphyodont dentition (Peyer, 1968) of spot is comprised of three

tooth rows. Premaxillary and dentary teeth within a tooth family (Peyer, 1968) are shorter toward the lingual aspect.

Villiform pharyngeal teeth begin to erupt on the paired medial certatobranchials of branchial arch 5 and on the pharyngobranchials of arches 2, 3, and 4 by 5.5 mm NL (Table 1). The complete complement of pharyngeal teeth is reached by complete transformation with villiform teeth on the anterior aspect of the ceratobranchials and pharyngobranchials 3 grading to conical teeth toward the posterior aspect.

Gut Contents

Laboratory reared specimens show no evidence of feeding as yolk-sac larvae. Green chyme, comprised presumably of dinoflagellates and algal cells, is seen during oil-globule absorption. Rotifers and copepod nauplii are taken soon after the absorption of the oil globule. The diet of wild larvae consists primarily of copepod nauplii and copepodites with some juvenile pelecypods and cladocerans. The size of preferred food organisms increases with the size of the larvae (Kjelson et al., 1975; Table 2), and, as the larva grows, adult calanoids enter the diet. During transformation, epibenthic harpacticoid copepods (*Phyllopodopsyllus* spp.) dominate the diet (Table 2). Juveniles feed on detritus and epifaunal invertebrates, mainly annelids (Parker, 1971; Peters and Kjelson, 1975; Chao and Musick, 1977; Sheridan, 1978).

Table 1. Tooth counts of larval Leiostomus xanthurus. Premaxillary and dentary teeth were counted on the left side and counts multiplied by two; pharyngeal teeth were observed but not counted.

Age	Notochord/ Standard Length	Premaxillary Teeth	Dentary Teeth	Epibranchial Teeth	Ceratobranchial Teeth
4	2.4	-	-	-	-
7	2.4	-	-	-	-
	2.7	-	-	-	-
	3.0	-	-	-	-
	3.7	-	-	-	-
	4.0	2	-	-	-
23	4.5	10	-	-	-
28	4.3	8	-	-	-
28	5.2	10	2	-	-
30	5.2	10	-	-	-
35	5.5	14	-	+	+
35	6.4	12	6	-	-
36	5.1	14	2	-	-
36	10.0	30	22	+	+
41	5.4	12	-	-	-
41	8.9	20	18	+	+
42	10.5	30	16	+	+
43	5.8	12	8	-	-
50	6.6	18	12	-	-
54	8.0	18	16	+	+
54	8.2	16	14	+	+
	12.6	32	26	+	+
	12.6	44	38	+	+
	12.9	40	32	+	+
	12.9	34	50	+	+
	13.2	52	58	+	+
	13.9	30	48	+	+
	14.5	90	62	+	+
	17.7	108	76	+	+
	18.5	116	78	+	+
	19.1	108	72	+	+
	19.4	116	88	+	+
	20.0	108	86	+	+
	20.0	76	72	+	+
	20.1	96	76	+	+
	20.3	106	78	+	+
	20.5	68	80	+	+
	21.0	108	86	+	+

Table 1. (concluded)

Age	Notochord/ Standard Length	Premaxillary Teeth	Dentary Teeth	Epibranchial Teeth	Ceratobranchial Teeth
	21.0	88	86	+	+
	21.5	102	98	+	+
	22.9	70	64	+	+
	23.9	64	62	+	+
	24.2	64	74	+	+

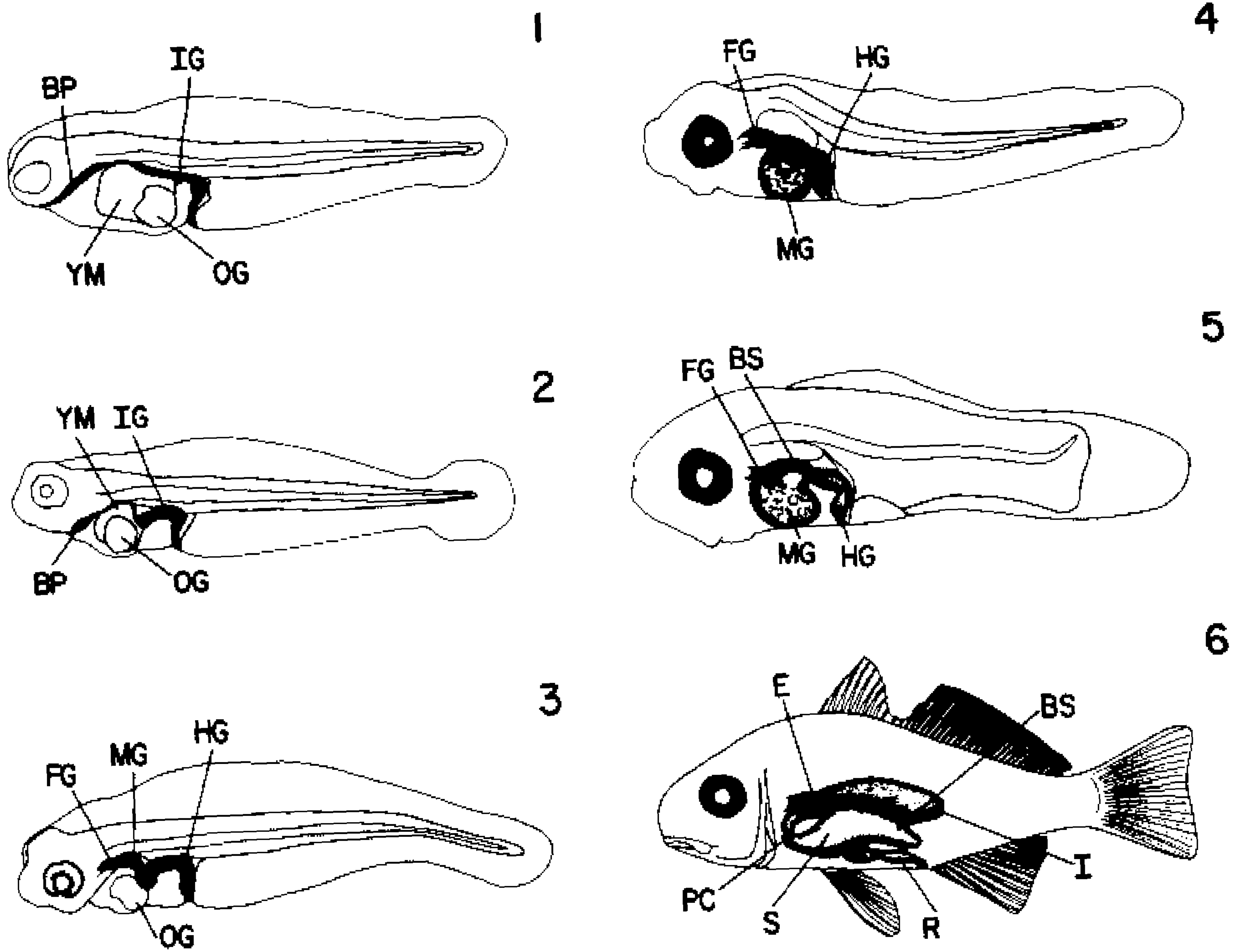
Table 2. Gut contents of larval spot, _Leiostomus xanthurus_ (percent occurrence).

Author Locality	This Study Continental Shelf, USA	This Study Continental Shelf, USA	This Study Continental Shelf, USA and James River, VA	Kjelson et al., 1975 Newport River, NC
No. Specimens	14	22	23	120
Length Range	2.4-4.5 mm	4.5-7.0 mm	7.0-19.5 mm	17-24 mm
% empty	39	44	4	Not Reported
Microzooplankton:				
Gastropoda	20			
Cladocera		17	6	
Copepoda				
Calanoida				10
Nauplii			12	
Copepodites	40	17	3	
Adults	20	34	38	
Centropages				28
Temora				21
Acartia				8

Table 2. (concluded)

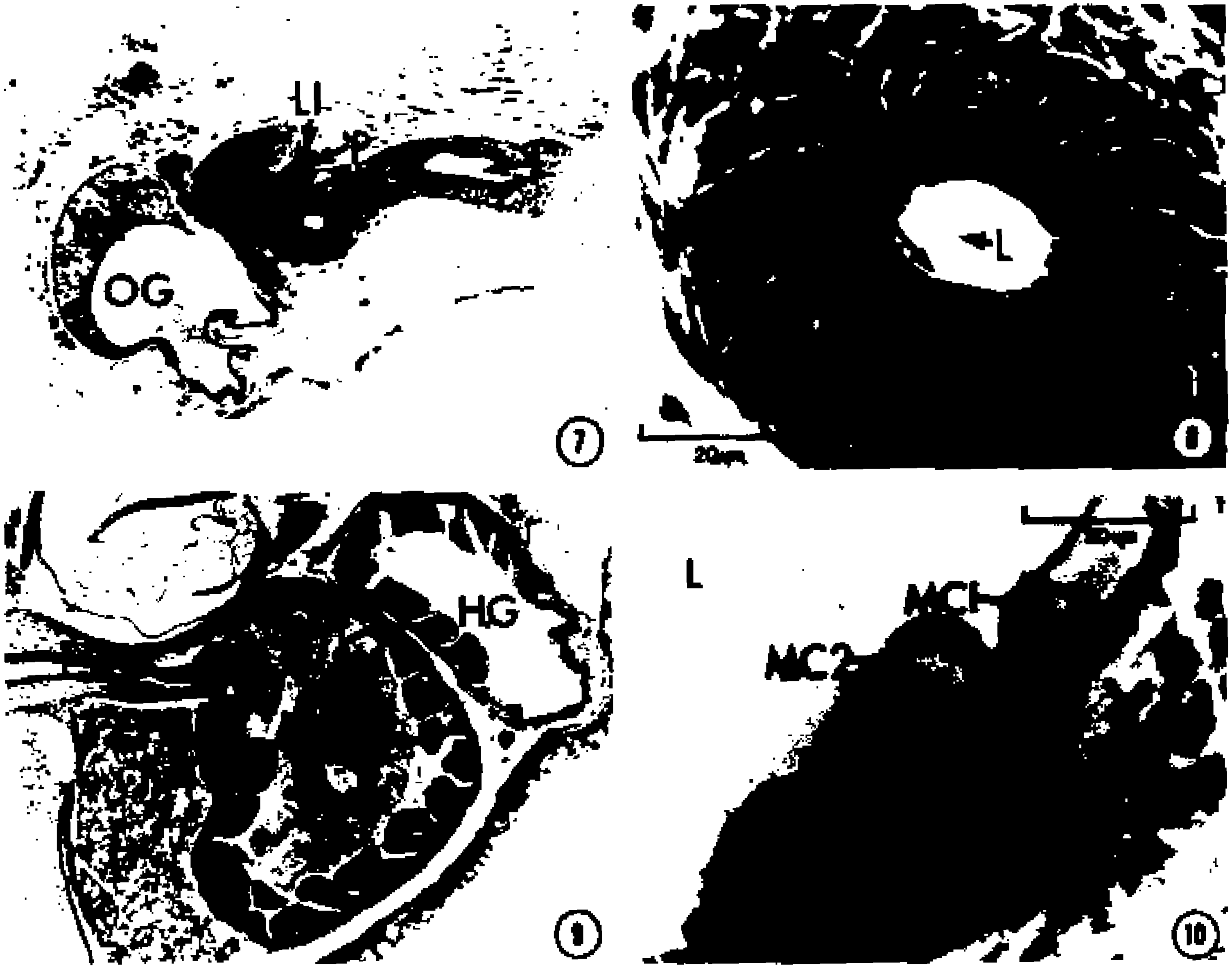
Author Locality	This Study Continental Shelf, USA	This Study Continental Shelf, USA	This Study Continental Shelf, USA and James River, VA	Kjelson et al., 1975 Newport River, NC
No. Specimens	14	22	23	120
Length Range	2.4-4.5 mm	4.5-7.0 mm	7.0-19.5 mm	17-24 mm
% empty	39	44	4	Not Reported
Invertebrate Eggs			6	
Others Remains	20	34		1
Epibenthos				
Amphipoda			3	
Copepoda				
Harpacticoida				32
Phyllopodopsyllus			14	
Others Remains			12	
Infauna:				
Gastropoda			3	
Vegetable Detritus			3	

Figure 1-6. *Morphological development of the alimentary canal of* Leiostomus xanthurus. 1. Incipient gut of a yolk-sac larva 2.2 mm NL. 2. Incipient gut of a yolk-sac larva 2.3 mm NL. 3. Incipient gut of a larva 2.8 mm NL in oil-globule absorption. 4. Alimentary canal of a larva 3.0 mm NL. 5. Alimentary canal of a transforming larva 7.6 mm SL. 6. Alimentary canal of an early juvenile 25.0 mm SL. Abbreviations: BP, bucco-pharynx; BS, blind sac; E, esophagus; FG, foregut; HG, hindgut; I, intestine; IG, incipient gut; MG, midgut; OG, oil globule; PC, pyloric ceaca; R, rectum; S, stomach; YM, yolkmass.

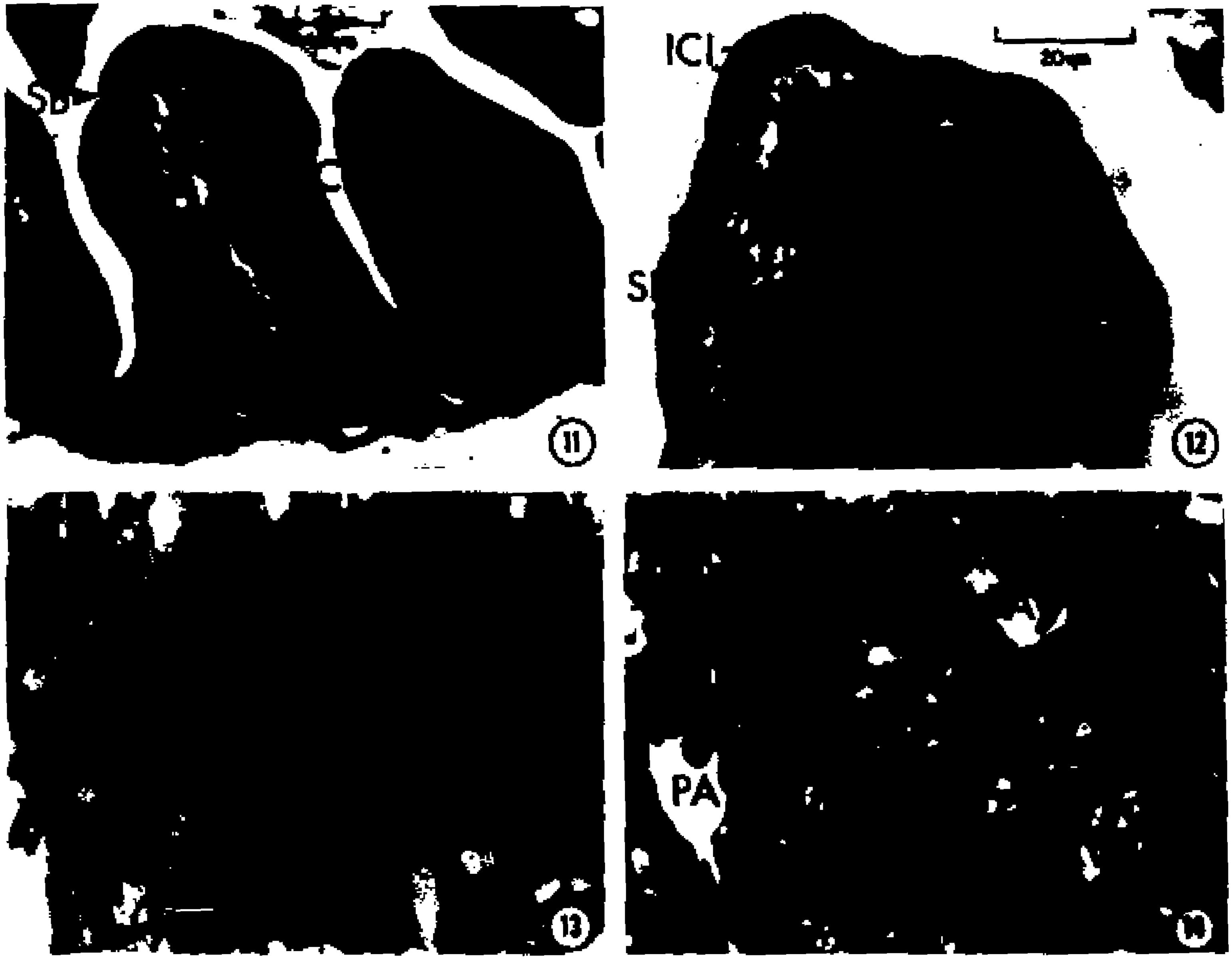

1
BP
IG
YM
OG
2
YM IG
BP
OG
3
FG
MG
HG
OG
4
FG
HG
MG
5
FG BS
MG HG
6
E
BS
I
PC
S
R

Figures 7-8. Photomicrographs of the incipient gut of a _Leiostomus xanthurus_ yolk-sac larva 2.3 mm NL. 7. Longitudinal section showing yolk mass, oil globule, incipient gut, liver, and pancreas. 8. Cross section showing mucosal epithelium and striated.

Figures 9-10. Photomicrographs of the alimentary canal of a _Leiostomus xanthurus_ larva 4.4 mm NL. 9. Longitudinal section showing foregut, midgut, hindgut, liver, and pancreas. 10. Longitudinal section showing two types of mucous cells in foregut. Abbreviations: CT, connective tissue; FG, foregut; EP, epithelium; HG, hindgut; IG, incipient gut; L, lumen; LI, liver; MG, midgut; MC1, mucous cell type 1, MC2, mucous cell type 2; OG, oil globule; P, pancreas; SB, striated border; YM, yolkmass.


LI
OG
7
L
20µm
8
HG
9
L
MC1
MC2
10

Figures 11-14. Photomicrographs of the alimentary canal of a *Leiostomus xanthurus* larva 4.4 mm NL. 11. Longitudinal section showing midgut. 12. Longitudinal section showing hindgut epithelium with striated border and intracellular inclusions. 13. Longitudinal section showing liver acini with liver sinus, hepatocytes, and glycogen vacuoles. 14. Longitudinal section showing pancreatic acini and zymogen granules. Abbreviations: EP, epithelium; GV, glycogen vacuoles; H, hepatocytes; ICI, intracellular inclusions; LS, liver sinus; PA, pancreatic acini; SB, striated border; ZG, zymogen granules.


SD
11
ICL
S
12
13
PA

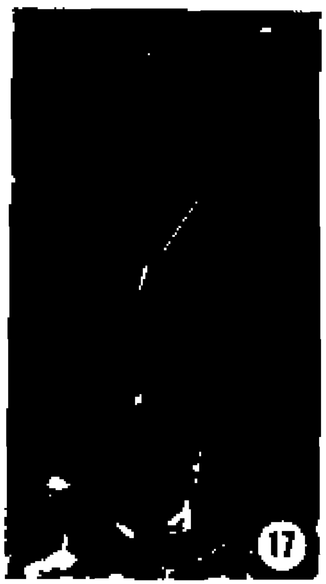
Figures 15-16. Photomicrographs of a transforming Leiostomus xanthurus larva 12.3 mm SL. 15. Longitudinal section showing junction of bucco-pharynx and esophagus with esophageal folds and increased number of mucous cells. 16. Longitudinal section showing the junction of the esophagus and blind sac with developing gastric glands.

Figure 17. Photomicrograph of an early juvenile Leiostomus xanthurus 18.1 mm SL. Longitudinal section of gastric corpus of a fish 18.1 mm SL showing uniform secretory cells of the gastric glands.

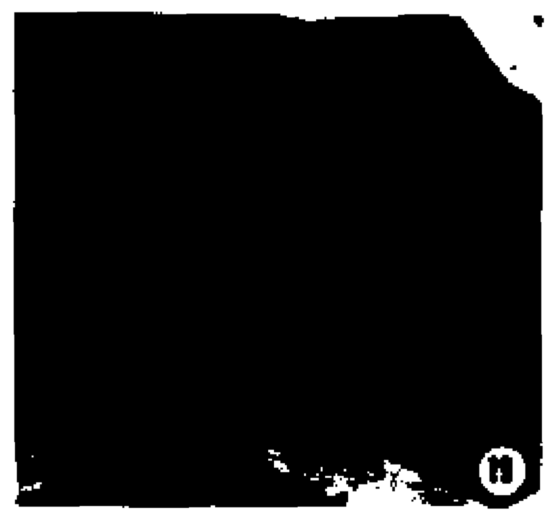
Figures 18-19. Photomicrographs of an early juvenile Leiostomus xanthurus 33.0 mm SL. 18. Longitudinal section of the intestine showing intracellular inclusions. 19. Longitudinal section of the rectum showing granular intracellular inclusions. Abbreviations: BP, bucco-pharynx; BS, blind sac; E, esophagus; GG, gastric glands; ICI, intracellular inclusions; MU, mucosa.


BP
T
E
15


GG
E


17


18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DISCUSSION

The ability to acquire, digest, and assimilate nutrients should be maximized by adaptation throughout the development of a fish. The numerous mitochondria and intracellular inclusions in the mid- and hindgut epithelium, as well as the histology of the liver and pancreas, suggest that the alimentary canal becomes functional during oil-globule absorption when first feeding may occur. There is no elaboration of cell types from first feeding until transformation, though the surface area increases. The liver expands rapidly in the larva and is active in glycogen storage. Ehrlich (1974a, b) has found that protein and carbohydrate are transferred to body tissues preferentially over trigylcerides in larval _Clupea harengus_ and _Pleuronectes platessa_. Growth is of prime importance to the pelagic marine larva in enhancing predator avoidance and feeding success. Protein deposition is undoubtedly manifested as larval growth, while high carbohydrate storage as hepatic glycogen possibly has a sparing effect on protein catabolism; Cowey et al. (1975) demonstrated such an effect in adult _P. platessa_. The bulbous midgut perhaps serves a function similar to a morphological stomach in allowing the ingestion and storage of many food items, an adaptation which is advantageous to a pelagic marine larva confronted with a patchy food supply.

The major changes in the development of the alimentary canal and associated structures are accompanied by changes in habitat and feeding regimen. The retrorse, conical, larval teeth are well suited for seizure of pelagic copepod nauplii and copepodites while the mucous cells of the bucco-pharynx and foregut aid in ingestion. The movement of transforming larvae into estuarine nursery areas (Chao and Musick, 1977) is concomitant with the differentiation of the esophagus, stomach, intestine, and rectum, as well as the appearance of the adult complement of villiform jaw and molariform pharyngeal teeth. This corresponds with a diet shift to epi- and infaunal invertebrates. The functional development of taste buds must aid in the selection of these food items while the pharyngeal teeth and the increase in mucous cells aid in mastication and ingestion. Complete development of gastric glands presumably aid with preliminary proteolysis.

The sequence of the alimentary canal development in spot is similar to that described for other marine fishes (Harder, 1960; Fukusho, 1972; others below) with some notable specializations. The intortion of the alimentary canal to form a loop is common among paracanthopterygian and acanthopterygian larvae (elopomorph and protacanthopterygian larvae have a straight gut); but it is not always simultaneous with yolk-sac or oil-globule absorption (e.g., the case of *Trachurus symmetricus*: Theilacker, 1978). Goblet or mucous cells are present in the bucco-pharynx and foregut in spot as well as most other larvae (Tanaka 1969a, b; Tanaka 1971; Vu 1976). In contrast, O'Connell (1976) found them only at the pharyngeal port in *Engraulis mordax* and Vu (1976) found goblet cells in the mid- and hindgut of larval Dicentrarchus labrax. The occurrence of two types of mucous cells in the larval foregut concurs with Reifel and Travill (1977) who described six different esophageal mucous cell types in adults with pairs of types associated with five particular families of freshwater teleosts. The apparent absence of Type 2 mucous cells in juvenile spot suggests that these cells are either transient larval structures or simply Type 1 cells that have expelled their contents and are being sloughed off. Taste buds appear in the bucco-pharynx of larval spot, but are presumed not functional until the early juvenile phase. In many teleosts with demersal eggs, however, taste buds are found in yolk-sac larvae (Tanaka, 1969a). The controversial rodlet or pear-shaped cell (see reviews in Kapoor et al., 1975; Morrison and Odense, 1978), also identified as the sporozoan Rhadbospora thelohani (e.g., Bannister, 1966), has been found in the alimentary canal of many fishes including the young of laboratory cultured Scophthalmus maximus (Anderson and Roberts, 1976). Smallwood and Smallwood (1931) referred to intestinal secretory cells, possibly identical to these pear-shaped cells (Barrington, 1957), in larval Cyprinus carpio. Alkaline blue 6B is particularly useful in demonstrating these parasites (Ruddell, personal communication), yet they were not found in larval or early juvenile spot.

The mechanism of lipid and protein digestion in larval and juvenile fishes may warrent further research. The epithelial cells of spot's larval mid- and hindgut are similar cytologically to those of six species described by Iwai (1969). He concluded that the vacuolar inclusions of the midgut contain lipids, resynthesized after hydrolysis and absorption, and that the granular inclusions of the hindgut contain protein, the result of pinocytosis from the lumen. Kawai and Ikeda (1973) and Tanaka et al. (1972) reported weak peptic activity in larvae before the differentiation of gastric glands while tryptic activity appeared shortly after hatching and increased gradually with growth. The pancreas of spot and other larvae (e.g. Umeda and Ochiai, 1973; O'Connell, 1976; Vu, 1976) contains conspicuous zymogen granules presumably comprised of trypsin. Tryptic activity alone might not completely hydrolyze proteins to amino acids without preliminary peptic digestion, and Yamamoto (1966) and Stroband (1977) have made speculative correlations between the lack of peptic digestion in stomachless adult teleosts and the incidence of pinocyctotic proteinaceous, intracellular inclusions. Accordingly, pinocytosis of protein compensates for incomplete proteolysis as might be the case in larval fishes (Iwai, 1969). While the location of absorption agrees with adult fishes (Fange and Grove, 1979), one might suspect complete proteolysis after the development of gastric glands. Yamamoto (1966) has suggested this based on the lack of pinocytotic invaginations and granular intracellular inclusions in adult _Salmo irideus_. Yet, acidophilic intracellular inclusions occur in early juvenile spot even after complete stomach differentiation. O'Connell (1976) also reports findings that contrast Iwai's hypothesis.

The alimentary canal and associated structures of spot, similar to that of other marine teleosts, becomes functional at first feeding and change little during the larval phase. Other than the expansion of alimentary canal surface area, there are no morphological or histological changes that could effect major changes in digestion or assimilation prior to transformation. The complete development of a stomach with functional gastric glands during transformation may influence digestion and assimilation and is accompanied by changes in habitat and feeding regimen.

EXPERIMENTAL RADIOTRACER STUDIES OF THE SHORT-TERM CARBON ASSIMILATION

INTRODUCTION

Our understanding of larval fish starvation and growth and the relationship of these processes to stock recruitment has been recently advanced by the application of physiological energetic principles. In physiological energetic models developed by Ivlev (1939) and Winberg (1956) and later refined by Paloheimo and Dickie (1966a,b) and Warren and Davis (1967), a fish's energy budget can be expressed as:

$$Q_{+} = Q_{*} + Q' + Q_{-} \qquad (1)$$

where = Q_{+} = energy of food consumed (ingesta)

Q_{*} = energy of waste products in feces and urine (egesta and excreta)

Q' = energy of growth

Q_{-} = energy of metabolism.

Rearranging gives:

$$Q_{+} - Q_{*} = Q' + Q_{-} \qquad (2)$$

or

$$bQ_{+} = Q' + Q_{-} \qquad (3)$$

(symbols following Laurence, 1977). The coefficient, b, represents assimilation and is generally expressed as either a coefficient, the coefficient of utilization (Winberg, 1956); or as a percentage, absorption efficiency (Kapoor et al., 1975). Assimilation, defined as that part of the food intake which is utilized (Brett and Groves, 1979) is difficult to measure directly in aquatic animals largely because it is difficult to separate egesta from excreta (Johannes and Satomi, 1967; Conover, 1978). The coefficient of utilization represents the fraction of metabolizable energy available after losses in feces (the result of incomplete absorption from the alimentary canal) and in urine or through the gills (largely the result of deamination of proteins). Absorption efficiency, the difference between the matter-energy in ingesta and egesta expressed as a percentage, has been customarily used synonomously with assimilation efficiency. Such estimates might be biased, however, if some egesta is confounded with excreta. The coefficient of utilization differs from absorption efficiency and is easier to measure as defecatory and excretory losses are combined. Because teleosts are ammonotelic (Forster and Goldstein, 1969), assimilation should be the dominant process encompassed by the coefficient, especially if carbon is used as a measure.

As originally formulated, these equations used energy units (calories), but others have substituted total wet or dry weight, weights of carbohydrates, fats, and proteins, or weights of total carbon and nitrogen (see reviews in Brett and Groves, 1979; Conover, 1978). Matter and energy are thus used interchangeably, though direct analogies are not always valid (e.g., Wiegert, 1968).

Important progress has been made in understanding the biological processes represented by the various terms of energetic models with larval fishes (Table 3), but most of these studies dealt with freshwater species with precocious larvae. Furthermore, few attempts have estimated energetic parameters of marine larvae or assessed changes in these terms during larval development; though it is well known that respiration and excretion rates, and conversion efficiencies can change with body weight (see review by Brett and Groves, 1979). Laurence (1977), an exception, estimated rates of ingestion, metabolism, and growth in a larval marine fish and used these estimates to simulate changes in assimilation with development. Rosenthal and Hempel (1970) and Nishikawa (1975) suggested that digestion improves with larval age as the result of the morphological development of the alimentary canal, but there is little direct evidence to support this.

This study was undertaken to estimate short-term carbon assimilation in discrete age cohorts of larval spot, Leiostomus xanthurus, to assess changes in assimilation, and to relate such changes to development. The coefficient of utilization and absorption efficiency were used herein to assess carbon assimilation.

Table 3. Bioenergetic components of larval fishes on various diets. K_1, gross conversion efficiency; K_2, net conversion efficiency; Q^-, respiration; b, coefficient of utilization.

Species	Age, Length, or Weight	Food	Food Concentration	Method of Determination	K_1 %	K_2 %	Q^- %	Assimilation or b %	Author
Abramis brama	12-18 mm	*Bromia*	20,200,2000 organism/l	Radiotracer	46-56	60-74 (/day WW)	9.1-19.2	74-83	Sorokin and Panov (1966)
Pleuronectes platessa	0-45 days	Terrestrial Oligochaete	Maintenance Intermediate	Calorimetric	21-37	28.4-46.2	16.0-31.9	96.2-99.4	Calculated from Edwards et al. (1969)
Limanda limanda	0-32 days	Oligochaete	Unlimited		33-34	99	63.4-99.0	99.0-99.3	Calculated from Edwards et al. (1969)
Micropterus salmoides		Zooplankton	200-1200 organism/l	Calorimetric	20	30-60		80.0 (b)	Laurence (1971)
Perca fluviatilis	3.5 mg	Zooplankton		Gravimetric	22-42	14.8-62.2			Kudrinskaya (1970)
Lucioperca lucioperca	2.1 mg	Zooplankton			36-50	19.6-59.5			Kudrinskaya (1970)
Cyprinus carpio	1-5 mg DW	Zooplankton	10-100 mg/l	Calorimetric	35.5-36.0	45.9-47.0	32.5-45.4	74.0-86.6	Filatov (1972)
Archosargus rhomboidalis	2-16 days	Zooplankton		Calorimetric	23.9-30.6				Stepien (1976)
Lucioperca lucioperca		Zooplankton		Gravimetric	21-43	26-54	79.1-80.5		Kudrinskaya (1976)
Perca fluviatilis		Zooplankton		Gravimetric	20-38	26-48	79.4-83.3		Kudrinskaya (1976)
Abramis brama		Zooplankton		Gravimetric	33-45	35-41	80.1-80.2		Kudrinskaya (1976)
Rutilus rutilus		Zooplankton		Gravimetric	35-41	43-47	80.1-80.3		Kudrinskaya (1976)
Pseudopleuronectes americanus	16.4-667.6 (ug DW)	Zooplankton	2-3 Nauplii/(ml)	Calorimetric	5-33				Laurence (1977)

CONCEPTUAL FRAMEWORK

Radiotracer methodology, utilizing carbon-14 was used to compute two parameters that were taken as relative indices of the coefficient of utilization and absorption efficiency. Carbon retention, the index of the coefficient of utilization, was computed from the ^{14}C body burden retained in larvae and corrected for respiratory loss after ingestion and complete alimentary canal evacuation. Carbon absorption, the index of absorption efficiency, was computed from ^{14}C ingested and egested as particulates. Computations followed Sorokin (1966):

$$C = a \cdot r \tag{4}$$

where

C = total carbon transferred (mg C)

a = reciprocal of the food substrate specific activity $(\frac{\text{mg C}}{\text{DPM}})$

r = radioactivity in larvae or particulate egesta $(\frac{\text{DPM}}{\text{mgDW}})$

Carbon retention and carbon absorption were considered relative indices because use of Equation (4) to estimate carbon transfer has some methodological problems. Carbon-14 can enter several physiological processes with different rates within an animal and be lost through respiration or excretion. If this occurs during ingestion or alimentary canal evacuation, estimates of total carbon ingested or retained calculated with Equation (4) will be biased (Conover and Francis, 1973). To correct this bias, Lampert (1977) collected respired $^{14}CO_2$ and added these DPM to the body burden of his animals. This correction is not entirely adequate, however, because the specific activity of carbon lost as CO_2 fluctuates during of the experiment: it increases with time after ingestion as more ^{14}C is assimilated. Because the specific activity of carbon lost is unknown, the absolute amount of total carbon retained within an animal cannot be calculated. Such calculations require multicompartmental analysis (Robertson, 1956; Atkins, 1969; Jacquez, 1972); but data necessary to support these models are difficult to collect (Conover and Francis, 1973). Nonetheless, estimates of carbon assimilation using equations similar to Equation (4), compare well with calorimetric estimates, especially if estimates are adjusted for respiratory loss (Lampert, 1977). Moreover such estimates of carbon assimilation are comparable among age cohorts if any bias is constant.

Use of Equation (4) to estimate and assess changes in these indices is justified by the following assumptions, all addressed herein: (1) the food substrate is uniformly labeled, i.e., the biochemical fractionation of ^{14}C is constant; (2) the specific activity of the food substrate is constant during the feeding interval; (3) respiratory loss does not occur during the feeding interval (no corrections for such bias could be made); (4) the estimates of carbon retention and carbon absorption were taken at complete alimentary canal evacuation; and (5) any biases are constant among age cohorts.

MATERIALS AND METHODS

Experimental Animals

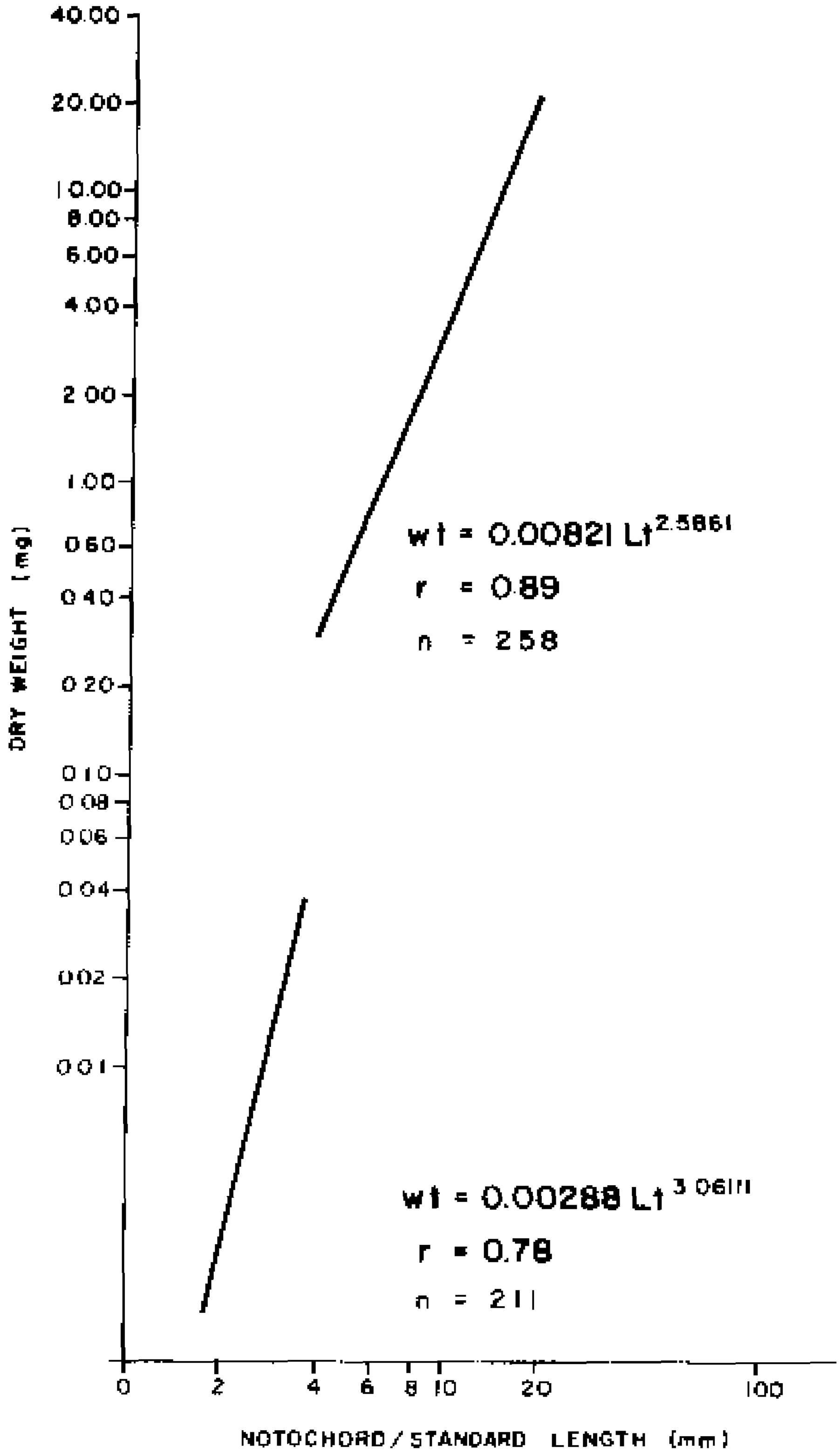
Spot larvae were obtained from laboratory cultured populations[2]. Artificially spawned and fertilized eggs were cultured in tanks at 20 ± 2°C and 30-35 ‰ salinity. This approximated conditions encountered in nature (see Early Life History section). After hatching larvae were maintained at concentrations of < 10/l with a photoperiod of 12 h light and dark and fed sequentially with the dinoflagellate, Gymnodinium splendens, the rotifer Brachionus plicatilis, and wild plankton at densities of 5 to 25 particles/ml. Larvae were removed from the culture tanks the evening before an experiment, placed in an experimental feeding tank, and fasted overnight. Statistics summarizing development of larval spot age cohorts are presented in Table 4. Condition factor, often used as an indicator of larval growth and robustness (Hempel and Blaxter, 1961; Blaxter, 1971), varied among cohorts. The growth exponents of weight and length regressions of larvae used in experiments (Figure 20) are somewhat lower than those reported by Laurence (1979). Larvae were generally healthy, however, and showed no histological signs diagnostic of starvation (Umeda and Ochiai, 1973; O'Connell, 1976; Theilacker, 1978).

Table 4. Summary of statistics characterizing development of discrete age cohorts of larval *Leiostomus xanthurus* used in carbon assimilation experiments.

Age (days from hatching)	Mean Notochord or Standard Length (mm)	Mean dry weight (mg)	Condition[a] Factor
7	1.8	0.022	37.7
8	2.5	0.050	32.0
9	2.2	0.034	31.9
14	2.8	0.075	34.1
15	3.1	0.048	16.1
16	3.2	0.140	42.7
31	6.6	1.330	46.3
34	6.8	1.505	47.9
43	11.4	4.426	29.9
47	9.2	2.320	29.8

[a] Condition factor = $\frac{\text{Mean dry weight}}{\text{Mean notochord length}^3} \times 10^4$

Figure 20. Weight and length regressions for Leiostomus xanthurus larvae used in carbon assimilation experiments. Upper line for postflexion larvae; lower line for preflexion larvae (Ahlstrom, 1968).


40.00
20.00
10.00
8.00
6.00
4.00
2.00
1.00
0.60
0.40
0.20
0.10
0.08
0.06
0.04
0.02
0.01
DRY WEIGHT (mg)
$wt = 0.00821\ Lt^{2.5861}$
$r = 0.89$
$n = 258$
$wt = 0.00288\ Lt^{3.0611}$
$r = 0.78$
$n = 211$
0
2
4
6
8
10
20
100
NOTOCHORD / STANDARD LENGTH (mm)

Brachionus plicatilis was chosen as the experimental food organism because it is amenable to mass culture in the laboratory (Theilacker and McMaster, 1971) and is an adequate nutritional source for larval fishes (May, 1971; Solangi and Ogle, 1977). Preliminary experiments suggested that it could be readily labeled with ^{14}C and that larval spot consumed them from first feeding through transformation; though the behavioral mode of ingestion changed with development. This allowed the use of the same food substrate among experiments with differing age cohorts of larvae. The major nutritional components of *Brachionus*, cultured by the following protocol, were determined and values compared with the literature (Table 5). Total protein and lipid extractions followed Lowry et al., (1951), Bligh and Dyer (1959), and Hanson and Olley (1963). Carbohydrates were extracted by precipitating proteins from *Brachionus* homogenates with trichloroacetic acid, dissolving lipids in ethanol, and precipitating carbohydrates with NaCl. Extractions were assayed following Lowry et al., (1951), Barnes and Blackstock (1973), and a modification of the anthrone method (Strickland and Parsons, 1972).

Experimental Design

The experimental design included labeling of *Brachionus* and monitoring changes in its specific activity, estimating carbon ingested by feeding larvae, and estimating carbon retained and lost during alimentary canal evacuation (Figure 21). Experiments were conducted in $20 \pm 1°C$, 30-35 ‰ salinity, 0.45 μm filtered seawater "sterilized" by ultraviolet irradiation. Experiments began at the

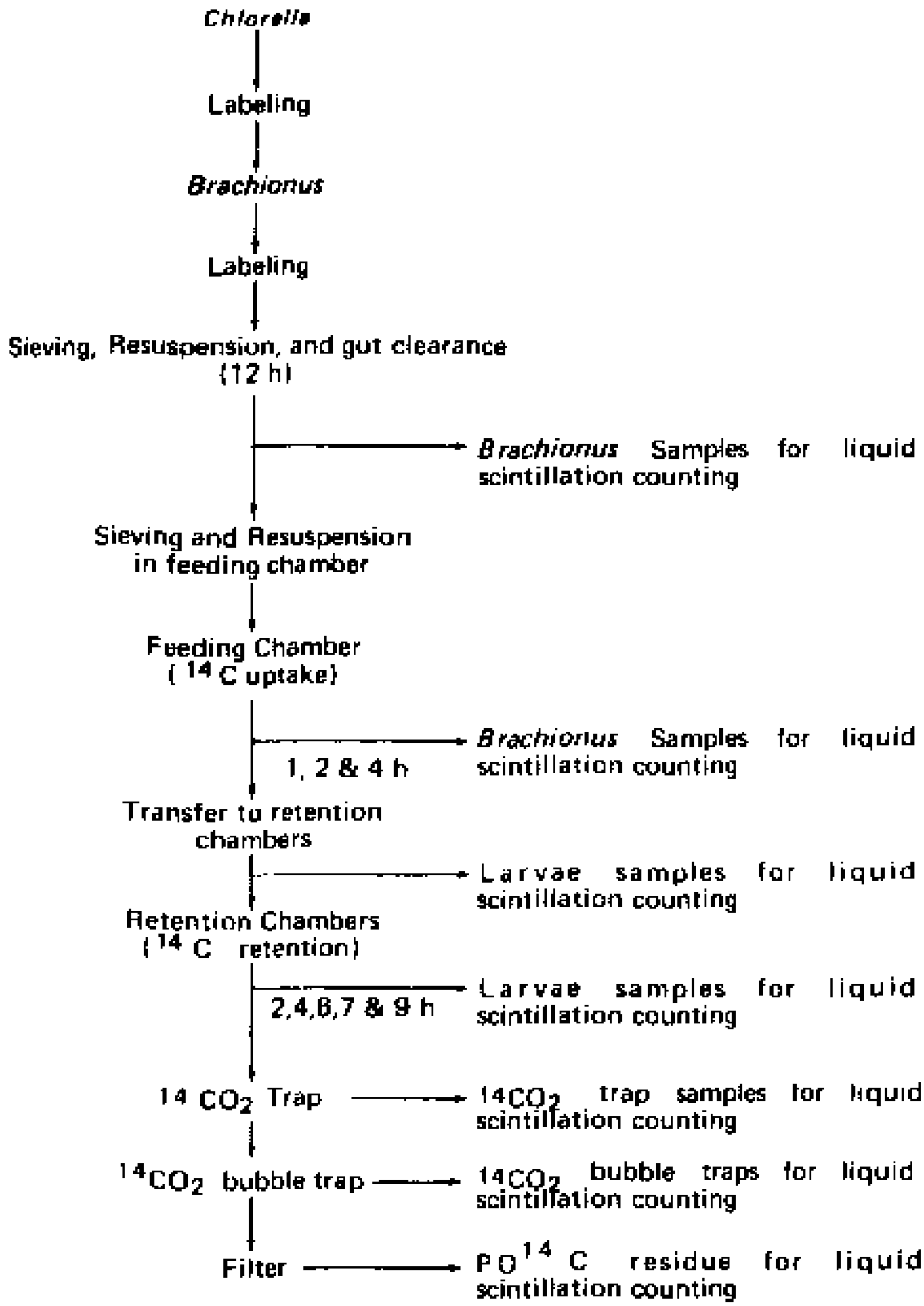
Table 5. Major nutritional components of *Brachionus plicatilis* expressed as a percentage of total dry weight.

Component	Present Study	Ehrlich (1972)	Scott and Baynes (1978)	Ogino and Watanabe (1978)
Protein	38.9		53.6[b]	58
Lipid	1.7	4.6[a]	11.2[b]	10
Carbohydrate	1.9	8.5	6.0[b]	6
Total carbon	41.7	33.1		
Total nitrogen	9.0	8.0		
C/N ratio	4.6	4.2		
Ash	7.0	8.5		

[a] Determined as total triglycerides.

[b] Calculated as grand mean of their experimental values.

Figure 21. Experimental design of carbon assimilation with age cohorts of larval *Leiostomus xanthurus*.



Chlorella
Labeling
Brachionus
Labeling
Sieving, Resuspension, and gut clearance (12 h)
Brachionus Samples for liquid scintillation counting
Sieving and Resuspension in feeding chamber
Feeding Chamber (^{14}C uptake)
1, 2 & 4 h
Brachionus Samples for liquid scintillation counting
Transfer to retention chambers
Larvae samples for liquid scintillation counting
Retention Chambers (^{14}C retention)
2,4,6,7 & 9 h
Larvae samples for liquid scintillation counting
$^{14}CO_2$ Trap
$^{14}CO_2$ trap samples for liquid scintillation counting
$^{14}CO_2$ bubble trap
$^{14}CO_2$ bubble traps for liquid scintillation counting
Filter
$PO^{14}C$ residue for liquid scintillation counting

beginning of the 12 h light photoperiod. Larvae were fed Brachionus at high densities (250-1000/ml) to allow the alimentary canal to fill and provide physiologically equivalent rations among age cohorts.

Brachionus was labeled following Sorokin (1966). Two l cultures (ca. 3.0×10^7 cells/ml) of the uncellular algae Chlorella sp. (strain Va 52) were innoculated with 200 µci of $NaH^{14}CO_3$ and cultured under constant illumination for 2 weeks to allow maximum population growth and isotope fixation (ca. 8.0×10^9 cells/ml; 3.0×10^{-3} CPM/cell). The culture medium (Dupuy et al., 1977) was adjusted to pH 9 with NaOH. Generally, Brachionus was fed high concentrafons (ca. 8.0×10^9 cells/ml) of Chlorella for 5 days or through 2 to 3 generations before use in an experiment. Prior to an experiment, Brachionus was isolated by gentle sieving on a 35 µm nitex screen, washed with and resuspended in seawater, and fasted for 12 h to clear their guts of labeled algae.

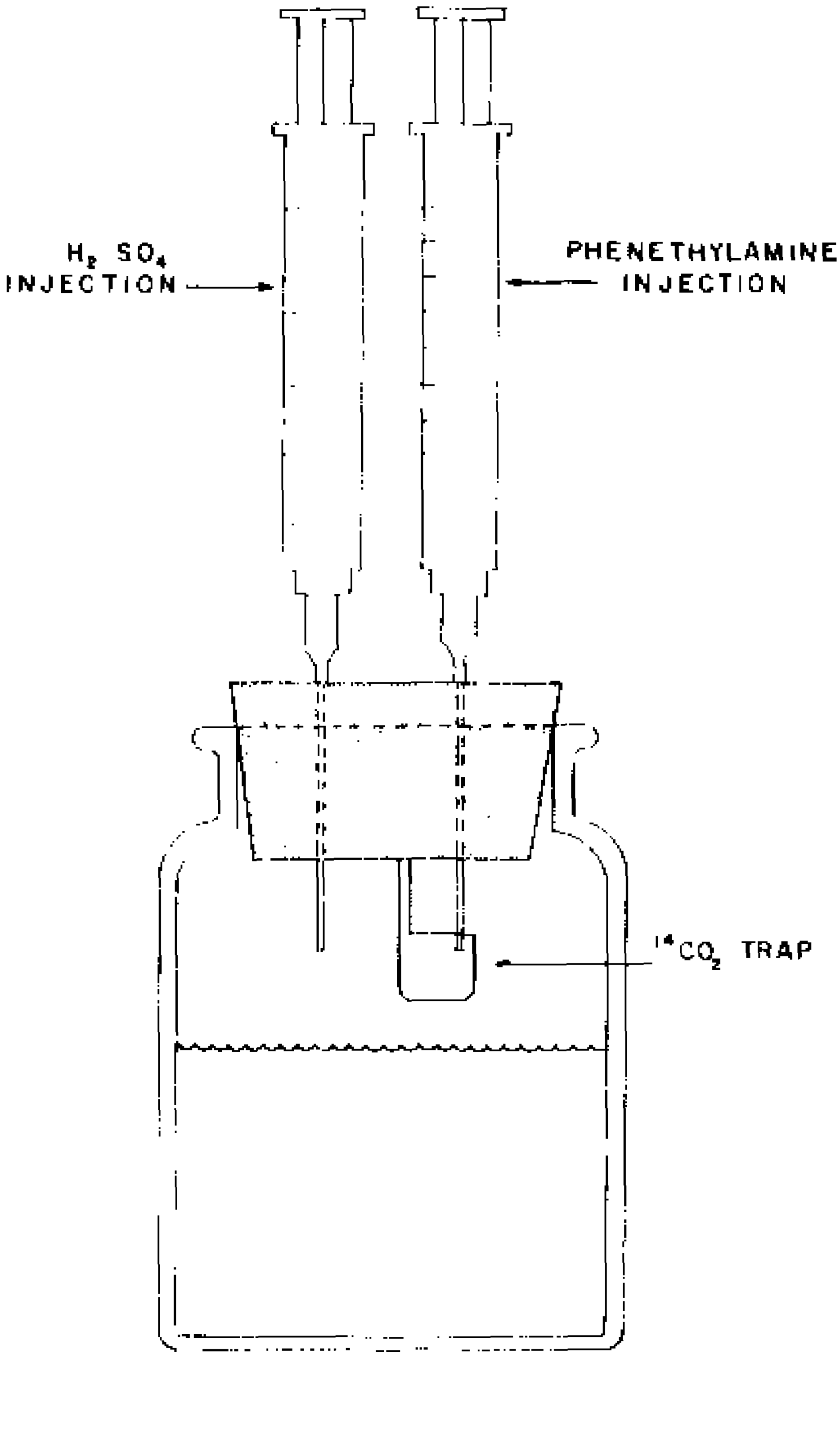
To assess label uniformity the distribution of ^{14}C in biochemical fractions in Brachionus was determined. Brachionus was labeled as described and samples collected after 2, 4, 6, and 8 days of labeling. Total proteins, lipids, and carbohydrates were extracted from these samples and the CPM of these extracts were expressed as ratios of total extracted CPM.

The specific activity of Brachionus was monitored by holding 1 l of labeled Brachionus in a beaker, sampling initially, and 1, 2, and 4 h thereafter. Triplicate aliquots were filterd through tared, glass-fiber filters, and washed twice with 0.5 N HCl and distilled water. They were then frozen, lyophilized, and stored within a desiccator in a freezer.

Carbon-14 uptake by larvae was estimated by adding labeled Brachionus to a feeding tank and sampling larvae at 1, 2, and 4 h. Triplicate samples were suffocated on a nitex screen, placed on tared, glass-fiber filters, and treated as described for Brachionus samples. Dead or moribund larvae were discarded.

Carbon-14 retention and loss by larvae was estimated by transferring larvae fed labeled Brachionus for 1, 2, and 4 h to a series of retention chambers (Figure 22) and sampling at 2, 4, 6, 7 and 9 h. Retention chambers were sealed with rubber stoppers fitted with $^{14}CO_2$ traps suspended in the atmosphere above the sea water. Traps consisted of a well with a 2.5 cm folded glass-fiber filter and two injection ports (Figure 22). Sampling involved injecting 200 µl of phenethylamine into the trap well and allowing any respired $^{14}CO_2$ in the atmosphere above the sea water to react for 5 minutes; the chamber was then opened and the larvae sampled as described. After removing larvae, the chambers were resealed and 0.3 ml of concentrated H_2SO_4 was injected into the water. Preliminary experiments indicated that this acidification lowered the pH below 4 and thus released $H^{14}CO_3$ as gaseous $^{14}CO_2$ to be collected in the suspended trap. Acidified chambers then were reopened. The phenethylamine and filter were removed, the trap well washed with 200 µl of 95% methanol, and the phenethylamine, filter, and washings placed in a scintillation vial for counting. To collect any remaining $^{14}CO_2$, the chambers were then

Figure 22. Schematic diagram of retention chambers used in carbon assimilation experiments with age cohorts of larval *Leiostomus xanthurus*.


H_2SO_4
INJECTION
PHENETHYLAMINE
INJECTION
$^{14}CO_2$ TRAP

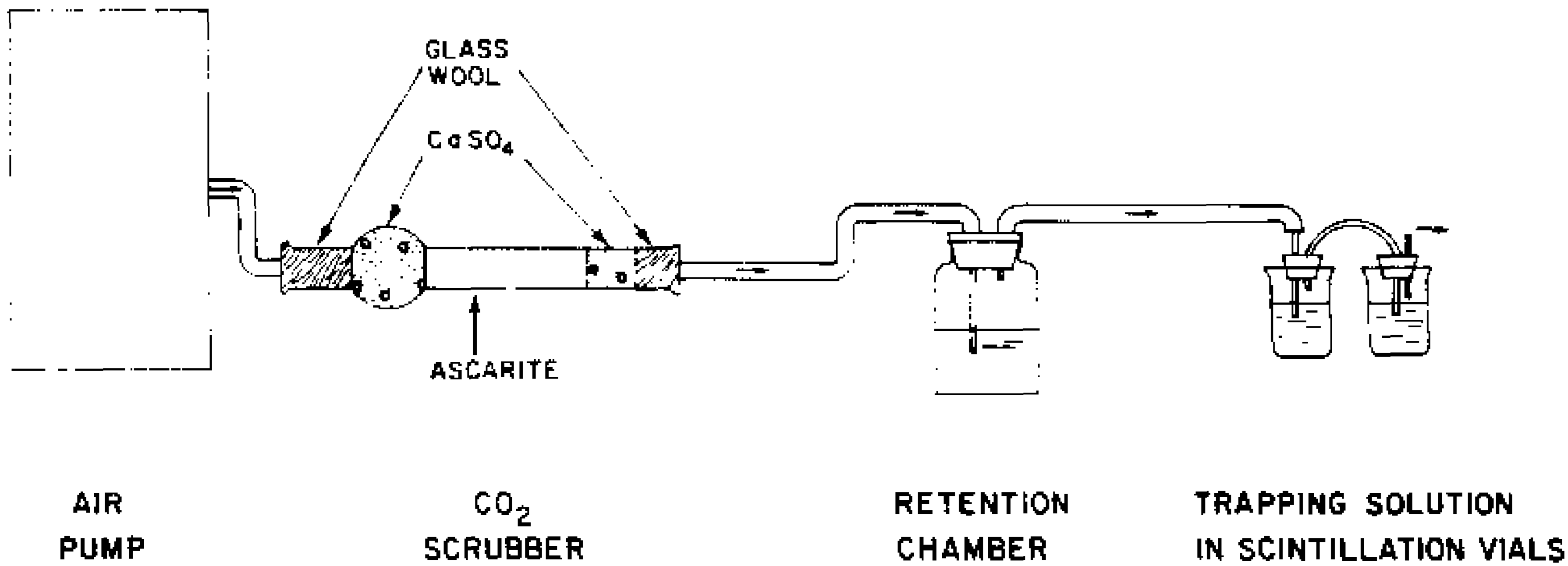
fitted to a bubble trap consisting of a series of two scintillation vials containing 5 ml of trap solution (Woeller 1961) and bubbled with CO_2-free air for 20 min to recover any remaining $^{14}CO_2$ (Figure 23). Following recovery of $^{14}CO_2$, the retention chamber sea water was assayed for ^{14}C egested as particulate organic carbon ($PO^{14}C$) by filtering through glass fiber filters. Filter samples were washed as described above.

Blanks, designed to assess ^{14}C contamination of retention chambers and larvae, showed that contamination was unimportant. Blanks consisted of a series of retention chambers to which the approximated maximum volume of water carried over during the transfer of larvae was added. Blanks were sampled for $^{14}CO_2$ and $PO^{14}C$ as described. To assess uptake of dissolved and particulate ^{14}C from the water, blank larvae were exposed for 1 h to feeding chamber water from which labeled *Brachionus* had been seived at the end of several experiments and sampled as described. Analysis of blanks showed that contamination of samples was variable but slight and averaged 9.7% ($^{14}CO_2$), 8.7% ($PO^{14}C$), and 0.3% (larvae). Because estimates were approximate and probably inflated, these errors were ignored.

Sample Processing

Samples of lyophilized *Brachionus* and larvae were removed from the freezer, brought to room temperature within the desiccator, weighed to the nearest μg on an electrobalance, placed in a glass scintillation vial, wetted with 300 μl of distilled water, and

Figure 23. Schematic diagram of bubble-trap for recovery of $^{14}CO_2$ in carbon assimilation experiments with age cohorts of larval *Leiostomus xanthurus*.


GLASS
WOOL
$CaSO_4$
ASCARITE
AIR
PUMP
CO_2
SCRUBBER
RETENTION
CHAMBER
TRAPPING SOLUTION
IN SCINTILLATION VIALS

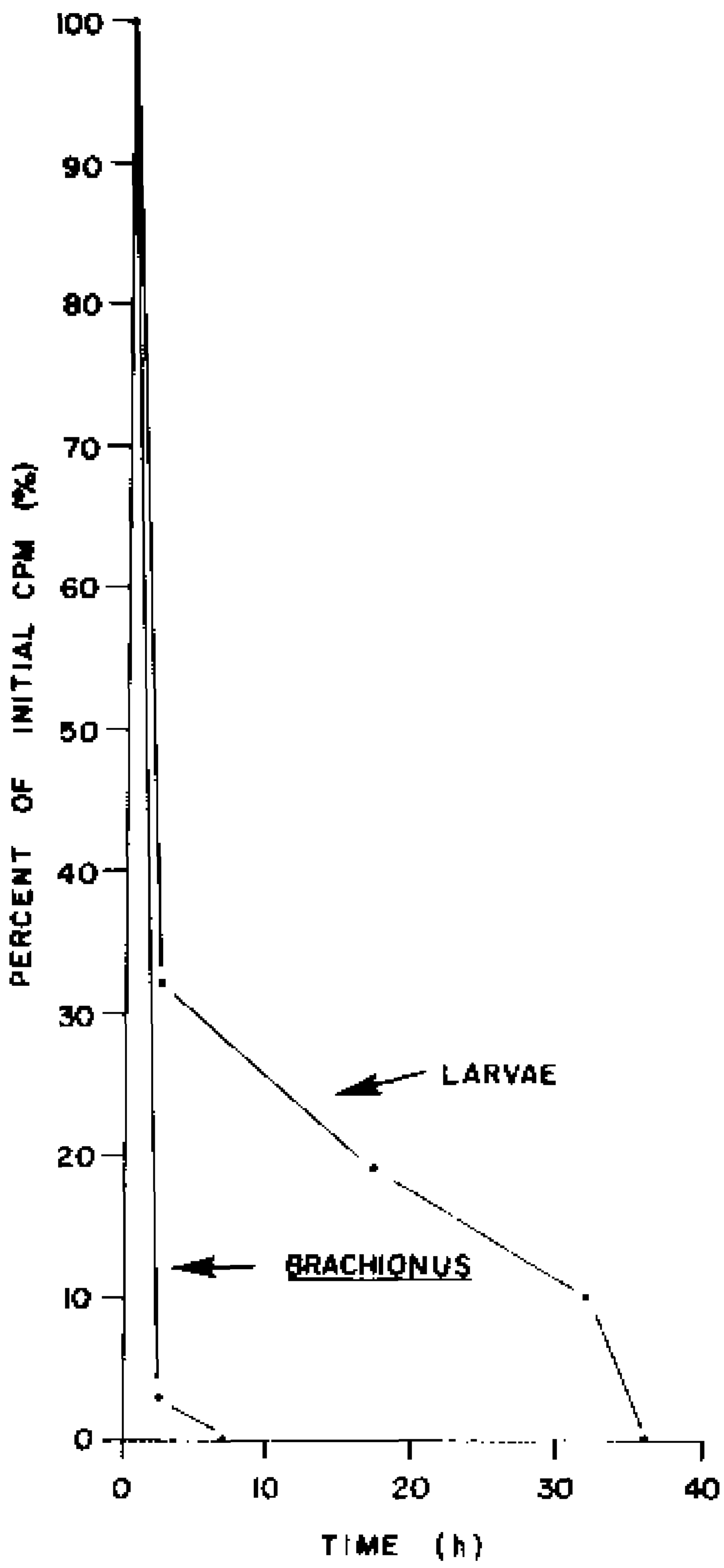
digested in 2 ml of NCS (Nuclear Chicago tissue solubilizer) for 1 to 2 days at 50°C. Particulate organic carbon-14 samples were treated similarly, but were not weighed. After digestion samples were then cooled to room temperature before adding scintillation cocktail (6 g POP, 0.05 g POPOP/l toluene). Brachionus, larvae, $PO^{14}C$, $^{14}CO_2$ trap, and $^{14}CO_2$ bubble-trap samples were combined with 15 ml of scintillation cocktail, held at room temperature in the dark for 36 h to decay chemiluminescence (Figure 24), and counted in a liquid scintillation counter for the entire ^{14}C pulse height spectrum at 2% error. Counts per minute (CPM) were corrected for counting efficiency and converted to disintegrations per minute (DPM) using quench curves (Figure 25). Mean counting efficiencies were used to correct trap (86.0%) and bubble trap (85.0%) samples.

Trap samples (DPM) were summed and corrected for recovery efficiency to give total ^{14}C respired by larvae. Recovery of total $^{14}CO_2$, determined in preliminary experiments with known quantities of $Na_2\,^{14}CO_3$, was 85% efficient (Table 6).

Notochord length was taken as the mean of a subsample in experiments with young age cohorts and as the mean of experimental samples for older cohorts.

Considerable variability was observed in the dry weight of larvae samples, especially in samples of smaller larvae. In experiments with larvae weighing <0.1 μg, the mean dry weight of the age cohort, calculated by winsorization, was used in body-burden calculations. In other experiments individual sample weights were used with values

Figure 24. Decay of chemiluminescent activity in larvae and *Brachionus* samples.



PERCENT OF INITIAL CPM (%)
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
TIME (h)
LARVAE
BRACHIONUS

Figure 25. Quench curves for larvae and *Brachionus* samples. Curves fitted by polynomial regression.

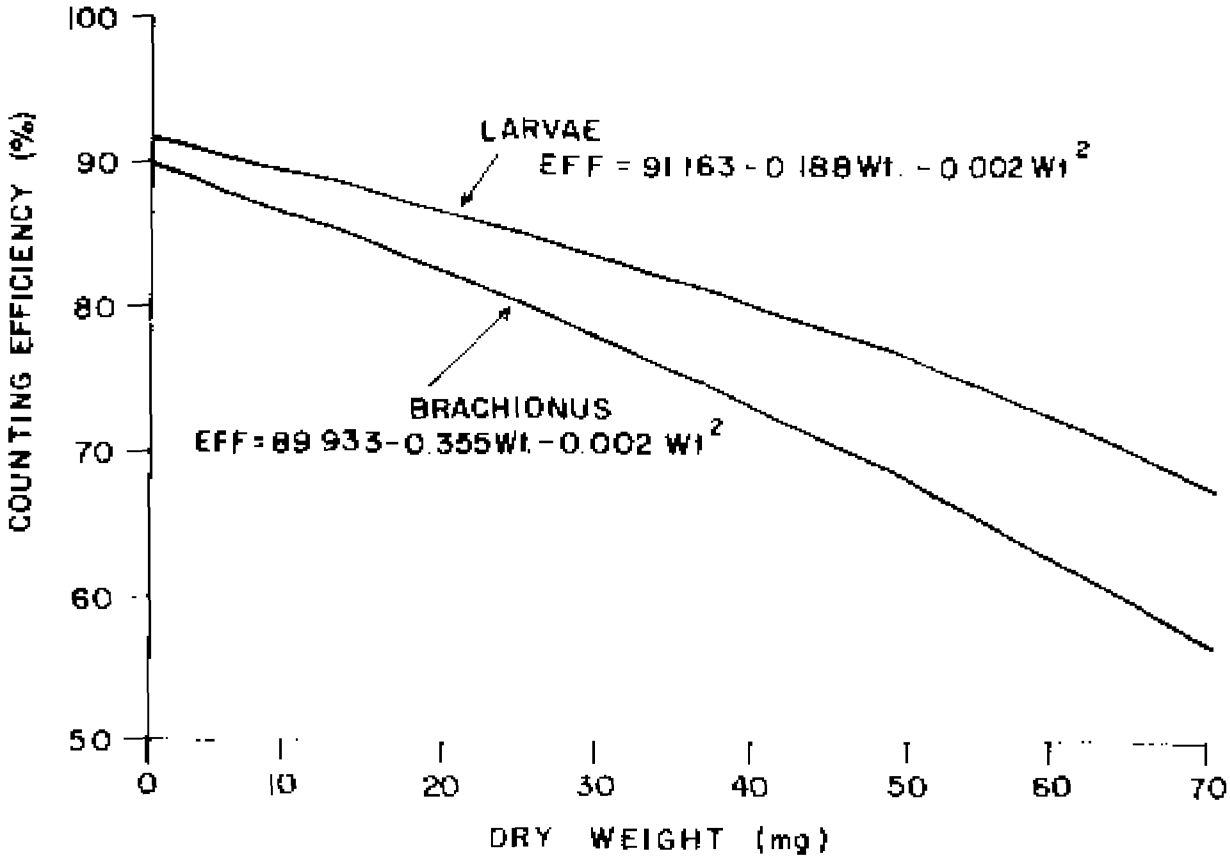

LARVAE
EFF = 91.163 - 0.188Wt. - 0.002 Wt^2
BRACHIONUS
EFF=89.933-0.355Wt.-0.002 Wt^2
COUNTING EFFICIENCY (%)
100
90
80
70
60
50
0
10
20
30
40
50
60
70
DRY WEIGHT (mg)

Table 6. Experimental recovery efficiency of $^{14}CO_2$. Summary of results with known quantities of $Na_2{}^{14}CO_3$.

DPM $Na_2{}^{14}CO_3$ added	DPM retention chamber trap	DPM bubble trap 1	DPM bubble trap 2	Total DPM recovered	Recovery efficiency (%)
138453.0	46544.7	51294.4	14231.4	112070.5	80.9
	54781.6	50874.9	9414.2	115070.7	83.1
	51466.7	53350.6	14717.5	119534.9	86.3
				mean	83.4
69226.5	23068.2	29966.1	8350.5	61384.8	83.9
	22386.9	29369.1	6303.8	58059.8	83.9
	13063.8	32035.4	7936.9	53036.1	76.7
				mean	83.1
34613.2	14162.9	13599.6	3193.9	30956.4	89.4
	12053.1	11290.0	5899.9	26243.0	75.8
	13520.1	11527.8	2818.7	27866.6	80.5
				mean	81.9
6922.6	2154.9	3042.5	1074.9	6272.3	90.6
	2132.1	4265.1	579.9	6977.1	100.0
	2734.2	3186.2	347.2	6267.6	90.5
				mean	93.7
		Mean of all observations			85.1

predicted from the weight and length regressions used for the few missing or suspect weights.

Mean total carbon content of Brachionus, (41.7%, Table 4), used to calculate specific activity (a of Equation 4), was determined by gas chromatography.

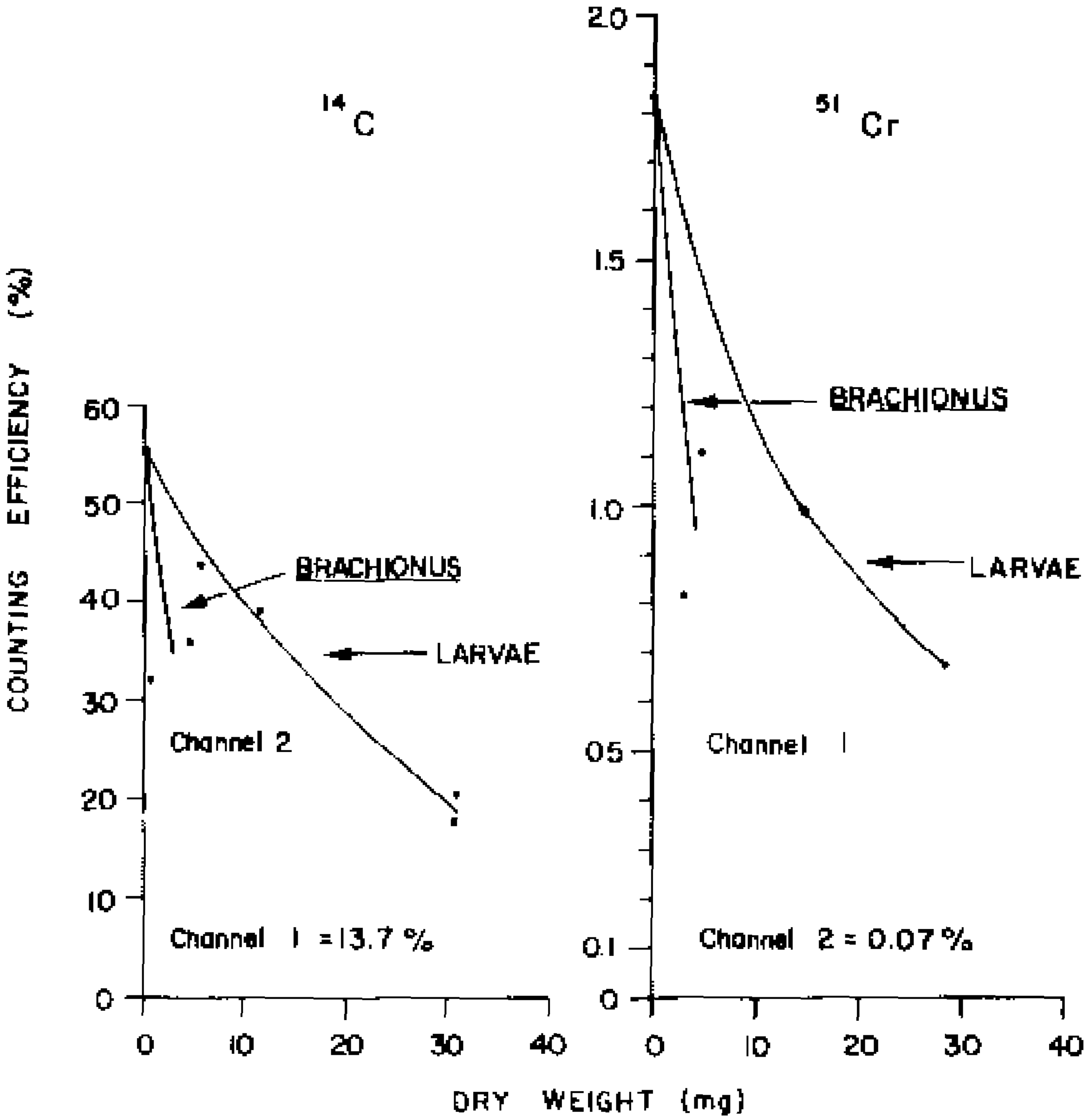
Data Processing

Carbon-14 uptake, retained, and lost, as well as carbon retention and absorption were related to development by stepwise-multiple regression. Percentage scores (transformed to the arcsine in degrees) were regressed on time (log transformed or polynomial functions), age, length, and weight (measures of development), and condition factor. In some cases the number of observations was low compared with the number of independent variables, but the matrix of simple correlation coefficients allowed assessment of associations.

Ancillary Experiments

Dual-tracer experiments were conducted to compare the behavior of an assimilated and metabolized tracer (^{14}C) against that of an unassimilated and inert tracer (^{51}Cr). This design also allowed an alternate calculation of absorption efficiency. Carbon-14 labeled Brachionus was suspended in 1 l of seawater containing ca. 200 μci $^{51}CrCl_3$ and were allowed to fix ^{51}Cr for 12 h. Experiments were conducted following general protocol. Samples were counted by the isotope exclusion method (Kobayashi and Maudsley, 1970; Sheppard and Marlow, 1971). Quench curves for ^{14}C and ^{51}Cr are shown in Figure 26.

Figure 26. Quench curves for larvae and *Brachionus* samples from dual label experiments. Curves fitted by eye.



COUNTING EFFICIENCY (%)
14 C
51 Cr
BRACHIONUS
LARVAE
Channel 2
Channel 1 = 13.7 %
BRACHIONUS
LARVAE
Channel 1
Channel 2 = 0.07 %
DRY WEIGHT (mg)

Results were used to calculate carbon retention, carbon absorption, and absorption efficiency by the indicator-ratio method (Calow and Fletcher, 1972; Wightman, 1975; Cammen, 1977).

Several experiments were conducted with a reduced, more environmentally realistic food concentration, because digestion and assimilation might possibly be less efficient under maximal feeding. Experiments were conducted as described above with several age cohorts using a Brachionus concentration of 1-10 rotifers/l.

A comparison was made between the number of rotifers ingested (calculated using the total carbon per dry weight of Brachionus, values of the weight of an individual rotifer, and values of carbon ingested) and visual counts of the number of rotifers in alimentary canals excised from larvae. Values used for the dry weight of an individual rotifer were 0.16 μg (Theilacker and McMaster, 1971) and 0.75 μg per rotifer (Scott and Baynes, 1978). The number of rotifers ingested per larva was estimated as follows:

$$\text{Number of rotifers} = \frac{\dfrac{\text{mg C ingested/larvae}}{\text{mg C/mg DW rotifers}}\ \text{(calculated by Equation [4])}}{\text{mg DW/rotifer}} .$$

These estimates were compared with counts of rotifers excised from a subsample of larvae thus serving as a check of experimental accuracy.

RESULTS

Carbon-14 Uptake, Retention, and Loss

Specific activity estimates of Brachionus were consistently high among experiments and highly variable both within and among experiments (Table 6). Regressions of Brachionus body burden on time indicated that the slope was never significantly different from zero (Table 7). Thus, the specific activity of Brachionus was considered constant; the mean of all Brachionus specific activity estimates within each experiment was used in calculations of carbon ingested, retained, and egested.

Experiments to assess the biochemical fractionation of ^{14}C in Brachionus indicated that maximum label fixation is complete within 2 days of labeling and that the fraction of ^{14}C in total extracted protein, lipids, and carbohydrates remains constant thereafter (Figure 27). Each experiment utilized Brachionus labeled for 4 to 11 days (at least 3 population doublings), thus the food substrate was considered uniformly labeled.

The magnitude of ^{14}C uptake by larval spot varied among experiments due to differences in Brachionus specific activity, but the pattern of uptake showed some trends among age cohorts (Figure 28). Multiple regression of ^{14}C uptake (expressed as a

Table 7. Regressions of _Brachionus_ body burden (DPM/mg DW) related to time (min) in carbon assimilation experiments with discrete age cohorts of larval _Leiostomus xanthurus_.

Age of Cohort (days)	Regression Equation	Correlation Coefficient	df	F	Mean Body Burden (DPM/mg DW)	s.d.
7	DPM/mg DW = 204,360 - 21.226 Time	0.08	1,7	0.040	200,987	31,110
8	DPM/mg DW = 322,499 - 210.998 Time	0.32	1,6	0.669	293,751	58,768
9	DPM/mg DW = 93,920 - 44.460 Time	0.21	1,10	0.503	88,860	22,792
14					576,322	477,324
15					721,458	114,070
16	DPM/mg DW = 40,201 + 1.265 Time	0.01	1,10	0.001	40,359	13,635
31	DPM/mg DW = 50,328 + 1.240 Time	0.01	1,10	0.002	50,494	8,900
34	DPM/mg DW = 163,491 - 145.954 Time	0.21	1,7	0.348	137,868	68,671
43	DPM/mg DW = 46,244 - 3.281 Time	0.11	1,10	0.126	45,795	3,005
47	DPM/mg DW - 721,859 - 2453.252 Time	0.47	1,7	2.026	351,146	553,452

Figure 27. Biochemical fractionation of ^{14}C in *Brachionus* during labeling.

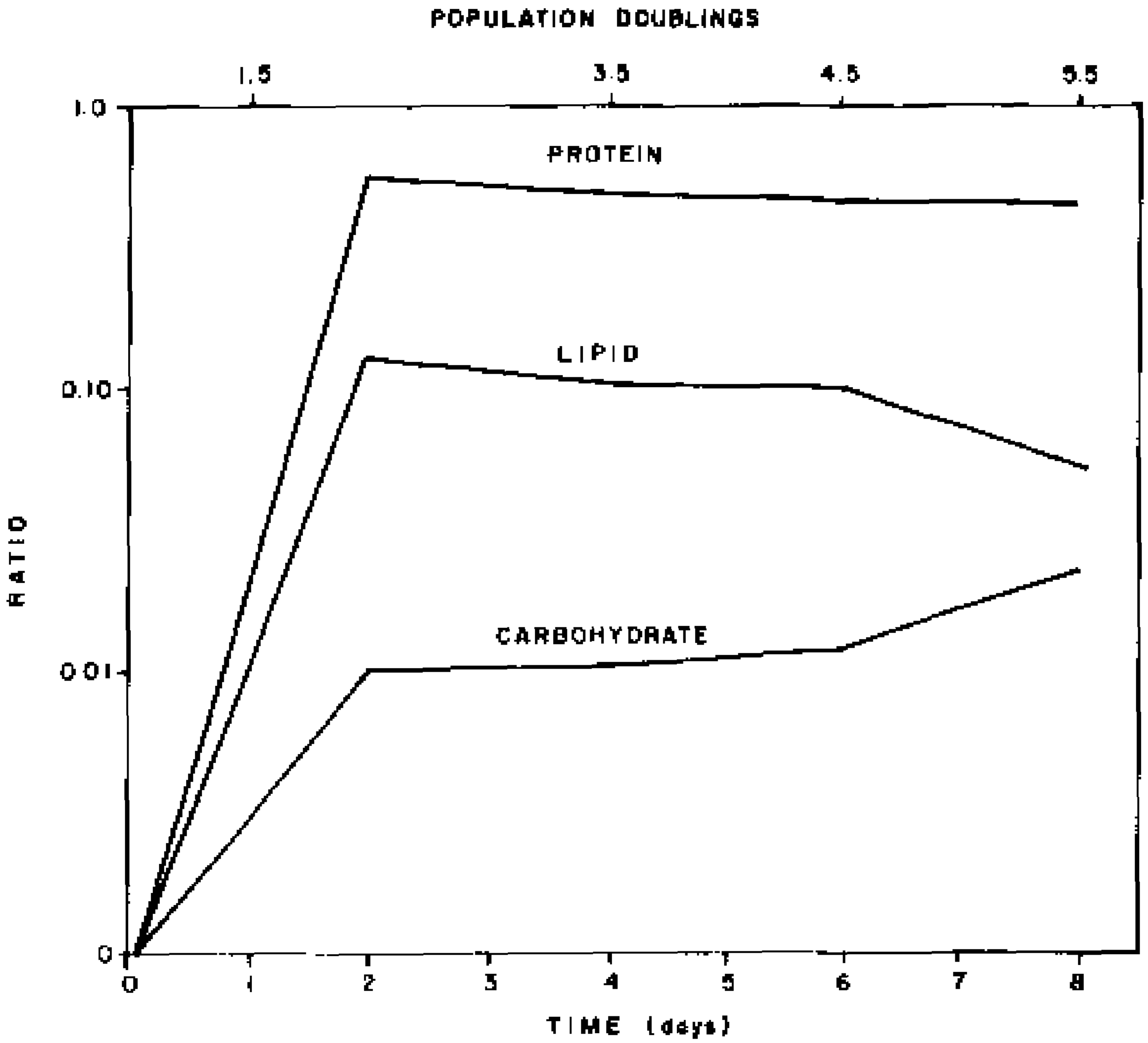
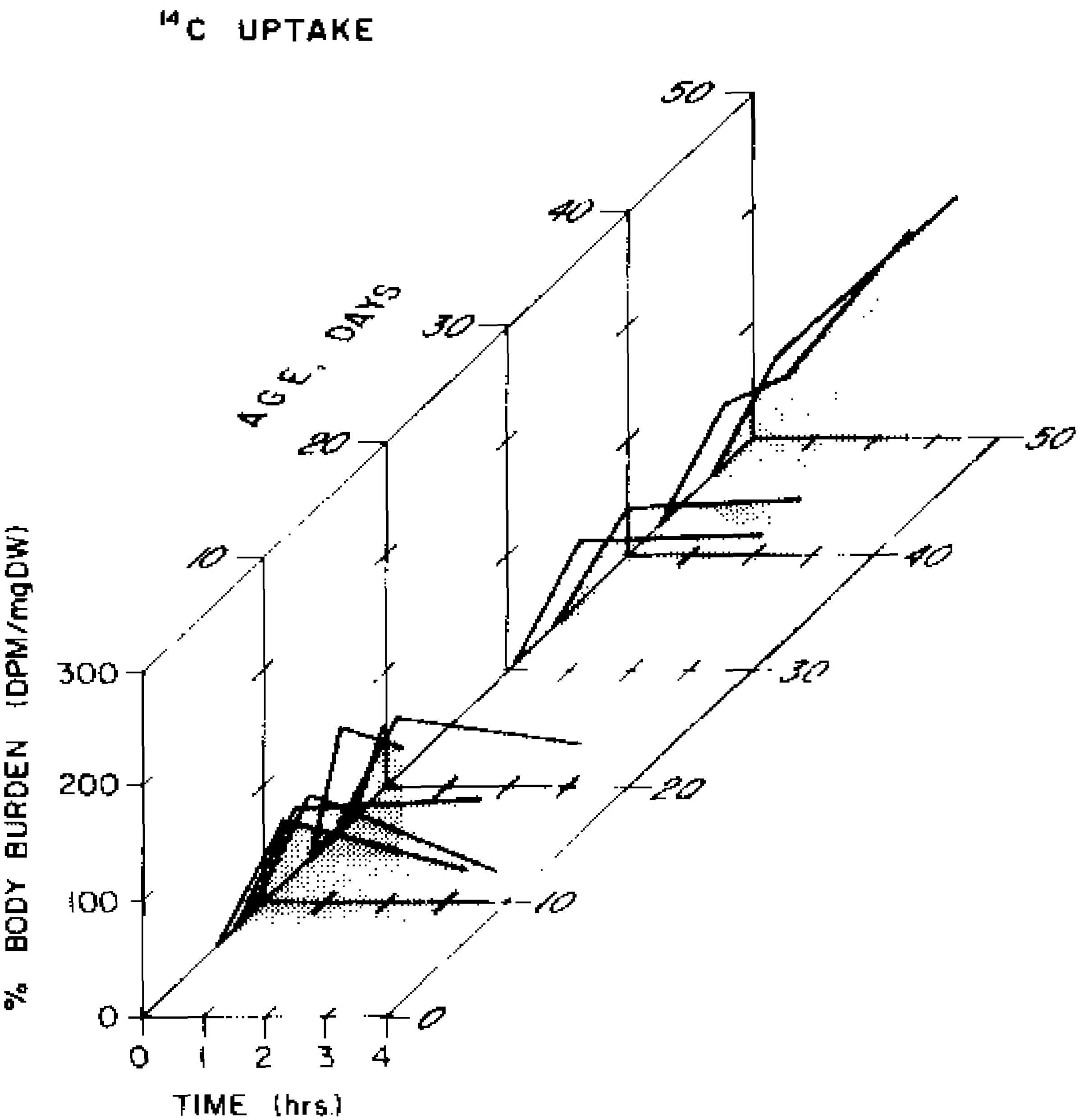

POPULATION DOUBLINGS
1.5
3.5
4.5
5.5
1.0
PROTEIN
LIPID
0.10
RATIO
CARBOHYDRATE
0.01
0
0
1
2
3
4
5
6
7
8
TIME (days)

Figure 28. Carbon-14 uptake by larval Leiostomus xanthurus in carbon assimilation experiments. Values expressed as a percentage of body burden (DPM/mg DW) at 1 h feeding.



^{14}C UPTAKE
AGE, DAYS
% BODY BURDEN (DPM/mgDW)
TIME (hrs.)
0
100
200
300
0 1 2 3 4
0
10
20
30
40
50

percentage of the 1 h estimate) on log time, length, weight, and age indicated that uptake was related to time and length ($P<0.10$; Table 8). Among all age cohorts uptake was rapid for the first hour and then slowed (Figure 28); the marked inflection in uptake at 60 min of feeding is probably due to the onset of egestion. Visual observations of feeding behavior and defecation agreed with this pattern. Because estimates of ingestion based on ^{14}C uptake would be confounded if egestion has occurred, ^{14}C retention and loss was followed for the 1 h feeding series only.

Carbon-14 retained and lost after 1 h of feeding showed a consistent pattern with time, but no trends with development (Figure 29). Pooled data for all experiments are shown in Figure 30. Multiple regressions of ^{14}C retained, respired, and lost as particulates (expressed as a percentage of the 1 h estimate) on time, time squared, length, weight, and age indicated that these processes were related to time squared and time, but not to any measure of development ($P>.001$; Tables 9, 10, and 11). Though ^{14}C loss is a composite of losses via egestion, respiration, and excretion, the pattern of ^{14}C retention and $PO^{14}C$ production indicated that egestion dominates this loss during the early minutes following transfer of larvae to a food free environment. Egestion was complete by 6 or 7 h after feeding stopped (Figure 30). A percentage of newly-assimilated ^{14}C entered quickly into short-term, metabolic processes and was lost as $^{14}CO_2$. It appears from these data that respiratory loss of the label began as early as 1 h following ingestion; thus any bias of carbon ingestion calculations due to respiratory loss of label during

Table 8. Stepwise multiple regression table of ^{14}C uptake (%) related to time (min), age (days), length (mm), and weight (mg) in carbon assimilation experiments with discrete age cohorts of larval *Leiostomus xanthurus*.

Regression Equation	Simple r	df	Partial F	Multiple F
Arcsine Percent Uptake =				
+9.620 + 36.212 Lg Time	0.84	1,41		94.81 (<.001)
+0.074 + 37.126 Lg Time			102.05 (<.001)	
+ 1.812 Length	0.03	2,40	2.93 (<.10)	51.09 (<.001)
+0.621 + 37.179 Lg Time			99.66 (<.001)	
+ 2.950 Length			0.43 (<.75)	
+ 0.252 Age	0.04	3,39	0.07 (>.75)	33.29 (<.001)
-14.481 + 37.259 Lg Time			98.36 (<.001)	
+ 14.202 Length			0.56 (<.75)	
- 1.469 Age			0.44 (>.75)	
- 14.176 Weight	0.02	4,38	0.37 (>.75)	24.66 (<.001)

Table 9. Stepwise multiple regression table of ^{14}C retained (%) related to time (min), age (days), length (mm), and weight (mg) in carbon assimilation experiments with discrete age cohorts of larval Leiostomus xanthurus.

Regression Equation	Simple r	df	Partial F	Multiple F
Arcsine Percent Retained =				
-31.000 + 1.202 Time	-0.35		65.60(<.001)	
- 0.004 Time^2	-0.30		63.43(<.001)	
- 0.000 Time^3	-0.33	3,64	59.51(<.001)	27.25(<.001)
-32.704 + 1.200 Time			65.59(<.001)	
- 0.417 Time^2			63.43(<.001)	
+ 0.394 Time^3			59.48(<.001)	
+ 1.746 Weight	0.11	4,63	1.32(<.75)	20.87(<.001)
-25.303 + 1.198 Time			65.30(<.001)	
- 0.004 Time^2			63.15(<.001)	
+ 0.000 Time^3			59.26(<.001)	
+ 7.528 Weight			1.43(<.25)	
+ 2.645 Length	0.08	5,62	0.89(<.50)	16.84(<.001)
-10.560 + 1.202 Time			65.74(<.001)	
+ 0.004 Time^2			63.44(<.001)	
+ 0.000 Time^3			59.84(<.001)	
+21.910 Weight			1.87(<.10)	
+15.067 Length			1.34(<.25)	
+ 1.446 Age	0.06	6,61	0.95(<.50)	14.18(<.001)

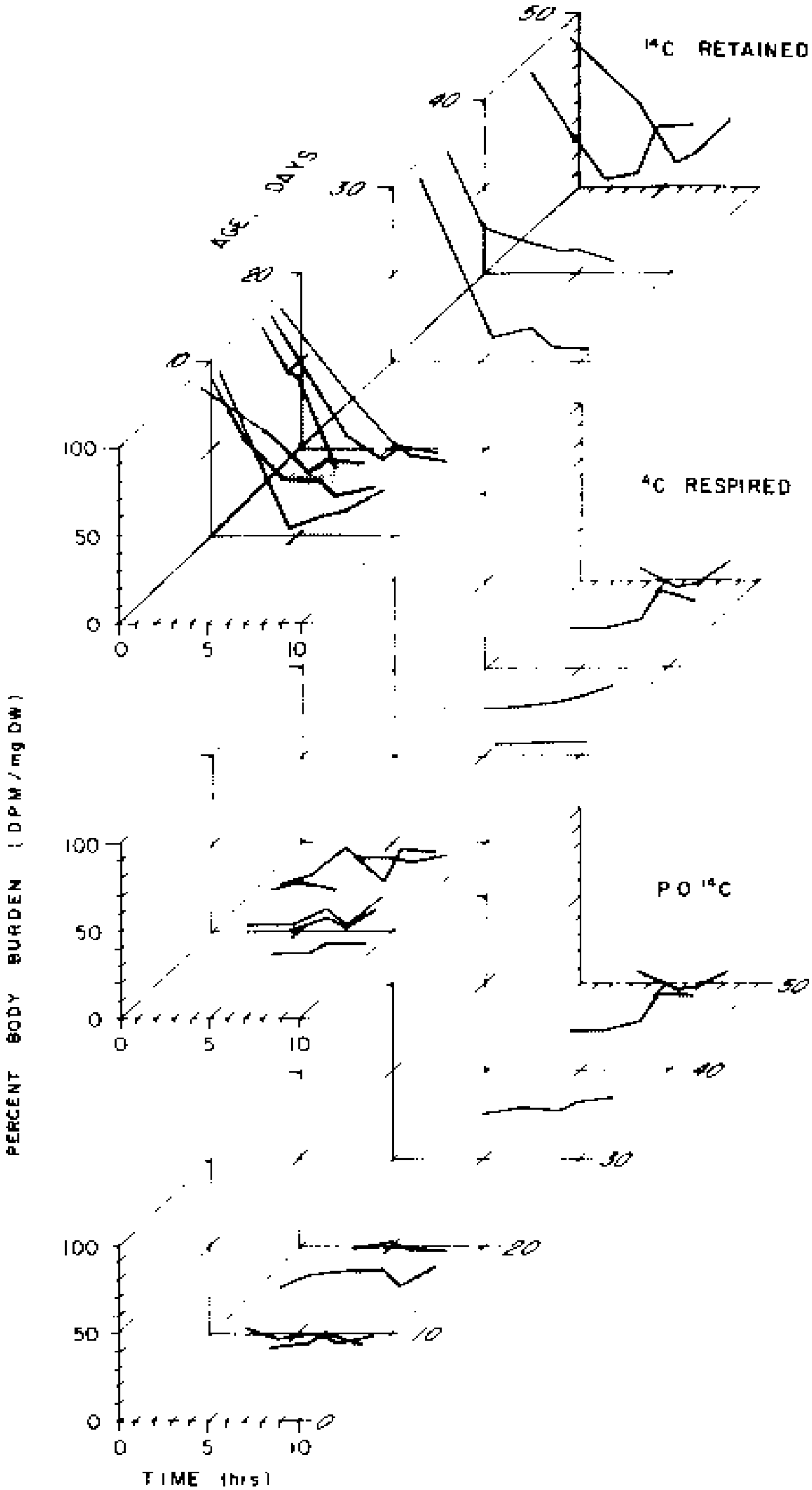
Table 10. Stepwise multiple regression table of ^{14}C respired (%) related to time (min), age (days), length (mm), and weight (mg) in carbon assimilation experiments with discrete age cohorts of larval *Leiostomus xanthurus*.

Regression Equation	Simpler	df	Partial F	Multiple F
Arcsine Percent Respired =				
218.281-72.036 Log Time	-0.58	1,68		35.04(<.001)
F - Level is insufficient for further calculation.				

Table 11. Stepwise multiple regression table of $PO^{14}C$ (%) related to time (min), age (days), length (mm), and weight (mg) in carbon assimilation experiments with discrete age cohorts of larval *Leiostomus xanthurus*.

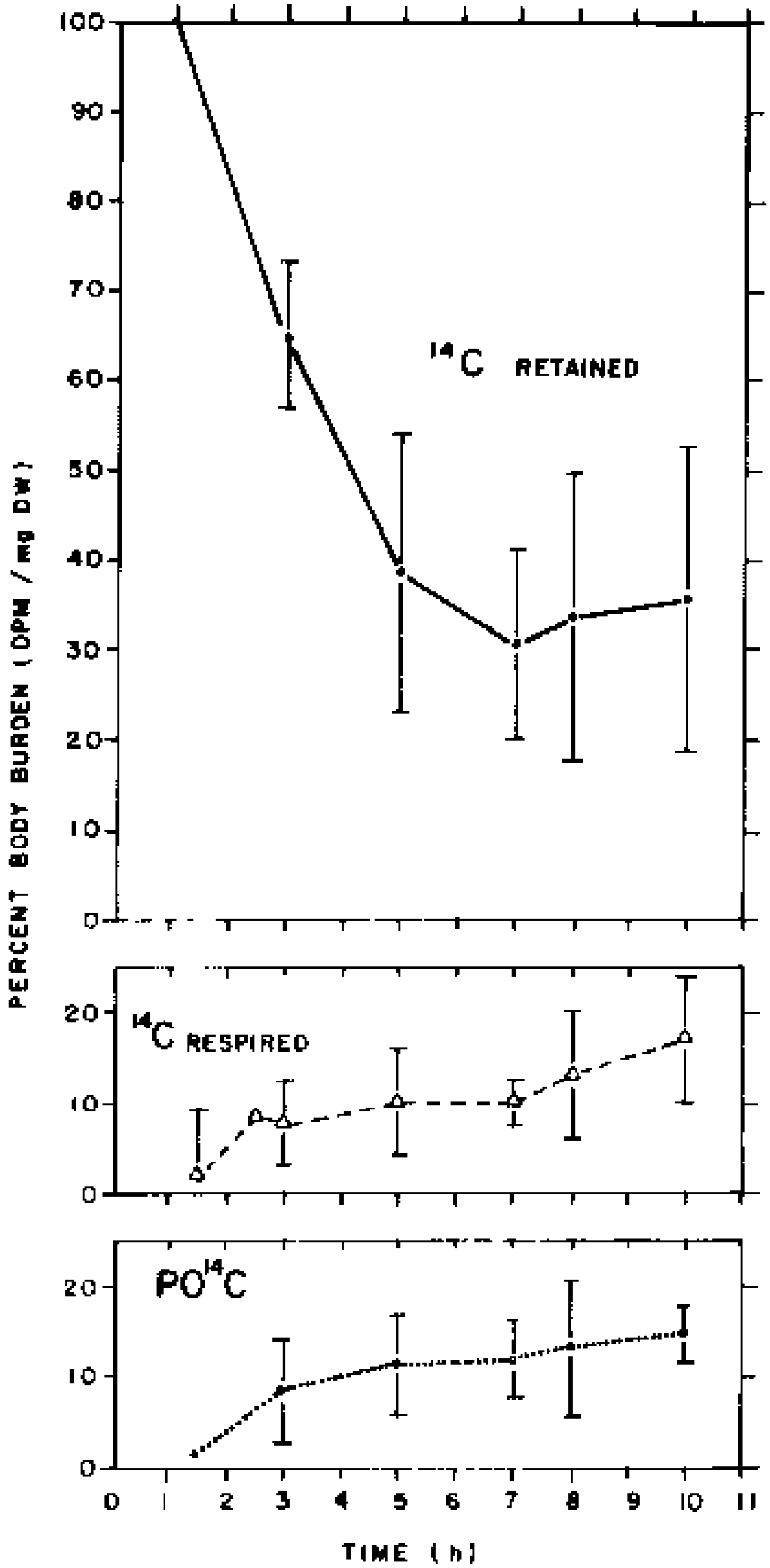
Regresson Equation	Simple r	df	Partial F	Multiple F
Arcsine Percent $PO^{14}C$ =				
216.485 - 74.176 Lg Time	-0.71	1,40		39.63(<.001)
211.882 - 74.176 Lg Time			39.42(<.001)	
+ 0.190 Age	0.10	2,39	0.78(<.50)	20.09(<.001)
212.284 - 74.176 Lg Time			38.71(<.001)	
- 0.612 Age			0.59(<.75)	
- 1.962 Length	0.08	3,38	0.30(>.75)	13.26(<.001)
247.214 - 74.176 Lg Time			39.20(<.001)	
3.286 Age			1.98(<.25)	
- 27.628 Length			1.67(<.25)	
32.436 Weight	0.07	4,37	1.48(<.25)	10.44(<.001)

Figure 29. Carbon-14 retained, respired, and lost as $PO^{14}C$ by larval *Leiostomus xanthurus* in carbon assimilation experiments. Values expressed as percentage of body burden (DPM/mg DW) at 1 h feeding.



¹⁴C RETAINED
¹⁴C RESPIRED
PO ¹⁴C
AGE, DAYS
PERCENT BODY BURDEN (DPM / mg DW)
TIME (hrs)

Figure 30. Carbon-14 retained, respired, and lost as $PO^{14}C$ by larval <u>Leiostomus</u> <u>xanthurus</u> in carbon assimilation experiments. Data pooled from all experiments; brackets indicate 95% confidence intervals. Values expressed as a percentage of body burden (DPM/mg DW) at 1 h feeding.



PERCENT BODY BURDEN (DPM / mg DW)
^{14}C RETAINED
^{14}C RESPIRED
$PO^{14}C$
TIME (h)

feeding is probably minimal. The rate of $^{14}CO_2$ production, initially very rapid, slows after several hours (Figure 30). Calculations of carbon retained and egested were made by pooling data from the 6 and 7 h retention intervals.

Carbon Assimilation and its Relation to Development

Carbon retention (Table 12), the relative index of the coefficient of utilization, was not related to development but was negatively related to condition factor (Figure 31). Condition factor showed the highest correlation followed by weight in multiple regression of retention on length, weight, age, and condition factor. Although the partial regression coefficients were not significant, carbon retention decreases sharply with increasing condition factor (the simple regression of carbon retention on condition factor suggested that the probability of a zero slope is <0.25) (Table 13).

Carbon absorption (Table 12), the index of absorption efficiency, was negatively related to age as well as condition factor (Figure 32). Multiple regression indicated that the partial regressions coefficients of age and condition factor were both significant ($P<0.10$; Table 14).

No age-related biases in the indices of assimilation were evident or, if present, they were constant. Carbon retention was corrected for respiratory loss of ^{14}C, but this correction might itself be biased. The percentage of ingested ^{14}C lost through respiration was not associated with age and only weakly associated with condition

Table 12. Summary of values used to assess carbon assimilation in age cohorts of larval *Leiostomus xanthurus*

Age (days)	Carbon Ingested per dry weight of larvae (mg)	Carbon Retained per dry weight of larvae (mg)	Carbon Egested per dry weight of larvae (mg)	Carbon Retention (%)	Carbon Absorption (%)
7	0.0712	0.0407	0.0006	57.1	99.2
8	0.0116	0.0067	0.0002	57.9	98.0
9	0.0715	0.0186		26.0	
14	0.0049				
15	0.0302	0.0161	0.0006	53.2	99.0
16	0.0181	0.0063	0.0031	34.8	82.9
31	0.0098	0.0009		9.4	
34	0.0360	0.0140	0.0118	38.9	67.2
43	0.0099	0.0064		64.7	
47	0.0148	0.0066	0.0031	44.2	79.3
			Mean	42.9	87.6

Figure 31. Carbon retention, the index of the coefficient of utilization, related to development (A) and condition factor (B) of larval *Leiostomus xanthurus* age cohorts. Regressions calculated with arcsine (in degrees) transformed percentages.

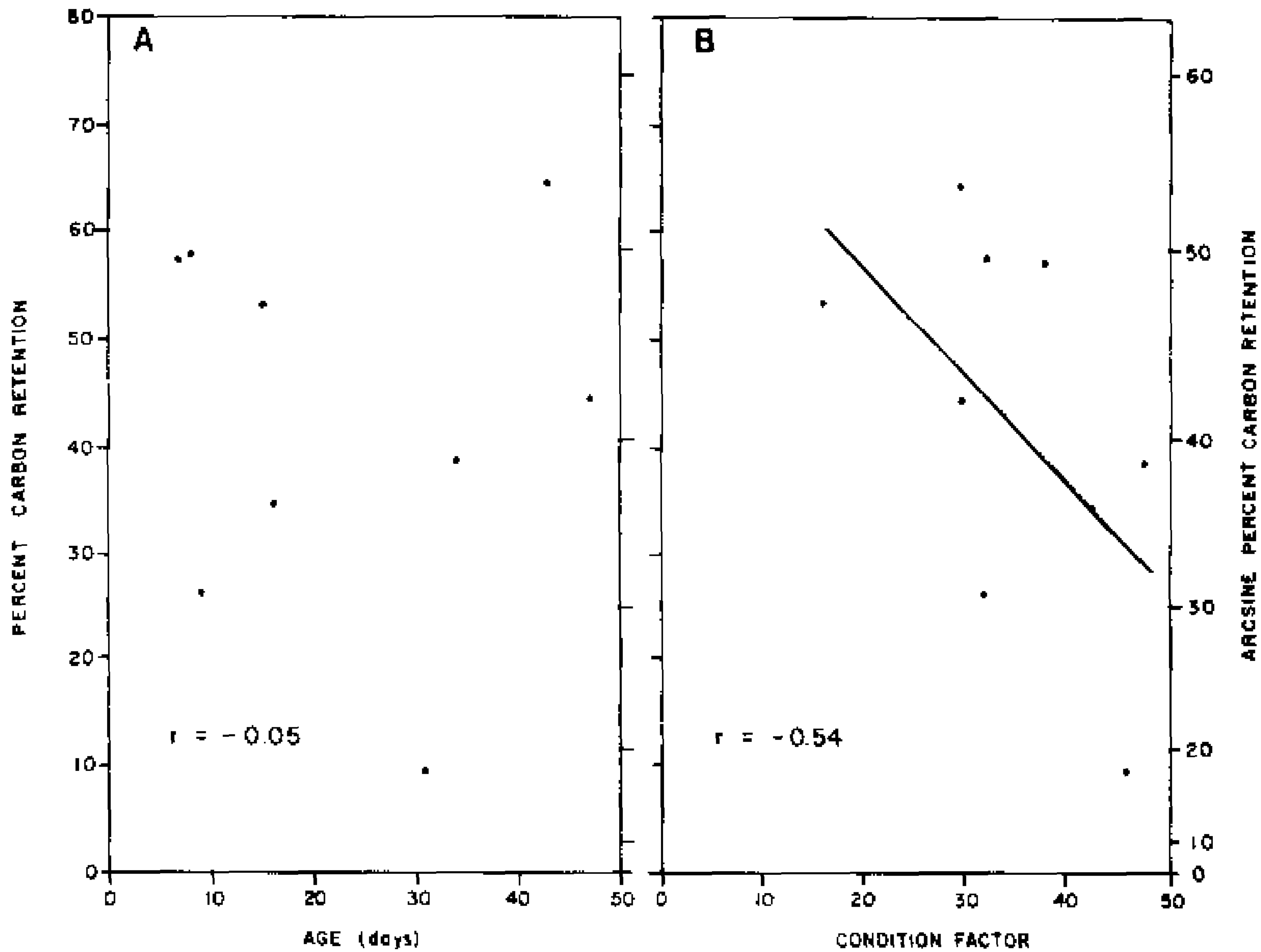

A
B
PERCENT CARBON RETENTION
ARCSINE PERCENT CARBON RETENTION
r = -0.05
r = -0.54
AGE (days)
CONDITION FACTOR

Table 13. Stepwise multiple regression table of carbon retention (%), the index of the coefficient of utilization, related to age (days), length (mm), weight (mg), and condition factor of Leiostomus xanthurus larval age cohorts.

Regression Equation	Simple r	df	Partial F	Multiple F
Arcsine Percent Carbon Retention =				
61.686 - 0.608 Condition Factor	-0.54	1,7		2.89(<.25)
60.003 - 0.609 Condition Factor			2.64(<.25)	
+ 1.555 Weight	0.21	2,6	0.40(<.75)	1.52(<.50)
71.357 - 0.504 Condition Factor			1.92(<.25)	
+13.560 Weight			2.02(<.25)	
- 5.421 Length	0.07	3,5	1.68(<.50)	1.69(<.50)
83.970 - 0.525 Condition Factor			1.74(<.50)	
+24.979 Weight			0.95(<.50)	
-15.437 Length			0.54(<.75)	
- 1.186 Age	0.05	4,4	0.24(>.75)	1.13(<.50)

Figure 32. Carbon absorption, the index of absorption efficiency, related to development (A) and condition factor (B) of larval Leiostomus xanthurus age cohorts. Regressions calculated with arcsine (in degrees) transformed percentages.

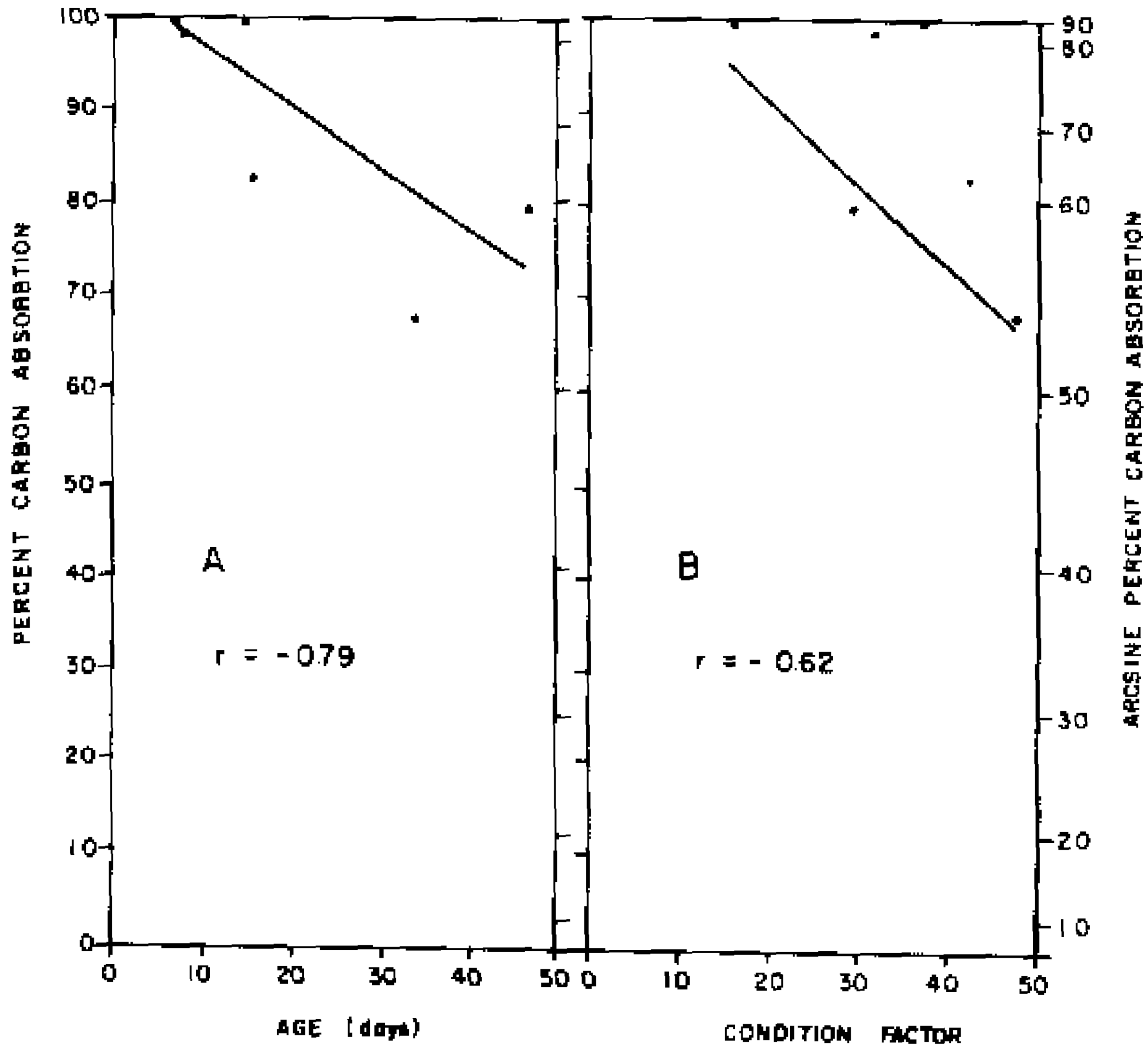

PERCENT CARBON ABSORBTION
100
90
80
70
60
50
40
30
20
10
0
A
r = -0.79
AGE (days)
0
10
20
30
40
50
B
r = - 0.62
CONDITION FACTOR
ARCSINE PERCENT CARBON ABSORBTION
90
80
70
60
50
40
30
20
10

Table 14. Stepwise multiple regression table of carbon absorption, the index of absorption efficiency, related to age (days), length (mm), weight (mg), and condition factor of *Leiostomus xanthurus* larval age cohorts.

Regression Equation	Simple r	df	Partial F	Multiple F
Arcsine Carbon Absorption =				
87.542 - 0.648 Age	-0.79	1,4		6.57(<.10)
110.530 - 0.596 Age			16.41(<.05)	
- 0.693 Condition Factor	-0.62	2,3	8.96(<.10)	14.31(<.05)
108.894 - 0.212 Age			0.86(<.75)	
- 0.762 Condition Factor			19.34(<0.5)	
- 4.467 Weight	-0.75	3,2	3.60(<.25)	*
79.086 - 2.803 Age			1.64(<.50)	
- 0.737 Condition Factor			4.21(<.25)	
-31.795 Weight			1.90(<.25)	
+22.958 Length	-0.78	4,1	1.47(<.50)	3.19(<.25)

* F - level insufficient for further calculation.

factor (Figure 33); thus any bias in carbon retention was considered constant. An age-related bias might be inherent in relationships between carbon assimilation indices and condition factor for the condition factor of larval fishes generally increases with length after yolk-sac absorption (e.g., Sameoto, 1972; Sekiguchi, 1975; Ehrlich et al., 1976). Condition factor of the age cohorts used, however, showed no association with age (Figure 34) and the correlation of condition factor with length was even weaker.

Ancillary Experiments

The pattern of ^{51}Cr retained and lost was similar to that of ^{14}C with some exceptions (Figure 35). A residue of ^{51}Cr remained within the larval alimentary canal longer than the experiment. Taken as a whole, however, these results support the inference that egestion is complete within 6 or 7 h following feeding. Carbon absorption, calculated by difference according to general experimental procedures, was 99% (15 day age cohort, Table 12); a similar value was calculated using the indicator-ratio method (Table 15).

The pattern of ^{14}C retained and lost in experiments with reduced *Brachionus* concentrations was similar to the main experiments with high concentrations and maximal feeding (Figure 36), but estimates of carbon retention were higher (Table 16). Results of these experiments were highly variable and only two of the six were successful. Variability was largely due to variable feeding success. The mean coefficient of variation of ^{14}C uptake samples was 78.0 compared to a mean of 62.8 for experiments with high *Brachionus* concentrations.

Figure 33. Percent $^{14}CO_2$ respired related to development (A) and condition factor (B) of larval *Leiostomus xanthurus* age cohorts. Regressions calculated with arcsine (in degrees) transformed percentages.

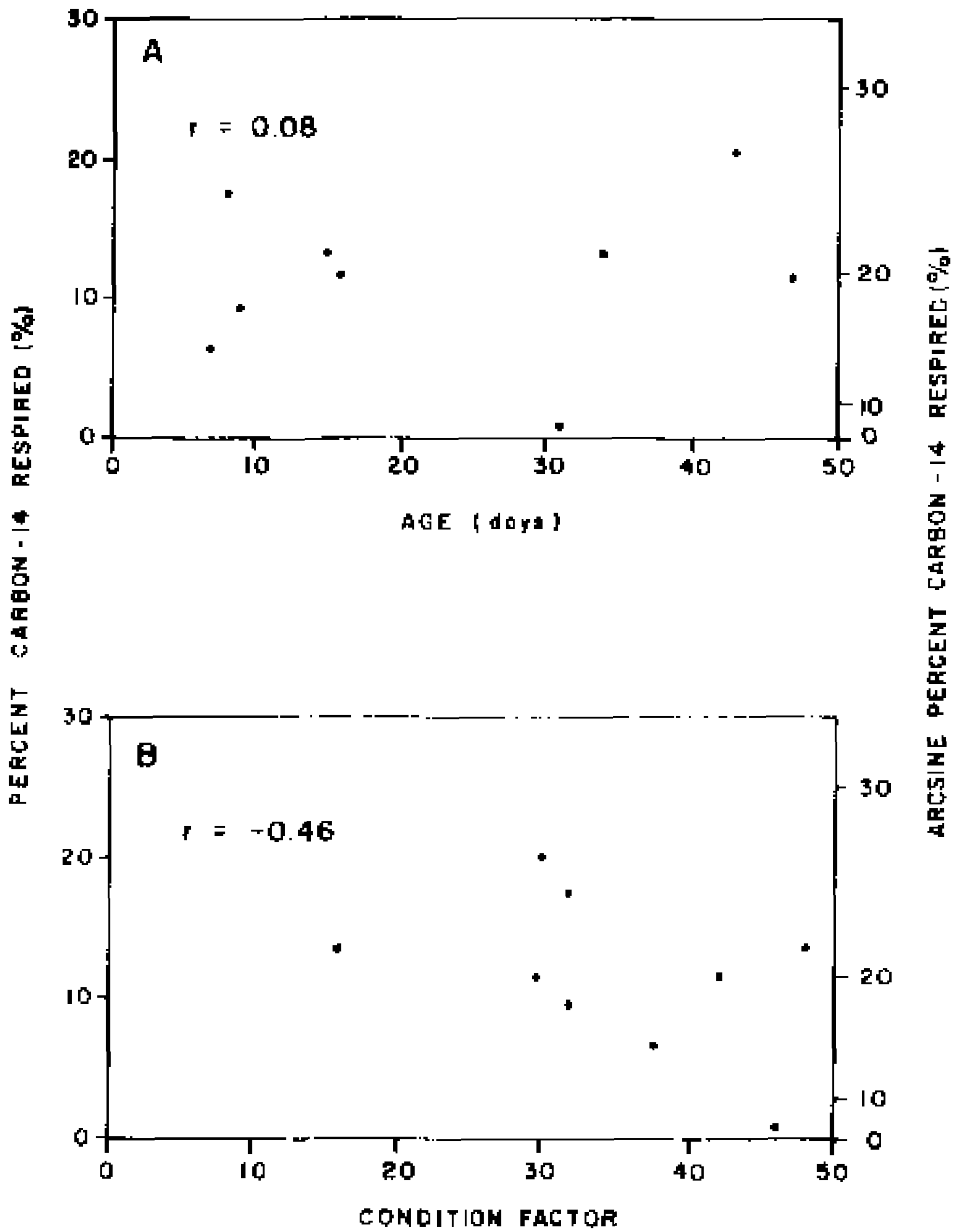
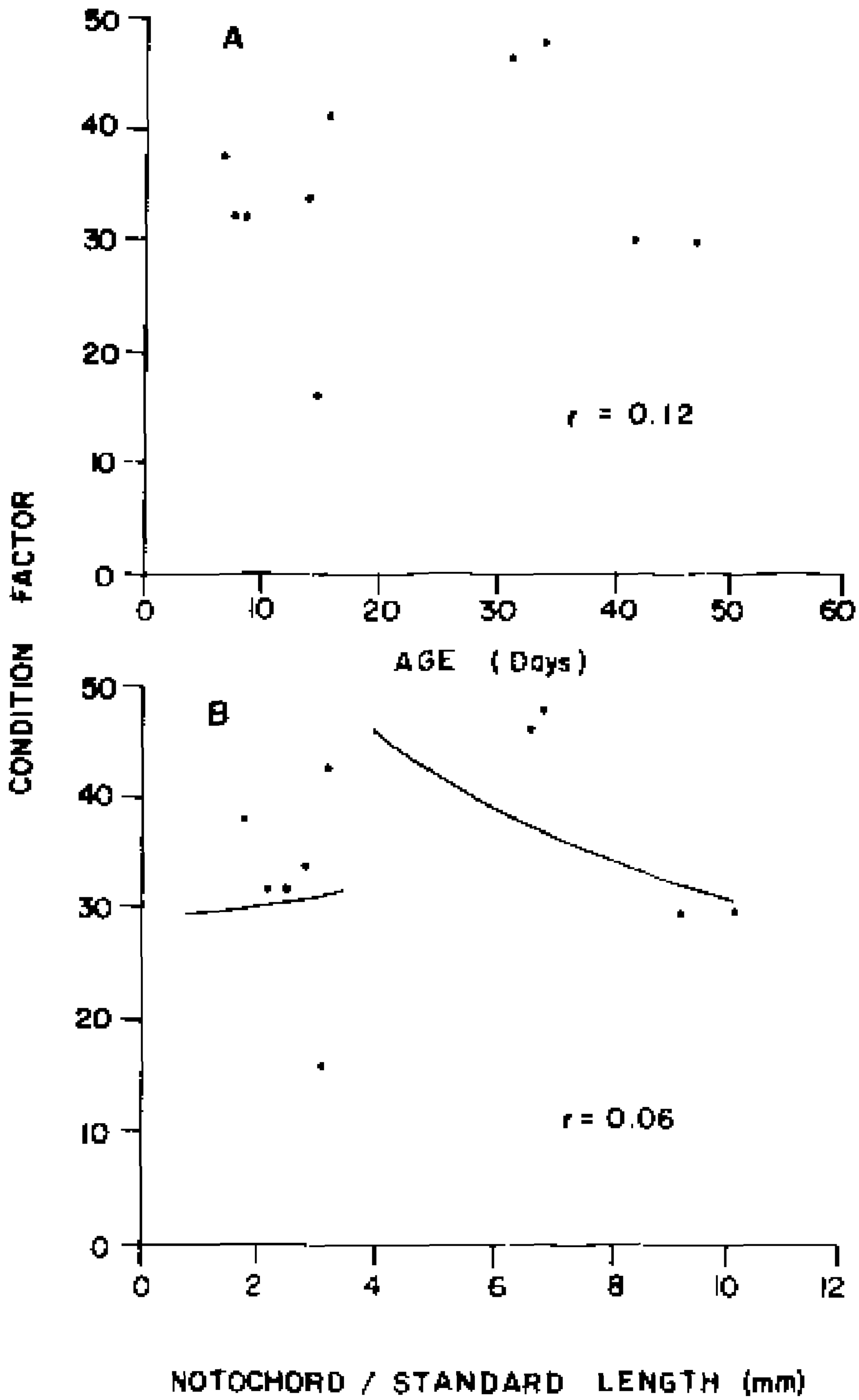

A
r = 0.08
AGE (days)
B
r = -0.46
CONDITION FACTOR
PERCENT CARBON-14 RESPIRED (%)
ARCSINE PERCENT CARBON-14 RESPIRED (%)

Figure 34. Condition factor related to age (A) and notochord or standard length (B) of larval *Leiostomus xanthurus* age cohorts used in carbon assimilation experiments. Lines (B) indicate predicted condition factor from weight and length regressions.



A
r = 0.12
AGE (Days)
B
r = 0.06
CONDITION FACTOR
NOTOCHORD / STANDARD LENGTH (mm)

Figure 35. Carbon-14 and ^{51}Cr retained and lost by larval *Leiostomus xanthurus* in a dual-tracer experiment with 15 day age cohort. Values expressed as a percentage of body burden (DPM/mg DW) at 1 h feeding.

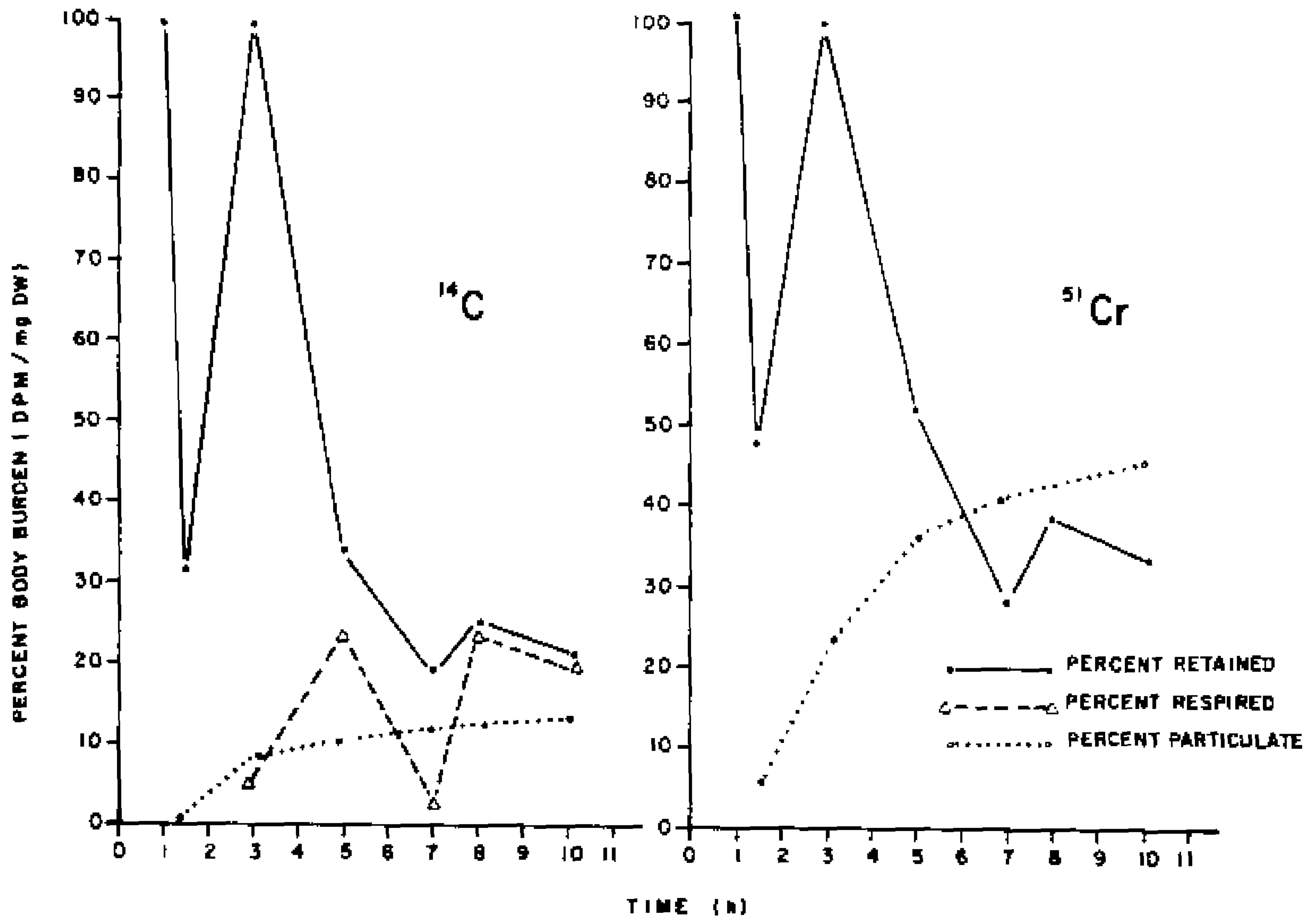
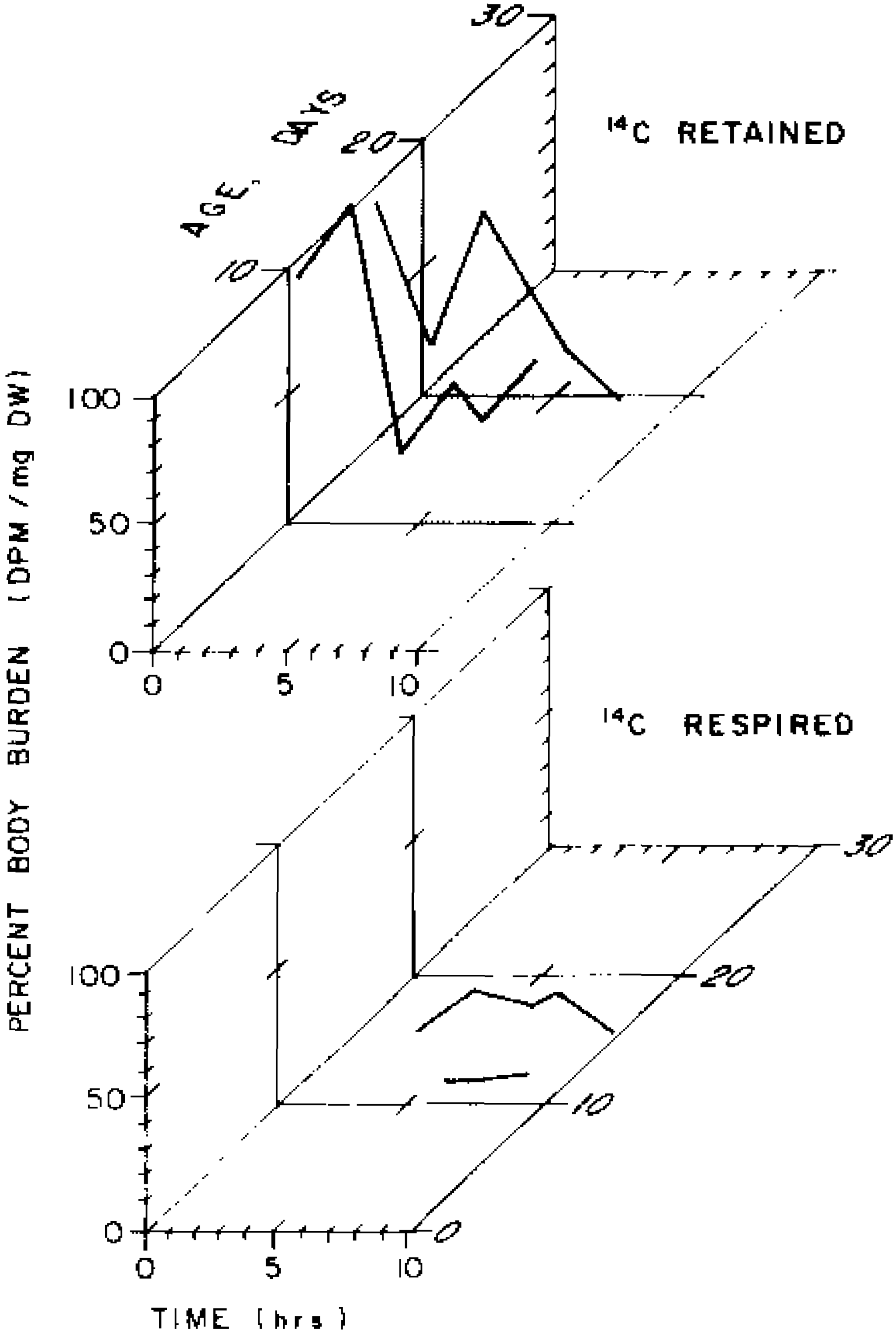

^{14}C
^{51}Cr
PERCENT BODY BURDEN (DPM / mg DW)
TIME (h)
PERCENT RETAINED
PERCENT RESPIRED
PERCENT PARTICULATE

Table 15. Summary of dual-tracer values used to calculated absorption efficiency by the indicator-ratio method.

	Body Burden of ^{14}C (DPM/mg DW)	Body Burden of ^{51}Cr (DPM/mg DW)	Ratio of tracers	Ratio of food/feces	Assimilation Efficiency
Food	543687	13055657	24.0	1.02	98.08
Feces	4400	13055657	23.6		

Figure 36. Carbon-14 retained and lost by larval *Leiostomus xanthurus* in carbon assimilation experiments with reduced *Brachionus plicatilis* concentrations (5-10/ml). Values expressed as a percentage of body burden (DPM/mg DW) at 1 h feeding.



¹⁴C RETAINED
¹⁴C RESPIRED
AGE, DAYS
PERCENT BODY BURDEN (DPM / mg DW)
TIME (hrs)
0
5
10
10
20
30
0
50
100

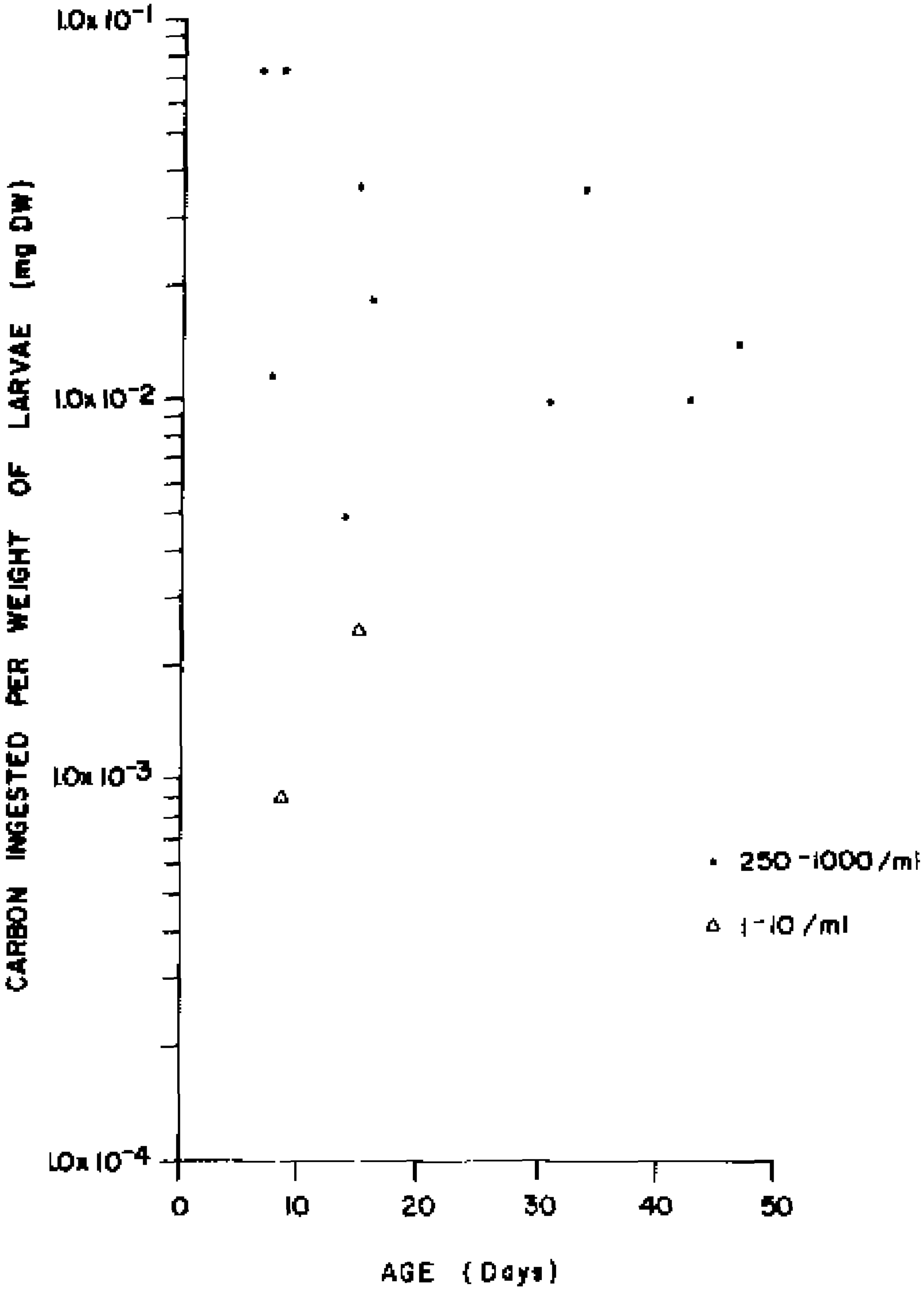
Table 16. Summary of values used to asses carbon assimilation in age cohorts of larval *Leiostomus xanthurus* fed with reduced *Brachionus* concentrations.

Age (days)	Carbon Ingested per dry weight of larvae (mg)	Carbon Retained per dry weight of larvae (mg)	Carbon Retention (%)
9	0.000903	0.000556	61.5
15	0.002578	0.002222	86.2
		Mean	73.8

The quantity of carbon ingested per dry weight of larvae in experiments with reduced Brachionus concentrations compared with carbon ingested at high concentrations was generally an order of magnitude lower (Figure 37).

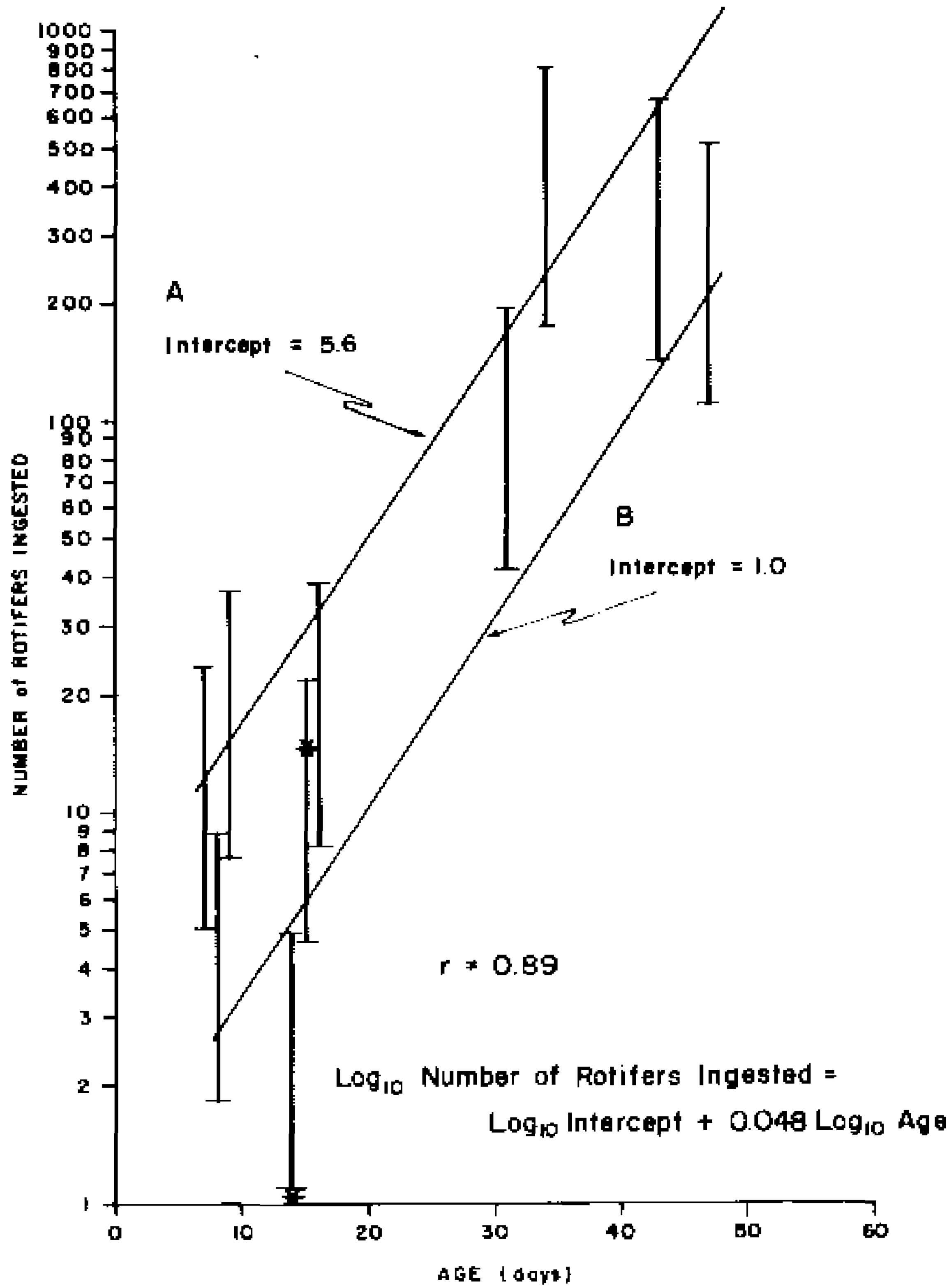
The number of rotifers ingested by a larva, calculated from ^{14}C uptake data, increased expontially with age and compares well with counts of the number of rotifers excised from alimentary canals (Figure 38). The count of rotifers that falls outside of the range of calculated values was based on a single specimen rather than a mean of three counts. The ^{14}C method of estimating ingestion used herein is valid.

Figure 37. Carbon ingested per dry weight of larval *Leiostomus xanthurus* fed high (250-1000/ml) and low (5-10/ml) concentrations of *Brachionus plicatilis*.



1.0x10⁻¹
1.0x10⁻²
1.0x10⁻³
1.0x10⁻⁴
CARBON INGESTED PER WEIGHT OF LARVAE (mg DW)
0
10
20
30
40
50
AGE (Days)
• 250-1000/ml
△ 1-10/ml

Figure 38. The number of rotifers (_Brachionus plicatilis_) ingested related to development of larval _Leiostomus xanthurus_. A. Regression calculated using dry weight value of Theilacker and McMaster (1971). B. Regression calculated using maximum dry weight value of Scott and Baynes (1978). Stars indicate mean number of rotifers counted in alimentary canals excised from larvae.


NUMBER of ROTIFERS INGESTED
AGE (days)
A
Intercept = 5.6
B
Intercept = 1.0
r = 0.89
Log10 Number of Rotifers Ingested =
Log10 Intercept + 0.048 Log10 Age
1000
900
800
700
600
500
400
300
200
100
90
80
70
60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
0
10
20
30
40
50
60

DISCUSSION

Conflicting evidence exists concerning the presence of carbon pools with rapid turnover rates in aquatic, poikilothermic animals (see review in Conover and Francis, 1973; also Streit, 1975; Lampert, 1977; Wetzel, 1976). In larval spot, carbon is assimilated rapidly after ingestion and at least some of it enters a short-term, metabolic pool. Sorokin and Panov (1966) reported rapid carbon loss through respiration following ingestion by larval freshwater bream, *Abramis brama*. Larval spot evidenced the same pattern.

Mean carbon retention, 42.9%, was somewhat lower than the expected coefficient of utilization for juvenile and adult fishes, about 70% (Ware, 1975). This difference may be due to a real shift in the coefficient of utilization after transformation or possible bias associated with tracer methodology. Carbon retention, as estimated herein, is a relative index of the coefficient of utilization because of the bias associated with respiratory loss of newly assimilated carbon. Alternatively, carbon retention could be reduced at high feeding levels. The results of experiments with reduced *Brachionus* concentrations were highly variable, but values for carbon retention (73.8) were close to that expected for adult fishes. Using a ^{14}C radiotracer design and environmentally realistic food concentrations, Sorokin and Panov (1966) found close agreement between carbon retention and carbon absorption.

Mean carbon absorption, 87.6%, approximated assimilation efficiencies reported for juvenile and adult fishes (see review in Conover, 1978; Brett and Groves, 1979) as well as those calculated for larval fishes (Table 3). Brachionus loricas contain either indigestible chitin (Donner, 1966) or scleroprotein (Hyman, 1951) and comprise a large portion of the particulate egesta of larvae. Carbon absorption may improve following transformation with the dietary shift that includes more soft bodied invertebrates (Part I).

Assimilation, as assessed herein, does not appear to improve with the larval development of spot prior to transformation. This is not surprising for there are no major changes in the alimentary canal or associated organs that could effect such a change (Part I). Surface area of the alimentary canal appears to increase, but this might simply keep pace with the size increase of the larvae. Sorokin and Panov (1966) found no major increase in assimilation during the larval development of A. brama. Yet Laurence's (1971, 1977) energetic models for M. salmoides and P. americanus simulate increases in assimilation with increasing body weight of first-feeding larvae.

Is the negative relationship between carbon absorption and development real or artifact? Weight-specific absorption efficiency might actually decrease with larval age if the percentage of carbon absorbed is constant or increases at a slower rate than the increase in larval weight. The negative relationship might be an artifact if younger larvae fill their alimentary canal and begin egestion sooner than older larvae with a greater alimentary canal capacity. Experiments with larval Anchoa lamprotaenia and Clupea harengus (Chitty, 1980; Werner and Blaxter, 1980), larvae with a morphologically straight rather than looped alimentary canal, suggest that egestion may begin within minutes after ingestion. If this occurred in experiments with young spot larvae, estimates of ingestion and consequently absorption would be inflated due to partial digestion and assimilation of food as it traversed the alimentary canal. Dissections of larvae, however, indicated that digestion of Brachionus takes longer than 1 h, and egested cuticular loricas were first observed several hours after the transfer of larvae to retention chambers. The accuracy of carbon absorption values calculated by difference was also supported by results of the dual-label experiment. The indicator-ratio method avoids problems associated with estimates of ingestion, yet gave a similarly high value for absorption efficiency.

The negative relationship between assimilation and condition factor has important implications to larval growth and survival. Larvae that have been feeding well enough to survive, but less than that required for maximum growth, apparently assimilate more nutrients from a ration than do larvae with a better growth history. Perhaps a larva with a lower condition factor is biochemically or cytologically better primed for assimilation of nutrients. Koslomarova (1962) reported that larval Esox lucius made up for growth delays by more efficient utilization of available food.

The present results, suggesting that first-feeding larvae are fully capable of digesting and absorbing nutrients, and that larvae with a poor growth history are more efficient in assimilating available food, are plausible in evolutionary terms. Larvae may mitigate the risk of a patchy food distribution by fully utilizing available resources. Such compensatory mechanisms may be of great adaptive significance to pelagic marine larvae.

EPILOGUE

The alimentary canal of spot becomes functional during the completion of oil-globule absorption when first feeding may occur, a period coincidental with Hjort's "critical period" (see Introduction). Most pelagic marine larvae begin to feed during yolk absorption (e.g., Blaxter and Hempel, 1963; Blaxter and Ehrlich, 1974; Houde, 1974). Whether these larvae are capable of digestion and assimilation before yolk-sac absorption is not known. If they are, they may store energy for utilization should they encounter inadequate food at first feeding. There is also evidence of thermal optima for efficient yolk utilization (Ryland and Nichols, 1967; Laurence, 1973); if yolk is absorbed too rapidly larvae die even in the presence of adequate food. Perhaps a lack of coincidence between first feeding and functional alimentary canal development is the cause. Preliminary experiments on the point-of-no-return (see review in May, 1974) in larval spot indicate that the duration of the "critical period" is about two days following yolk-sac and oil-globule absorption, after which irreversible starvation occurs[3]. Spot larvae, reared at 20°C, appear to be fully equipped to digest and absorb available food resources during the "critical period."

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[3] Powell, A. B. 1979. Effects of delayed feeding on first feeding spot (Leiostomus xanthurus) larvae: Survival and morphological changes. Ann. Rept. of the Beaufort Laboratory to the U.S. Dept. of Energy. pp. 411-421.

Two lines of evidence suggest that the assimilative abilities of larval Leiostomus xanthurus does not improve significantly prior to the completion of transformation. Taken separately the absence of both major developmental changes in the alimentary canal and discernible changes in carbon assimilation are equivocal, but taken together they are implicative.

The development of the alimentary canal is quite similar among teleosts with no major morphological or histological changes occurring prior to transformation or metamorphosis. Among pelagic marine larvae, clupeomorph and protacanthopterygian larvae have a straight alimentary canal; paracanthopterygians and acanthopterygians have a looped alimentary canal. The alimentary canal is histologically partitioned in a similar pattern in both groups (see review in Part I). While these groups might differ in the way food traverses the alimentary canal, they probably don't differ in the mechanism of digestion and assimilation; though some conflicting evidence remains concerning these mechanisms. Thus unchanging assimilative abilities of larval Leiostomus xanthurus may be a general phenomenon of teleostean development.

Comparative studies with other species, principally those with a straight and looped alimentary canals, and experiments that offer control over condition factor may be the next step in the analysis of starvation induced mortality in the early life history of fishes.

APPENDIX

Because laboratory reared fish larvae often differ biochemically, behaviorally, and morphometrically from wild larvae (e.g., Balbontin et al., 1973; Blaxter and Ehrlich, 1974; Arthur, 1976), one may expect physiological differences as well. Herein are data on the development of *Leiostomus xanthurus* at 20°C in the laboratory with comparisons with field caught specimens. Statistical comparisons were not made because of the possibility of differential shrinkage from preservation.

Figure 39. Length and age relationship of laboratory reared *Leiostomus xanthurus* larvae: Bars indicate ranges.

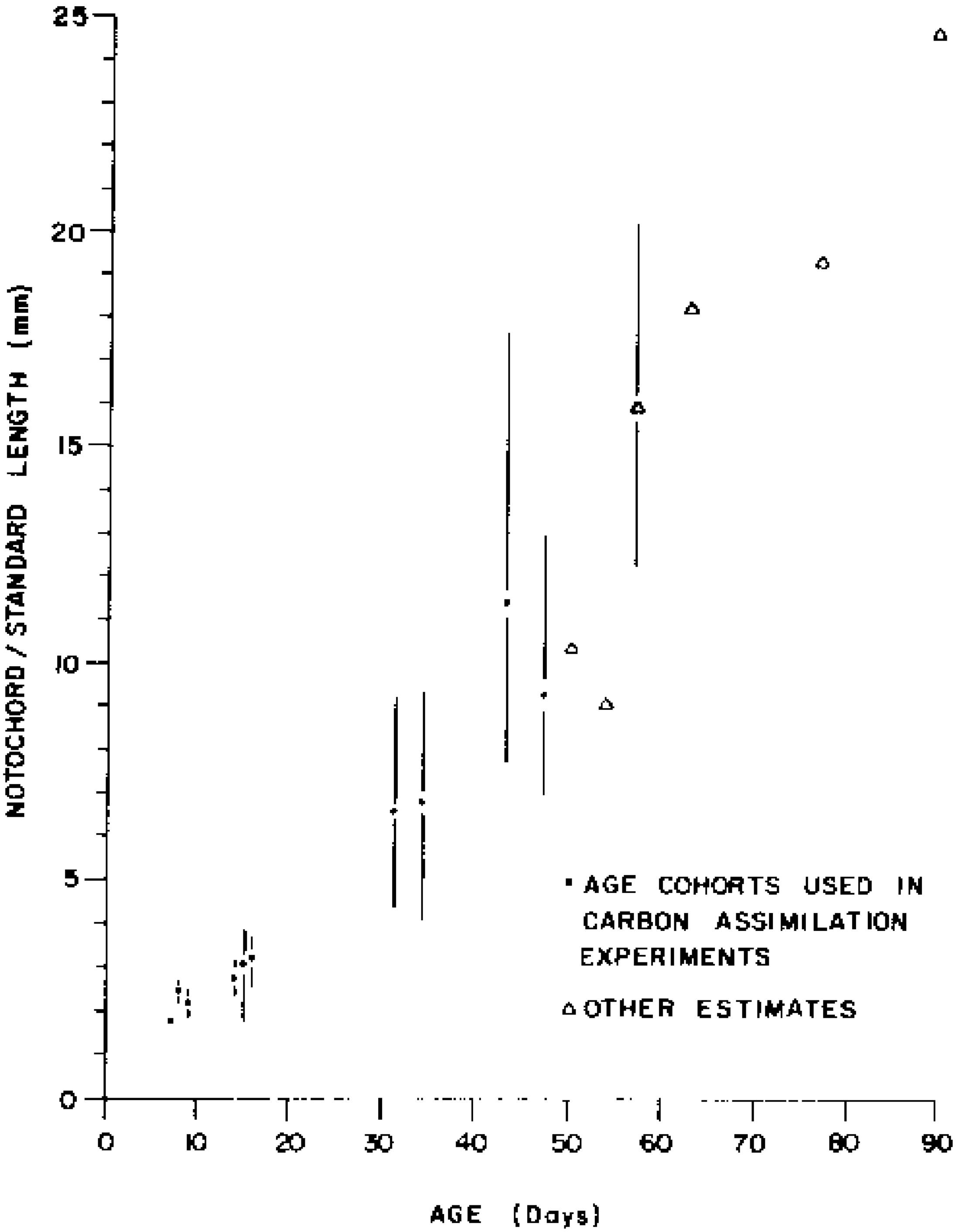
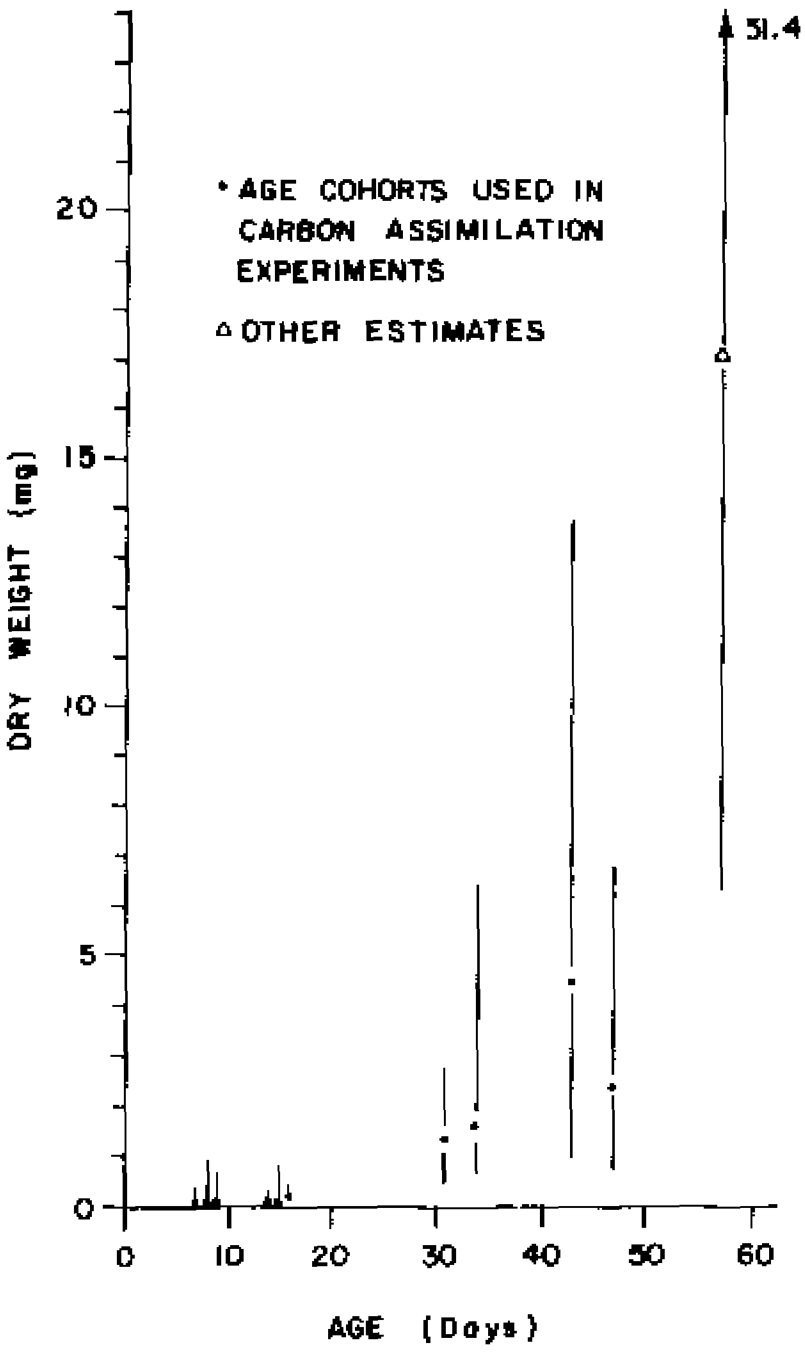

NOTOCHORD / STANDARD LENGTH (mm)
0
5
10
15
20
25
0
10
20
30
40
50
60
70
80
90
AGE (Days)
• AGE COHORTS USED IN CARBON ASSIMILATION EXPERIMENTS
△ OTHER ESTIMATES

Figure 40. Weight and age relationships of laboratory reared *Leiostomus xanthurus* larvae: Bars indicate ranges.



51.4
• AGE COHORTS USED IN CARBON ASSIMILATION EXPERIMENTS
△ OTHER ESTIMATES
DRY WEIGHT (mg)
0
5
10
15
20
0
10
20
30
40
50
60
AGE (Days)

Figure 41. Schematic summary of the development of laboratory reared *Leiostomus xanthurus* larvae.

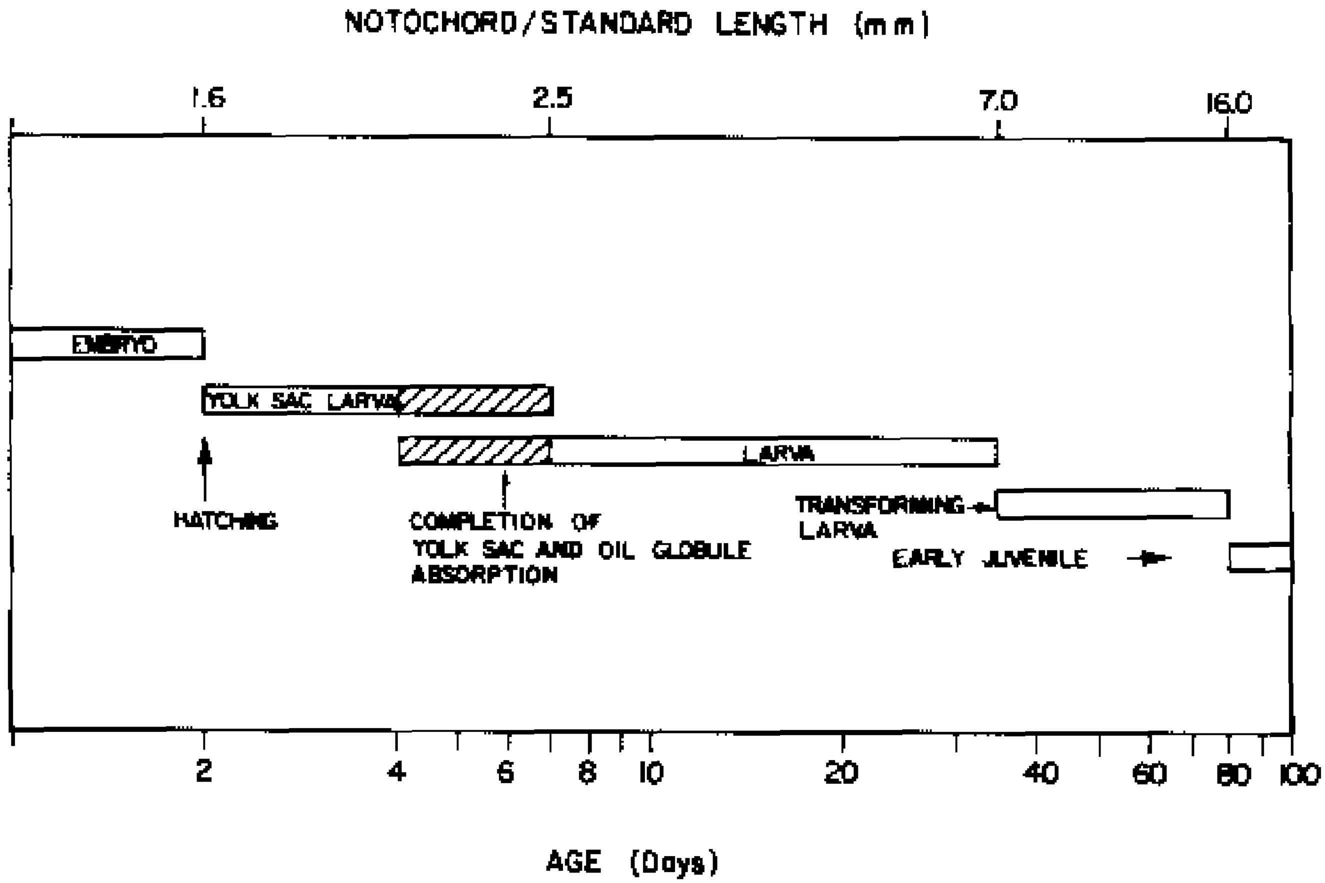
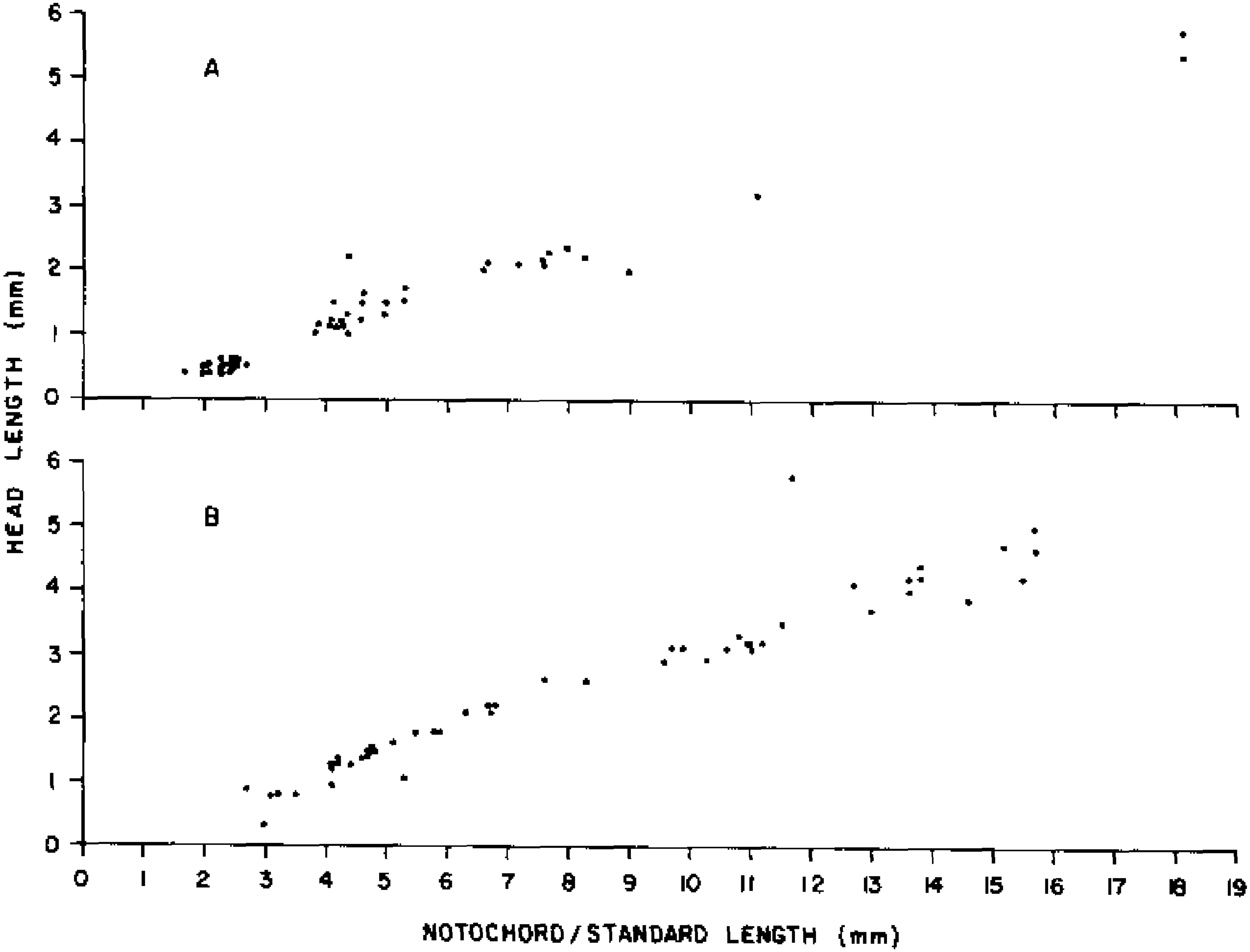

NOTOCHORD/STANDARD LENGTH (mm)
1.6
2.5
7.0
16.0
EMBRYO
YOLK SAC LARVA
LARVA
HATCHING
COMPLETION OF
YOLK SAC AND OIL GLOBULE
ABSORPTION
TRANSFORMING
LARVA
EARLY JUVENILE
2
4
6
8
10
20
40
60
80
100
AGE (Days)

Figure 42. Head length and notochord or standard length relationships of laboratory-reared and wild _Leiostomus xanthurus_ larvae. Head length was taken as the distance from the tip of the snout to the intersection of the cleithrum and the notochord.



A
B
HEAD LENGTH (mm)
0
1
2
3
4
5
6
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
NOTOCHORD / STANDARD LENGTH (mm)

Figure 43. Head height and notochord or standard length relationships of laboratory reared and wild *Leiostomus xanthurus* larvae. Head height was measured following Ehrlich et al. (1976).

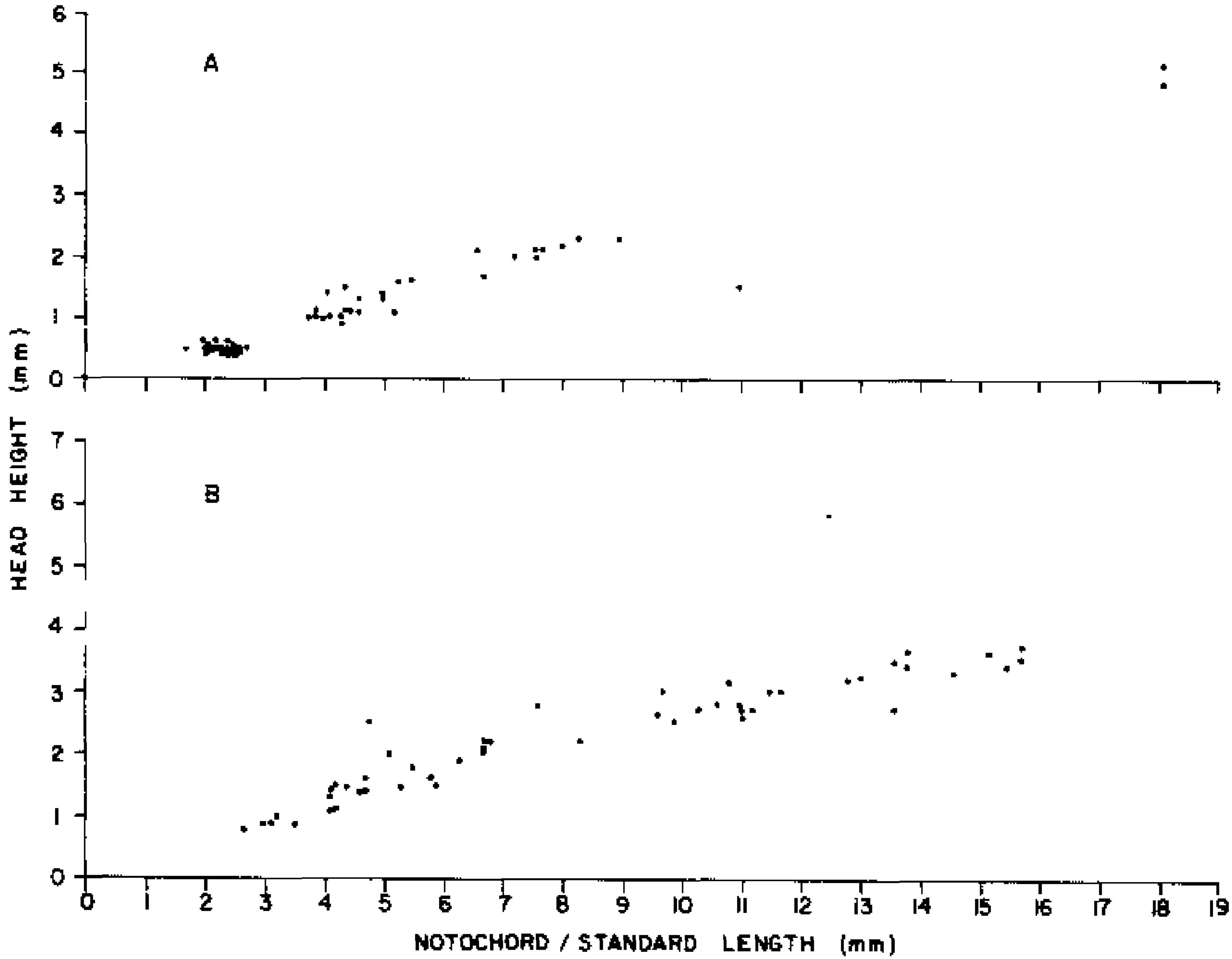

A
B
HEAD HEIGHT (mm)
NOTOCHORD / STANDARD LENGTH (mm)

Figure 44. Eye diameter and notochord or standard length relationships of laboratory reared and wild *Leiostomus xanthurus* larvae. Eye diameter was measured following Richardson and Joseph (1973).

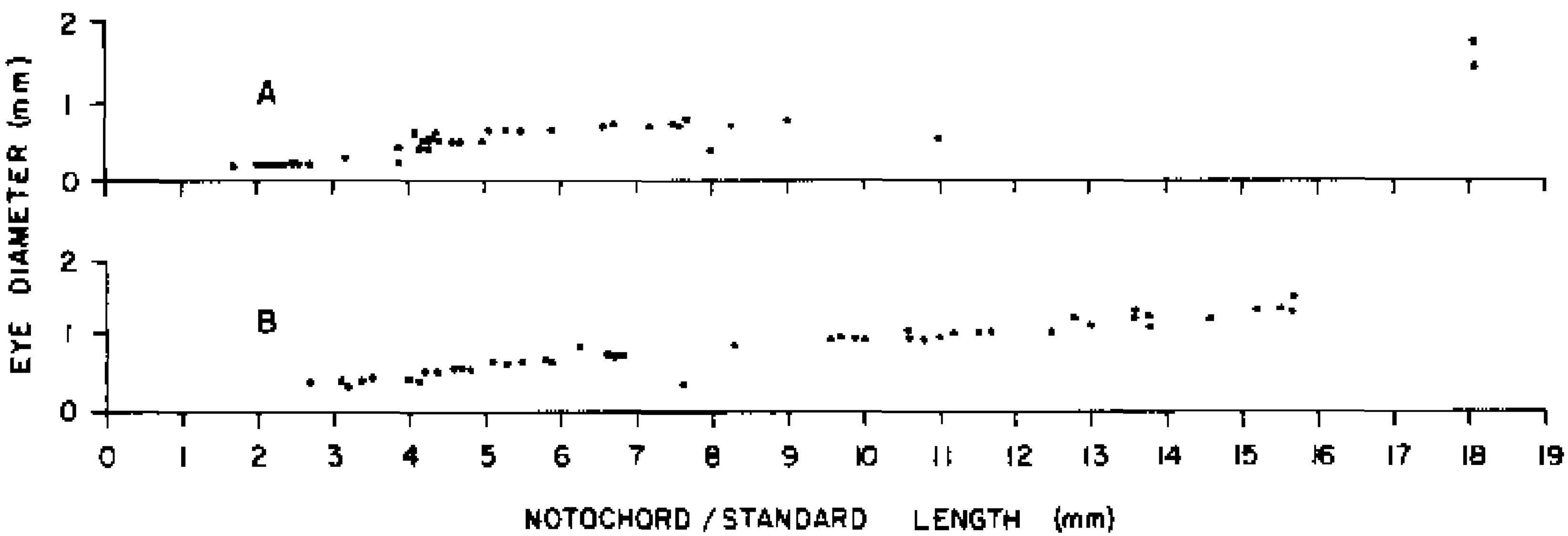

A
B
EYE DIAMETER (mm)
0
1
2
0
1
2
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
NOTOCHORD / STANDARD LENGTH (mm)

Figure 45. Eye height and notochord or standard length relationships of laboratory reared and wild *Leiostomus xanthurus* larvae. Eye height was measured following Ehrlich et al. (1976).

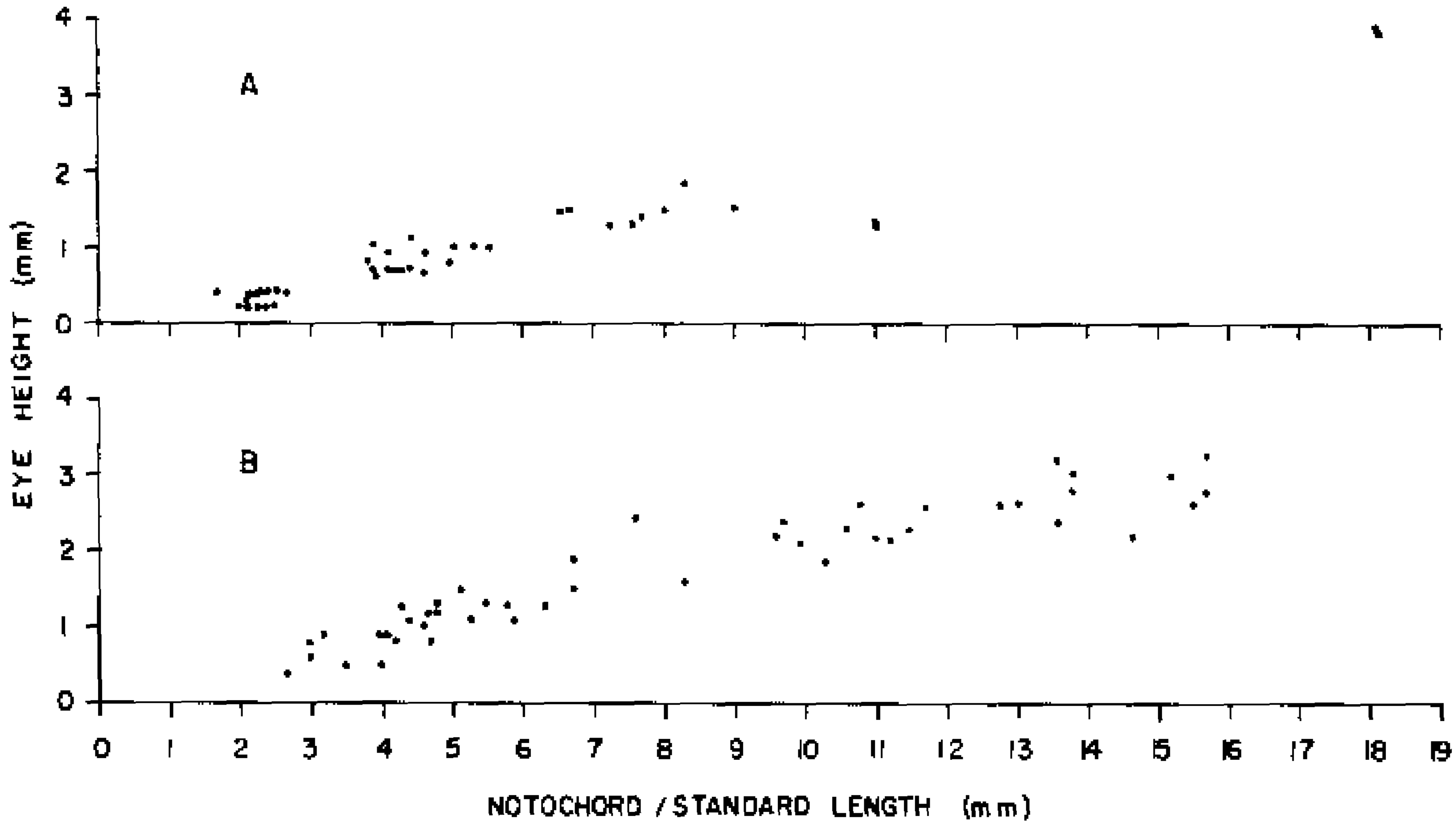
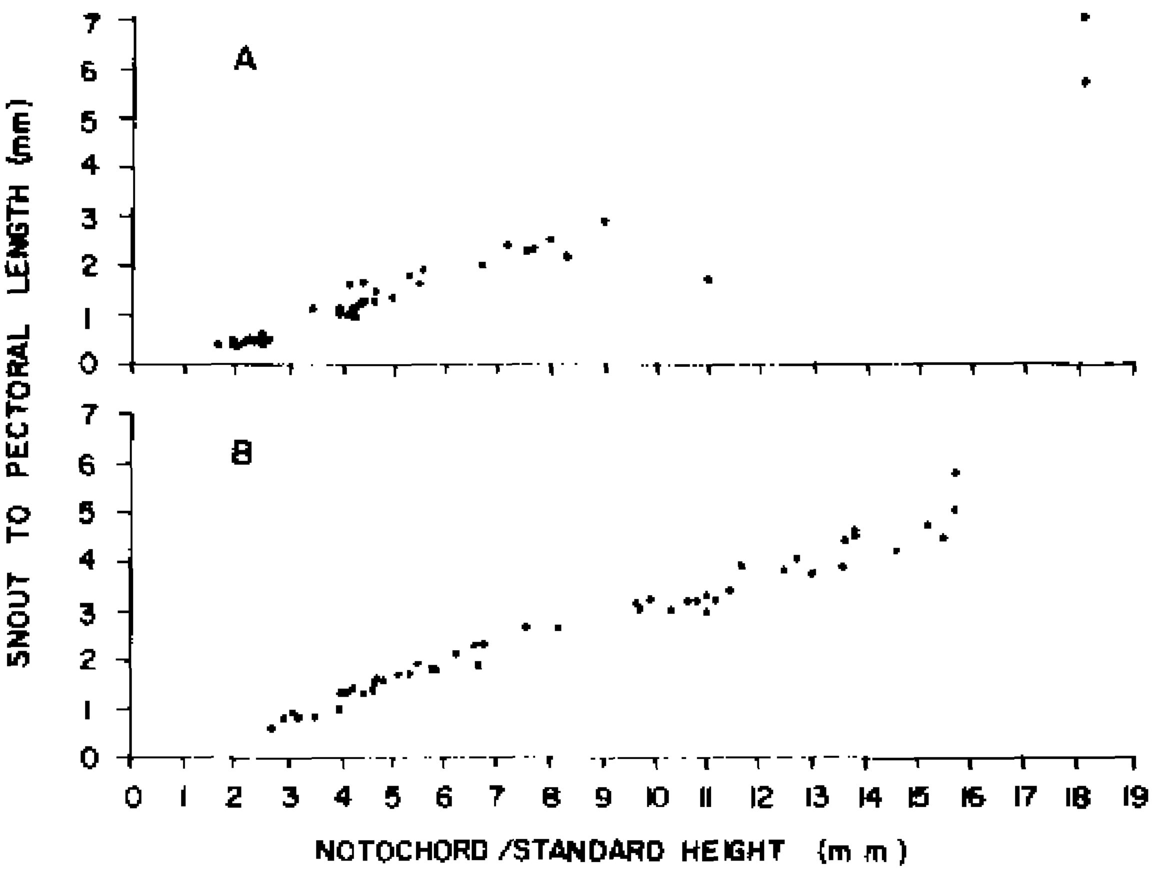

A
B
EYE HEIGHT (mm)
NOTOCHORD / STANDARD LENGTH (mm)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19

Figure 46. Snout to pectoral length and notochord or standard length relationships of laboratory reared and wild *Leiostomus xanthurus* larvae. Snout to pectoral was measured following Mayo (1973).



A
B
SNOUT TO PECTORAL LENGTH (mm)
0
1
2
3
4
5
6
7
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
NOTOCHORD /STANDARD HEIGHT (m m)

Figure 47. Pectoral depth and notochord or standard length relationships of laboratory-reared and wild *Leiostomus xanthurus* larvae. Pectoral depth was measured following Mayo (1973).

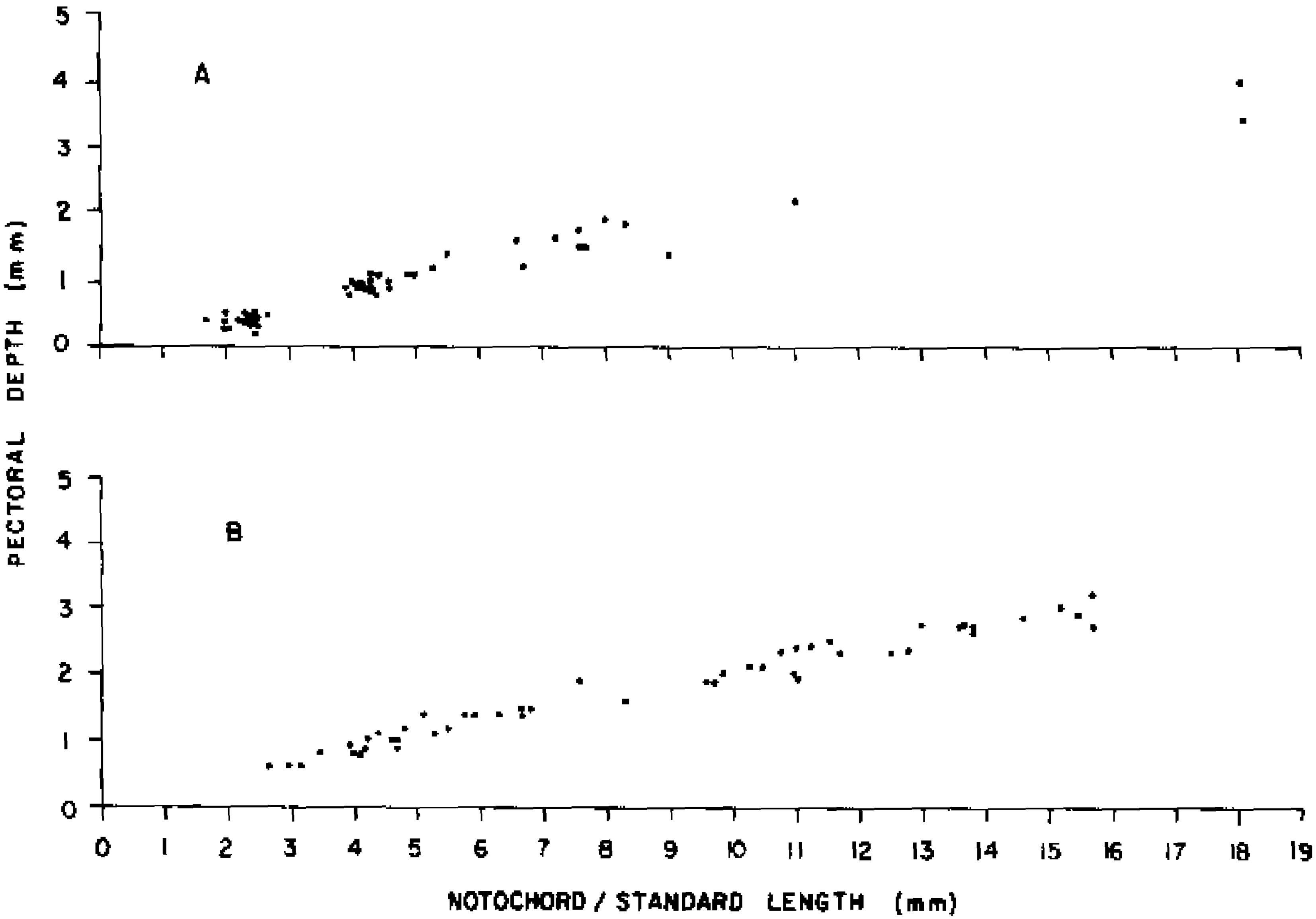

A
B
PECTORAL DEPTH (mm)
NOTOCHORD / STANDARD LENGTH (mm)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19

Figure 48. Preanal length and notochord or standard length relationships of laboratory-reared and wild *Leiostomus xanthurus* larvae. Preanal length was measured following Mayo (1973).

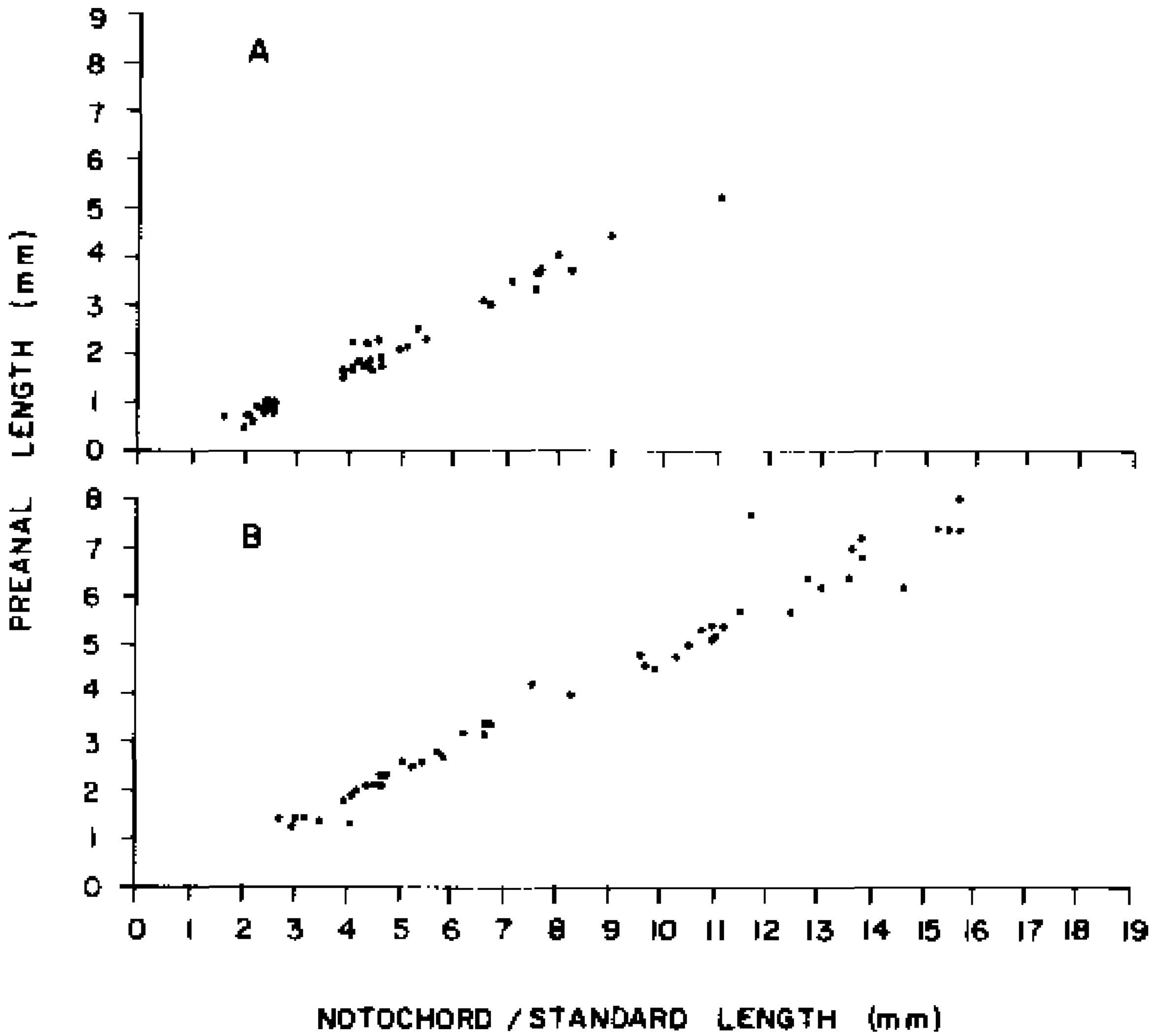
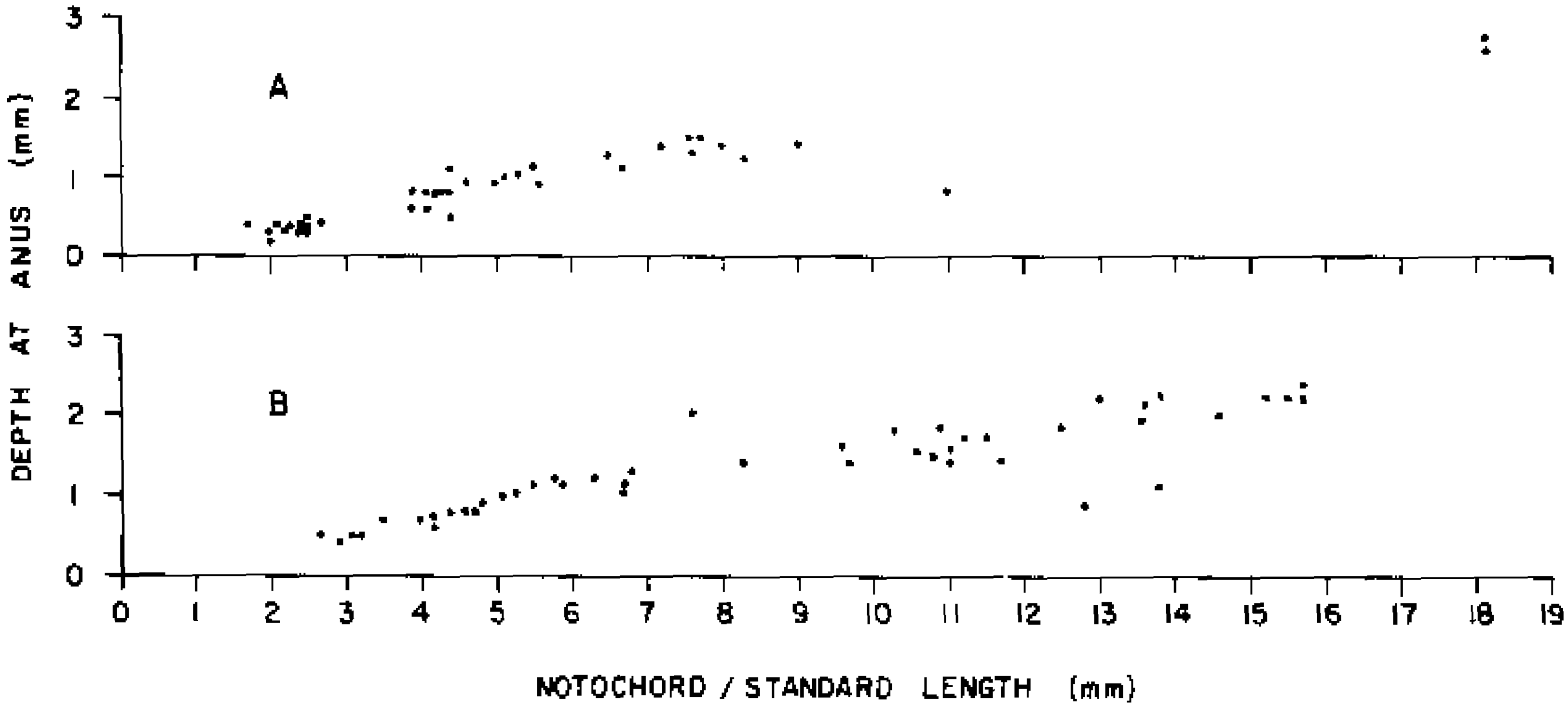

A
B
PREANAL LENGTH (mm)
NOTOCHORD / STANDARD LENGTH (mm)

Figure 49. Depth at anus and notochord or standard length relationships of laboratory-reared and wild *Leiostomus xanthurus* larvae. Depth at anus was measured following Mayo (1973).


A
B
DEPTH AT ANUS (mm)
0
1
2
3
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
NOTOCHORD / STANDARD LENGTH (mm)

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