Lily Ann Kohlweiss POPULATION DYNAMICS OF THE INTERNAL PARASITES OF LEPOMIS GIBBOSUS (LINNAEUS) (CENTRARCHIDAE) FROM MALLARD CREEK, NORTH CAROLINA. (Under the direction of Dr. James S. McDaniel) Department of Biology, April 1971.

Studies of the dynamics of fish-parasite relations are still relatively few, although effective management and utilization of freshwater fish as a food resource requires a closer examination of this association. The sunfish under study herein, *Leppmis gibbosus* (L.), from eastern North Carolina, harbored *Leptorynchoides thecatus* (Acanthocephala) and *Barbulostomum cupuloris* (Trematoda: Digenea) in the small intestine, and some *Proteocephalus* sp. cestodes and acanthocephalan cystacanths in the liver. Throughout the study, all fish taken were infected with at least one species of parasite. Five-year-old fish were the most heavily infected, and infections declined in older fish. None of the fish showed any external manifestations of their infection, although ulcer-like lesions were observed in the small intestine about the attachment organs of the acanthocephalans. The absolute number of parasites was higher in female fish than in male fish. This may be related to the breeding cycle of the sunfish. A seasonal cycle was indicated for the Acanthocephala and Trematoda by the presence of a peak month of infection, followed by a decline in intensity. Only three species of parasites were obtained, possibly indicating a response to the stresses imposed by the euryhaline environment.

POPULATION DYNAMICS OF THE INTERNAL PARASITES OF *LEPOMIS GIBBOSUS* (LINNAEUS) (CENTRARCHIDAE) FROM MALLARD CREEK, NORTH CAROLINA

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by

Lily Ann Kohlweiss

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Dedicated

to

My Parents

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INTRODUCTION

The steady growth of world populations is gradually forcing nations to expand their search for new sources of protein. Man has systematically harvested the land and the sea, but thus far has paid less attention to fresh-water resources. Most fresh-water fish, with the exception of carp and very recently catfish, are not farmed and harvested on a large scale. Instead they are considered game fish, and only the individual angler or small-operation commercial fisherman takes them. Increasing numbers of people may make it necessary to undertake more large-scale rearing and harvesting of these fish, and any attempt to cultivate fish requires a knowledge of parasites and their relations to the fish.

Surveys of fish parasites have been carried out over the years by numerous workers; but then, as now, they were limited primarily to the enumeration and description of the parasites found. This was, and still is, necessary, in view of the numbers and new species continually being found. Recently, however, through the efforts of such workers as Chappell (1969), Chubb and Awachie (1964), and scientists in the U. S. S. R. (Dogiel, 1961), the actual dynamics of the relationship between fish and parasite are being examined. This field is still relatively unexplored, but it is of vital importance if man is to successfully manage this resource.

The object of this thesis is to determine the relationship between a member of the family Centrarchidae (sunfishes) and its internal parasites. The sunfish under study was Lepomis gibbosus, the pumpkinseed sunfish. Fish were taken from Mallard Creek, a minor tributary of the Pamlico River in Beaufort County, North Carolina (Plate 1). The area is popular as a fishing place and is relatively easy of access. In addition to sunfish, it supports a number of game fish such as pike, yellow perch, and bass. Surrounded by low swampy land, relatively shallow, with an abundance of submerged vegetation and made somewhat brackish by its proximity to the tidal Pamlico River, Mallard Creek appeared excellent for the maintenance of a variety of parasitic infections (Meyer, 1954).

The pumpkinseed sunfish is a popular fish with weekend anglers, small boys, owners of farm ponds, and fish hatcheries. They are hardy and prolific and quite easily caught. Besides making a tasty meal for man, this sunfish serves as prey for the larger carnivorous game fish. They are popular in fish hatcheries as a self-perpetuating food supply for maturing game fish, although if not held in check they can become so numerous as to be detrimental to the hatchery (Davis, 1956). Parasitic infections in sunfish, therefore, may have serious implications. The sunfish may serve either as an intermediate or as a final host for many parasites, since its major food is small invertebrates such as snails, insects, amphipods, copepods, other arthropods, and worms. Larger fish may become infected by preying on the sunfish.

In a crowded situation, low-grade or chronic infections could become acute and assume epizootic proportions, with disastrous results. Fish with unsightly infections, especially in skin or muscles, are usually wasted when caught, even though thorough cooking would remove the potential danger to man (Meyer, 1954). Most fish parasites are not hazardous to man unless the fish is eaten raw (Hoffman and Sindermann, 1963).

The association between fish and parasites involves many things. This thesis will attempt to evaluate the relationship on the basis of the age and sex of the host fish, its physiological state, and its feeding habits. Seasonal parasite loads were determined and an attempt was made to relate them to current environmental conditions.

This survey is a part of an extensive study of Mallard Creek begun in 1969 by the faculty and graduate students of the biology department of East Carolina University. Work is underway to determine the age and growth of the resident fish populations and their feeding habits, the organisms of the bottom sediments, physical characteristics of the bottom, the invertebrates associated with the floating vegetation, the algae, and the periphyton and perizoon of the overhanging vegetation. All of these studies will aid in expanding our knowledge concerning the coastal waters of North Carolina and their potential for expanding the economic picture of the State.

MATERIALS AND METHODS

Mallard Creek, the site of these collections, is located on the north shore of the Pamlico River (Estuary) approximately 12 miles east of Washington, Beaufort County, North Carolina. From Washington, proceed approximately 8 miles on U.S. 264 to the intersection of County Road 1334 (Jessama, N. C.). Continue along this road for 3.5 miles, then turning onto CR 1335, which terminates at Camp Leach, immediately east of the boat landing area at Mallard Creek (Plate I).

Specimens of *Lepomis gibbosus*, the pumpkinseed sunfish, were taken from Mallard Creek over a period of five months in 1969, June through October, and one month in 1970, May. A total of 80 fish were obtained.

All fish were taken by angling, and approximately 14 fish were collected each month. They were examined within a few hours of capture, or frozen for later study. Standard lengths, weight, sex, and condition of each fish was recorded before autopsy. Scale samples were taken and ages of the fish were obtained through interpretation of these by Mr. C. Grimes. As one complete annulus is formed by the end of each growing season, a fish with one annulus was said to be in age class I or its second growing season; a fish with three annulae was said to be in age class III or its fourth growing season, etc.

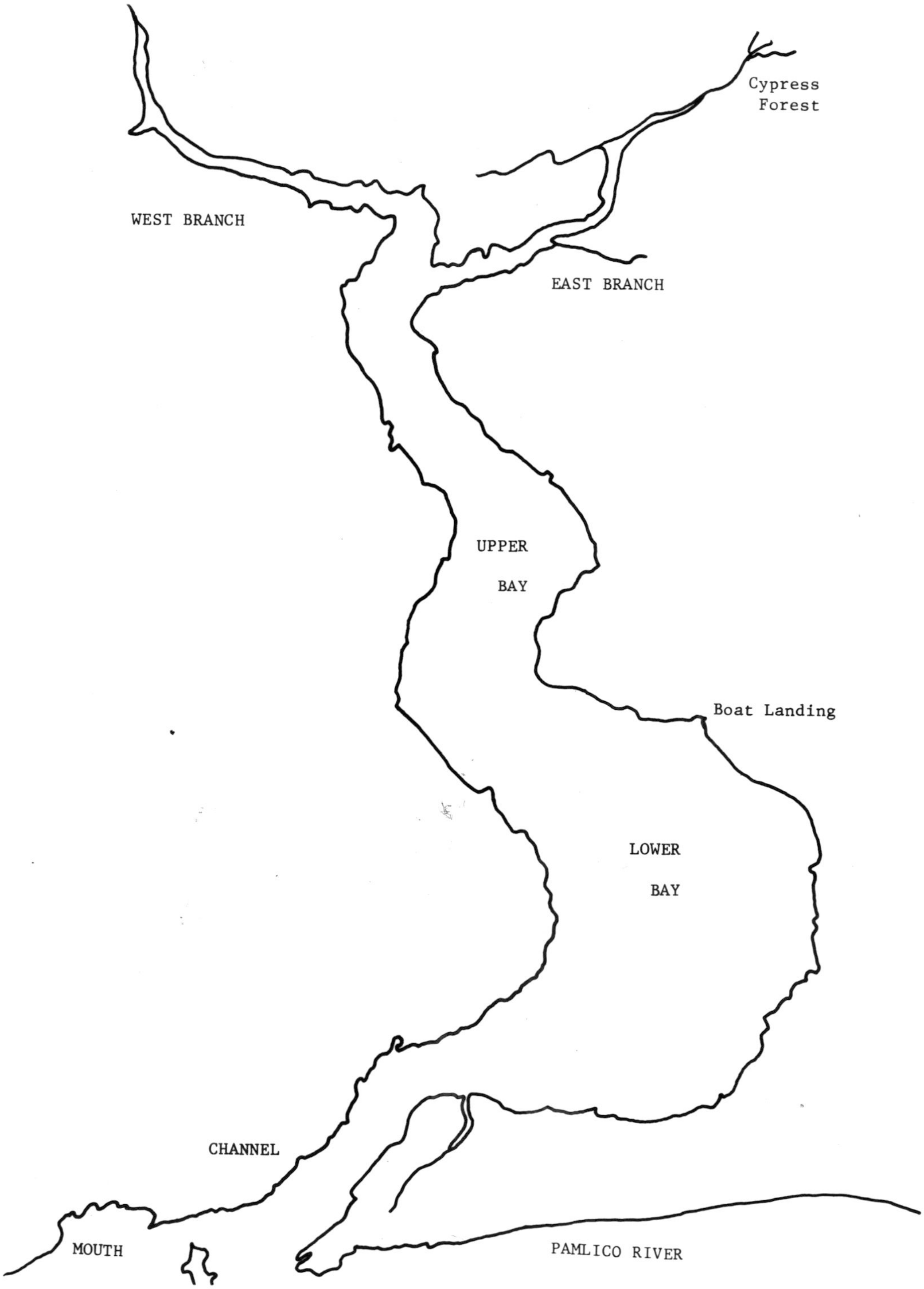
The intestines were opened in petri dishes filled with 0.7% NaCl (fresh hosts) or tap water (frozen hosts), and the worms were placed in

70% alcohol for storage. Some trematodes were placed first in warm AFA and later transferred to 70% alcohol for storage. Selected worms were stained in Mayer's paracarmine and mounted (wholemounts) in Permount. Standard histological techniques were used throughout the study.

Where applicable, data were subjected to appropriate statistical analyses.

Plate 1. Mallard Creek

X: Collection Sites


Cypress
Forest
WEST BRANCH
EAST BRANCH
UPPER
BAY
Boat Landing
LOWER
BAY
CHANNEL
MOUTH
PAMLICO RIVER

RESULTS

Parasites representing two phyla, Acanthocephala and Platyhelminthes were recovered from the sunfish (Table I). All fish harbored at least one type of worm, the locations of which were the small intestine (Acanthocephala and Trematoda) and the liver (Cestoda).

Acanthocephala *Leptorynchoides thecatus* (Linton, 1891)

The genus *Leptorynchoides* includes forms that parasitize marine fish as well as fresh-water fish. Lincicome and Van Cleave (1949a; 1949b) recognized *L. thecatus* as the single essentially fresh-water species of the genus for the North American continent. *Leptorynchoides thecatus* has been recorded from New England to Florida, and as far west as the Mississippi River. This species is capable of utilizing a large number of unrelated families of fish as definitive hosts, but appears to be associated predominantly, as far as numbers and degree of infection are concerned, with the family Centrarchidae (De Giusti, 1949).

All fish harbored adults of this form (Table I). A pronounced fluctuation was observed in numbers over the six-month sample period. The greatest total number of worms was found in August. Analysis of the data on host sex revealed that female fish were more heavily infected in June, while males reached their peak infection in August (Table II). Lesions from the proboscis hooks of the attached worms were observed in the small intestine.

Encysted larval Acanthocephala (cystacanths) were found in the livers of some of the fish, although not in great numbers (Table I). It was not possible to identify these as to genus or species, but it is likely that they were *L. thecatus*.

Trematoda *Barbulostomum cupuloris* Ramsey, 1965

The single digenetic trematode species harbored by *L. gibbosus* was identified as *B. cupuloris*, although with some reservations. It is undoubtedly of the Family Lepocreadiidae, which is composed of trematodes that infect both marine and fresh-water hosts (Hoffman, 1967). However, these specimens differed somewhat from the original description (Ramsey, 1965), as well as from the descriptions of *Homalometron armatum* and *H. pallidum*, which are very similar forms and members of the same subfamily Homalometroninae Manter, 1963, as described by Miller (1959) and Stunkard (1964), respectively.

The worms differ from *B. cupuloris* in the following respects: 1) size of eggs smaller on the average; 2) excretory bladder does not extend as far anteriorly; 3) worm smaller in size.

It differs from *H. armatum* in 1) the shape of the oral sucker; 2) shape of the excretory bladder; 3) extent of uterus; 4) extent of intestinal ceca. It differs from *H. pallidum* in 1) number and size of cuticular spines; 2) shape of the oral sucker; 3) size of the eggs; 4) size of the acetabulum.

Ramsey created this new genus and species on the basis of the cupuliform shape of the oral sucker and the presence of two ventro-lateral retractable papillae on it. In these two respects, *B. cupuloris* differs from the other members of the subfamily (Appendix C). The trematodes in my study had an oral sucker whose shape was subtriangular or cupuliform in nature, and possessed papillae on either side of this organ; hence, they were identified as *Barbulostomum*.

If this is indeed the proper taxonomic position for this worm, it apparently represents a new host record and an extension of the range. To my knowledge, no other descriptions of this worm have appeared, nor has any further elucidation of its life cycle been made.

Trematodes were recovered from fish beginning in late May (Table I) and highest infection was in the August sample of fish. None were recovered from the June sample of fish because they were not sought. All those found were free in the small intestine.

Cestoda Proteocephalus sp.

All cestodes found were plerocercoids, or immature larval forms (Table I). Presence of four suckers and a fifth apical organ indicated the genus, but further identification was not possible. All were found in the liver, but in no case did the liver appear unduly damaged.

Infection patterns in fish

The absolute number of parasites obtained was higher in female fish as opposed to males (Fig. 1; Table II). However, no statistical difference was seen in the numbers of parasites taken from male and female fish as determined by Student's "t" test (Table II; Appendix C).

Numbers of parasites found increased with increasing age of the fish from age class I to age class V. Few fish older than this age were taken, making accurate estimates of the trend in these older fish impossible (Fig. 2; Appendix A). No change in species or number of species was recorded throughout the lifetime of the fish.

Peak infection of all fish occurred in August (Figs. 1, 3; Table I).

Infected fish had lesions in the small intestine from the proboscis hooks of the acanthocephalans. Most fish were in breeding condition throughout the study, but none showed any obvious reduction in reproductive capacity or any external symptoms of the infections.

TABLE I

Seasonal Variation in Parasites of *Lepomis gibbosus* from Coastal North Carolina

	Months						Total	
Parasites	May	June	July	August	September	October	Number	%
Examined	17	11	14	17	15	6	80	
Infected	17	11	14	17	15	6	80	100
Leptorynchoides thecatus	668	642	725	765	321	185	3306	76
Acanthocephalan cystacanths	3	2	0	6	13	0	24	.05
Barbulostomum cupuloris	170	—	130	521	120	39	980	22
Proteocephalus sp.	0	0	7	19	7	1	35	.08
Total	841	644	862	1311	461	225	4345	100
% of Total	19	16	19	30	10	5	100	

TABLE II

Number of Parasites and Sex of Fish

Month	Parasites: *Leptorynchoides thecatus*		Cystacanths		*Barbulostomum cupuloris*		*Proteocephalus* sp.		Number of Fish	
	HOST SEX									
	M	F	M	F	M	F	M	F	M	F
May	200	468	2	1	49	121	0	0	6	11
June	80	562	0	2	—	—	0	0	3	8
July	345	380	0	0	30	100	5	2	7	7
August	620	145	4	2	354	167	9	10	13	4
September	79	242	2	11	46	74	5	2	4	11
October	50	135	0	0	14	25	0	1	3	3
Total	1374	1932	8	16	593	487	19	15	36	44
Average	38	43	4	2	13	4	2	3	6	7

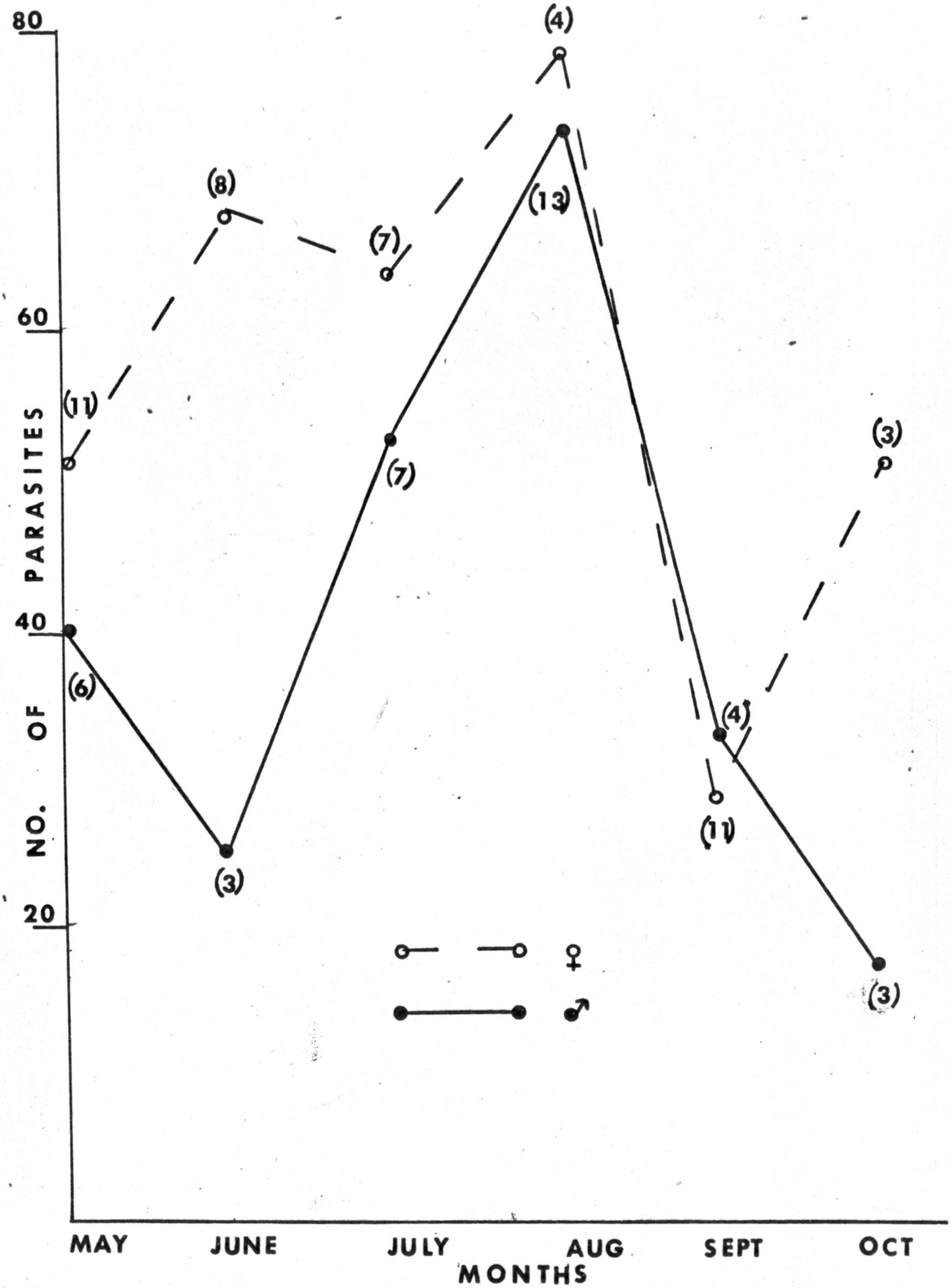


Fig. 1. The relationship of host sex to average parasite load. The numbers in parentheses represent the number of fish in the sample.

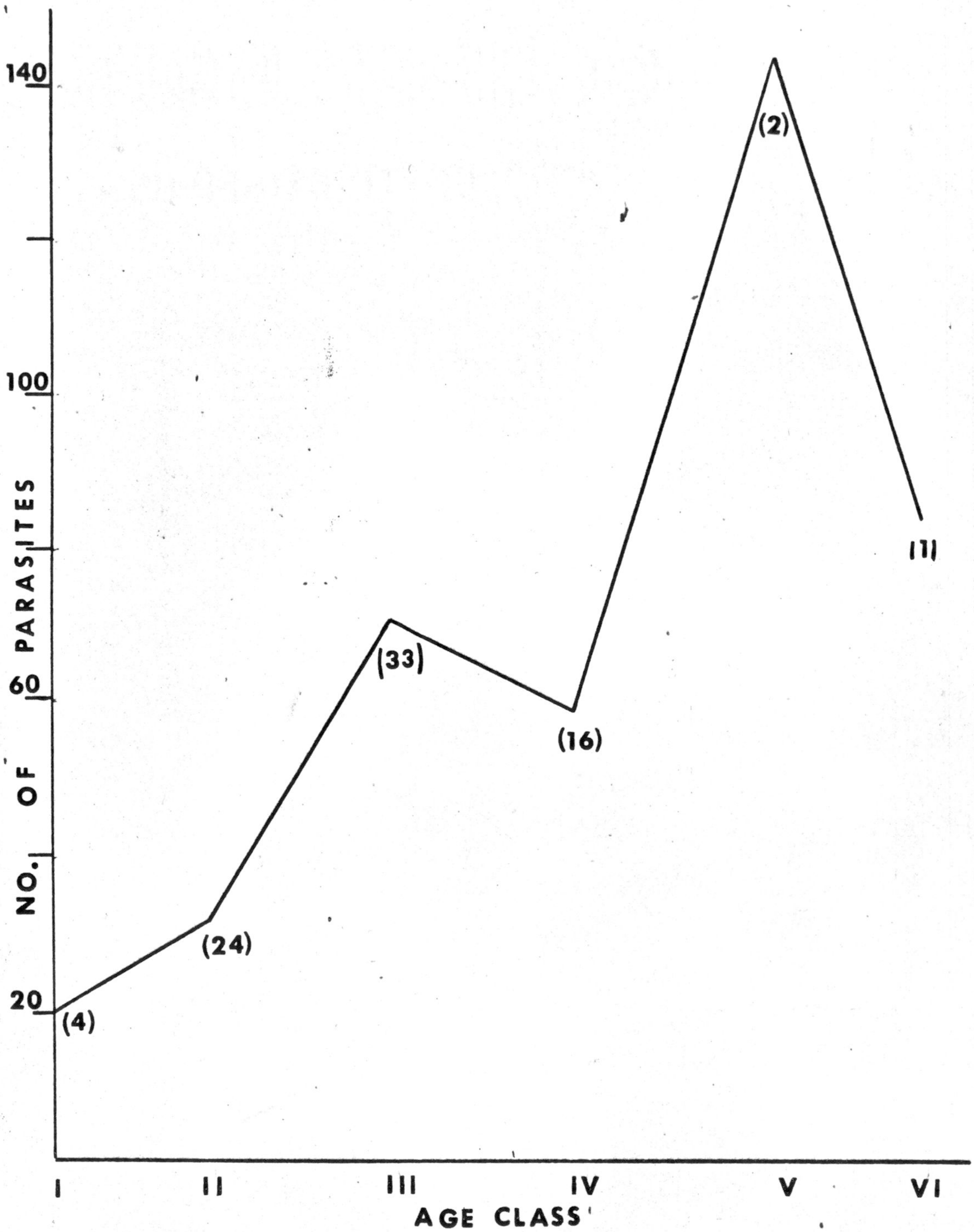


Fig. 2. The relationship of host age to average parasite load. The numbers in parentheses represent the number of fish in the sample.

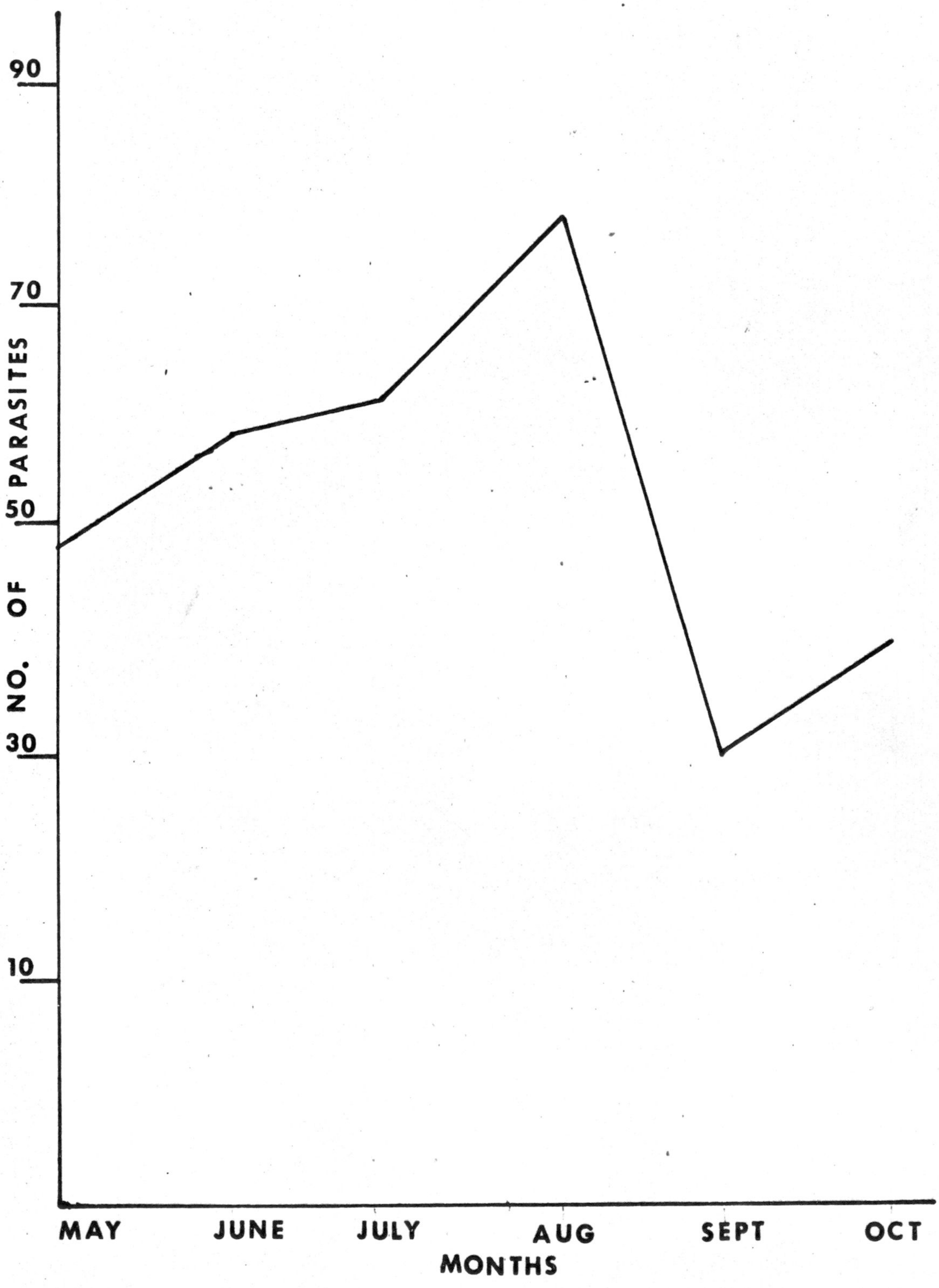


Fig. 3. The relationship of season to the average parasite load.

DISCUSSION

Interaction of biotic and abiotic factors influence the parasite fauna found in fish of different regions. The number and type of parasites found in fish may depend on the age and sex of the fish, its physiological state, its feeding habits, the density of the population, immune responses, the habitat, water temperature, and the season of the year (Northcote, 1957; Dogiel, et.al., 1961; Chappell, 1969).

In this study, 100% of the fish examined were infected with at least one species of parasite. Statistical tests showed no significant difference in the number of parasites harbored by male and female fishes (Appendix C); this agrees with Chappell's (1969) study of sticklebacks of a Yorkshire pond. Other workers have noted significant differences depending on the physiological state of the fish. According to Kennedy (1968) and Thomas (1964), females may be more heavily parasitized when in breeding condition than the males. Kennedy (1968) observed that very heavy infections of the tapeworm *Caryophyllaeus laticeps* occurred in female dace during spawning. He attributed this to a lowering of resistance in the females due to stress at this time, or to changes in hormonal balance. However, estrogens are supposed to increase resistance to parasite infection. Thomas (1964) hypothesizes that as the level of estrogen is depleted after spawning, the resistance of the fish may be decreased, thus resulting in higher infections.

The average number of parasites found in female fishes is seen to be generally higher than that of the males in this study, and infections show a somewhat different pattern (Fig. 1). The females tend to acquire their parasites in a stepwise manner, reaching a plateau in June and July and then increasing again through August. Males appear to acquire their inhabitants in a rather dramatic leap. Following the peak month in August, the parasite loads of both sexes drop precipitously. This is followed in October by a rise in parasite numbers in females, roughly to the same level as that encountered in May. Males continue to show a decline. This temporal difference in parasite loads may be related to the breeding behavior of the fish. Male sunfish are known to construct nests and engage in courtship rituals (Walden, 1964), after the chosen female has spawned, the male guards nest and fry until they are old enough to fend for themselves. This period is one of stress for the male. According to Thomas (1964), the level of stress due to the courting activity of the male brown trout results in a release of adrenal glucocorticoids that may decrease the immune response and result in greater infections.

Sunfish are capable of spawning twice in one season (Walden, 1964); thus the small increase in incidence found in females in October may be correlated with a second brood of eggs. It would appear from the data that the cycle begun in May was about to repeat itself in October, although I would suspect that, because of the approach of autumn and colder weather, this second cycle would not be as extended as the first one, and possibly the number of parasites not as great. Further collections of fish from Mallard Creek during the winter months are indicated.

The age and size of fish may be correlated with both the number and species of parasites present. Studies by other workers appear to show two major trends. Many fish show an increase in parasite levels, both number and species, as they grow older (Dogiel et al., 1961; Walkey, 1967; Chappell, 1969). In 1961, Dogiel postulated a rule stating that increase in age of fish was correlated with an increase in intensity and incidence of infection. More recently, however, a second trend has been observed. In this case, the abundance of parasites increases up to a certain age, at which point the increase is halted or reversed (Thomas, 1964; Awachie, 1966; Chappell, 1969). For example, Awachie (1966) in his observations on the cestode Cyathocephalus truncatus in trout (North Wales), showed that there is a tendency for this worm to increase with increasing length and age of the fish up to 3+ (in the fourth growing season) year-old fish. In the 4+ year-old fish there is a downward trend. The two trends may be due to the fact that some parasites are acquired seasonally and others cumulatively.

Dogiel's rule was based on the fact that older and larger fish have more parasites because they have been exposed to the chance of infection over a longer period of time and eat more, larger, and more varied prey in accordance with their size. Larger fish often tend to have different dietary habits than smaller ones, and this might explain the change or increase in parasite species with age. The possibility of age immunity developing in fishes is still uncertain. Dogiel (1964) stated that it could occur in birds and mammals but not in the lower vertebrates or invertebrates. Chappell (1969) indicated that an immune response was possibly indicated in sticklebacks, and Cross (1935) thought that age resistance might be a possible factor in the ability of some fish to withstand certain parasites. However, the development of resistance with age, if present, does not involve all the parasite species in one host to the same degree.

The fish of Mallard Creek appeared to follow the second trend of decreasing numbers of parasites as they approach a certain age. The number of parasites increased fairly steadily to age class V, which exhibited the highest average number of parasites. The number of parasites declined sharply in fish older than age class V. These results may indicate several things. As the species found did not vary with the age of the fish, it might be assumed that the dietary habits change very little with increasing size and age. Although carnivorous, the pumpkinseed sunfish rarely, if ever, attains a size that would enable it to eat anything larger than small invertebrates. However, larger specimens do require more food, and this may account for the increase in intensity of infections.

Age classes V and VI were poorly represented, probably indicating that older fish do not exist in great numbers in Mallard Creek. Sunfish have a life span of 5-6 years (Walden, 1964). The advanced age of these older fish may restrict their activity sufficiently so that they are easy prey for larger fish. Not enough fish of these age classes were taken to determine whether acquisition of parasites reaches a peak in age class V, thus following the second trend previously discussed. The decrease in parasites in age class VI may have been due to the small sample.

The small standard errors of the mean (Appendix A) in the age classes where there were enough representatives to warrant analysis would tend to indicate that age classes V and VI would show a statistically different number of parasites.

None of the sunfish taken showed any external manifestations of the infection; the breeding capacity did not appear to be impaired, the general state of health was good, and the rate of growth was typical. Parasitism often interferes with the growth of cold-blooded hosts, but there is no evidence that robbing the host of food is responsible (von Brand, 1966). Intestinal parasites must utilize the type of food that reaches them, and what is absorbed in the stomach is obviously not available to the intestinal worm. Thus, the character of the diet exerts an important influence on the types of parasites harbored by the host.

The small intestine of these fish, the site of most infection, showed mechanical damage dependent on the intensity of the infections. This damage was due to the proboscis hooks of the acanthocephalans, which Hyman (1951) observed to be the most injurious of all helminth attachment organs. Penetration of the intestinal lining by these hooks, which grapple into the tissue, causes tearing and ulcer-like lesions. Elimination of worms results in wounded tissue which may lead to inflammation, excess mucous, infection, and scarring. Mechanical injury also interferes with the absorption of food and the secretion of enzymes by reducing the effective surface area left to the fish.

Acanthocephalans absorb their food through the body wall; therefore, heavy infection could result in the worms presenting more surface area to the food than the host's intestine. Very often, however, no debilitating effects are evident in the host. Self and Timmons (1955) noted that injury by Neoechinorynchus prolixus to the carpsucker was negligible unless more than 80 worms were present. When this point was reached, partial occlusion of the gut was observed. Fox (1962) studied trout in Montana and found little relationship between parasite incidence and condition of the host. My study indicated that even heavy infection with Acanthocephala (130 worms in one age class III fish) did not result in outward evidence of injury or debilitation. It is conceivable that growth retardation could occur in smaller fish with very heavy infections, but we have no evidence for this in data on sunfish growth in Mallard Creek (Grimes, unpublished observations).

The incidence of infection of fish with parasites is a dynamic equilibrium between loss and gain throughout the year, a factor which is often overlooked (Hopkins, 1959). The seasonal variation in parasite levels has been demonstrated in several cases (Hopkins, 1959; Dogiel et al., 1961; McDaniel, 1961, 1969; Tedla and Fernando, 1969). Kennedy (1968) noted that the incidence of seasonal rhythms in maturation and incidence of parasitism of temperate zone fresh-water fish have often been reported. These fluctuations appear to be the result of changes in feeding activity on the part of the host (possibly due to its physiological condition), seasonal changes in numbers of intermediate hosts, and the presence of an annual life cycle in some parasites (Dogiel, et al., 1961). Both Dogiel and Hopkins show that acquisition of parasites in the summer is high. Studies by McDaniel (1961) on Buncombe Creek, Oklahoma, showed little seasonal change in total parasitism in four species of sunfishes. The numbers of parasites found were small, but the fish were infected at an early age and remained so. Later studies on Little River, Oklahoma, showed that the greater number of fish were infected in the winter months, and spring and summer infections were lower and more variable (McDaniel and Bailey, 1966; McDaniel, 1969). These results may be due to the nature of Oklahoma streams which tend to dry up into pools during dry summers. Van Cleave (1916) noted that in the Acanthocephala the seasonal distribution varies according to the species. For example, Neoechinorynchus gracilisentis is not found in gizzard-shad in Illinois in mid-summer, while N. longirostris in the same fish is not found from later winter to early summer.

Whenever there is a seasonal periodicity in parasite development there is a peak level of infection at one time of the year (Chubb and Awachie, 1964). This time may vary depending on the species encountered. Infection with the cestode Proteocephalus stizostehthi appears to reach its peak in the pikeperch of Lake Erie in the spring (Connor, 1953). Proteocephalus pearsei infections in Canadian perch are greatest in the summer (Tedla and Fernando, 1969), and observations relate to the breeding cycles of the parasites. Trematodes appear to be more prevalent in summer months and decline in the winter months

(Dogiel et al., 1961; Esch and Gibbons, 1967). Leptorynchoides thecatus has been shown by DeGiusti (1949) to have an annual life cycle with the highest infections in July and September. Immature forms are said to appear in fishes in May and June, eggs are deposited in the summer, and the adult worms die in the beginning of autumn. My study has shown a somewhat similar cycle for coastal North Carolina (Fig. 3). The peak month of infection is August, but larval forms were found as late as September. These may be from a new infection picked up in the course of the summer.

Hopkins (1959) showed that infections with Proteocephalus filicollis did not remain static in the stickleback, but rather was a cycle of loss and gain throughout the summer. Some worms do not mature, may not attach and are lost or die from unknown cause. Infections are picked up steadily throughout the summer and when conditions are most favorable for the worms, few are lost. This is the peak of infection. Adverse conditions, such as those encountered in the winter environment, cause a reduction in numbers, while the return of more favorable conditions in the spring accounts for the increase. An important consideration here is that in winter the host's intestine does not become a poor environment; rather the process of loss, which is already going on, is accelerated. Walkey (1967) observed that attachment organs may have an effect on the extent of infections. He noted that worms such as acanthocephalans, that can bury their proboscis hooks into the lining of the intestine, may have a greater chance of persisting longer and thus account for an increase in burden with age. Another factor that may contribute to the length of stay in the intestine is the fact that many worms tend to move to new positions (Chubb and Awachie, 1964). Free worms are obviously more easily lost than attached ones, which may account for a decrease in numbers. Loss in numbers of Acanthocephala may also be due in part to the fact that spent males die while females may live for a longer time, or vice versa. Loss of these worms at the end of a season when few new infections are being acquired would also account for a decrease in intensity of infection.

Water temperature appears to play a part in the seasonal periodicity of some acanthocephalans (Chubb and Awachie, 1964). The prevalent theory on the seasonal cycles exhibited by many worms is the influence of the water temperature on their life cycle. Many of the seasonal cycles appear to follow the same trends, that of immature forms in spring and autumn, gravid forms in late spring and summer, and disappearance of worms in winter (Walkey, 1967). This similarity leads many workers to feel that the reason behind the seasonal fluctuation is not so much the physiological state of the host, but rather an environmental factor: water temperature (Kennedy, 1970). Dace appear to have a temperature dependent rejection response to parasites that enable parasites to persist at lower temperatures but encourages resistance at higher ones (Kennedy, 1969). In addition, as the parasites are dependent on intermediate hosts for part of their life cycles, changing water temperatures may influence the abundance of these hosts, and thus the intensity of infections picked up by the fish. From my study and those of other workers I think it is not possible to entirely eliminate physiological and behavioral factors from consideration.

Water temperature may also be a factor in an immune response. A relatively high environmental temperature increases the intensity of an immune response. This appears to be related to the amount of antibody that can be released from cells, although it is not yet known whether lower temperatures inhibit antibody synthesis or higher ones increase it (Snieszko, 1969). Perhaps L. gibbosus is capable of resisting infections of most worms to a high degree, and only those worms which have a very widespread range (L. thecatus) or are most specific to the type of environment (B. cupuloris) are able to become established.

The list of parasites harbored by L. gibbosus from any one locality is usually quite long (Hoffman, 1967). However, my locality yielded only two intestinal parasites in this sunfish. The reasons for this are not clear. The euryhaline nature of Mallard Creek may create a more rigorous environment than one purely fresh-water or purely marine. Inhabitants must be able to tolerate certain extremes between fresh and brackish water, and also somewhat tidal fluctuations. Parasites incapable of utilizing brackish water intermediate hosts, or of withstanding the variation in saline content of the water in free-living stages of their life cycles, would naturally be excluded from this sort of environment. No evidence of monogenean trematodes was found on the gills of the fish, and with the exception of two leeches found on one fish, no external parasites, either larval or adult stages, were found. It is an ecological fact that those regions of the earth which provide variable and difficult living conditions such as the Arctic or desert areas, tend to have few species of organisms, but large numbers within the species. The opposite is true of those regions that are temperate and tropical, i.e. many species, but relatively fewer individuals within the species. Mallard Creek would appear to be a fairly productive area for vertebrates, harboring at least seven species of fish besides the sunfish. No stress due to crowding was observed as the fish of all species caught appeared to attain the sizes normal for them in the alloted time span. However, the data clearly implies that the number of species of parasites found in L. gibbosus inhabiting an estuarine environment is not additive as might be expected in an ecotone. Rather, the species appear to be only those best suited for this specific type of area. Suitability involves the adult parasite, its larval stages, or its intermediate hosts (Chubb, 1970).

SUMMARY

1. *Lepomis gibbosus* from Mallard Creek, North Carolina, harbored *Leptorynchoides thecatus* and cystacanths (Acanthocephala), *Barbulostomum cupuloris* (Trematoda: Digenea), and larval *Proteocephalus* sp. (Cestoda). All fish were infected with at least one species of worm.
2. The small intestine was the site of infection except for encysted cestodes and acanthocephalans in the liver. No serious damage was observed to either organ.
3. Five-year-old fish were most heavily infected. Greatest infection for all fish was in August.
4. Seasonal cycles are indicated for the acanthocephalan and trematode.
5. A question was raised as to the identity of the trematode and comparisons with other closely related species of the same subfamily are offered.

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APPENDIX

TABLE III

Age Classes of Fish and Number of Parasites.

Host Age Class	Number of Fish	Number of Parasites	Average Number of Parasites per Fish*	% of Total Parasites
I	4	85	22 ±19	2
II	24	759	32 ±4.5	17
III	33	2221	68 ±1.5	51
IV	16	939	59 ±2	21
V	2	269	135	6
VI	1	78	82	2
Totals	80	4347	54	99

*±Standard error of the mean where number of observations permit.

TABLE IV

Comparison of Taxonomic Characters of Members of the Subfamily Homalometroninae with *Barbulostomum cupuloris* Ramsey, 1965, from *Lepomis gibbosus* in Mallard Creek.

Characters	*Barbulostomum cupuloris* Mallard Creek, N. C.	*Barbulostomum cupuloris* Lake Ponchartrain, La.	*Homalometron armatum*	*Homalometron pallidum*
Size* Length x width	0.58-1.5 x 0.088-0.279	1.68-4.57 x 0.56-0.96	1.7-4.0 x 0.45-1.5	2.72 x 1.07
Oral Sucker Length x width	Cupuliform or sub-triangular; apex directed caudad. Papillae present 0.132 x 0.117	Cupuliform or sub-triangular; apex directed caudad; muscular papillae on ventrolateral edges of mouth .184-.260 x .179-.250	0.15-0.35 diameter	0.26 diameter
Acetabulum Length x width	0.080 x 0.066	0.153-0.240 x 0.173-0.257	0.18-0.51 diameter	0.29 diameter
Ovary	dextral or mesial; between acetabulum and anterior testis; diameter 0.058	dextral or mesial; midway between acetabulum and anterior testis diameter 0.117-0.216	dextral; between acetabulum and anterior testis 0.10-0.27 x 0.12-0.24	0.21 diameter

*All measurements in millimeters.

TABLE IV - Continued

Characters	*B. cupuloris* Mallard Creek, N. C.	*B. cupuloris* Lake Ponchartrain, La.	*H. armatum*	*H. pallidum*
Uterus	extending to level of ovary	ascending intercecally	confined to area between anterior testis and genital pore	
Eggs Length x width	few (4-5), large, operculate 0.058-0.102 x 0.044-0.073	few (1-9), large, minutely operculate, 0.092-0.117 x 0.058-0.082	few, large 0.071-0.115 x 0.048-0.069	0.11 x 0.07
Testes	two, tandem, intercecal 0.088-0.102 diameter	two, tandem, intercecal 0.143-0.362 diameter	two, tandem, intercecal 0.10-0.42 anterior 0.14-0.47 posterior	0.33 x 0.39
Intestinal ceca	bifurcate, simple; extend almost to posterior end of body; end blindly	bifurcate midway between pharynx and acetabulum; extend almost to posterior end of body; end blindly	bifurcation well anterior to acetabulum; extend to posterior end of body	simple, elongate

TABLE IV - Continued

Characters	*B. cupuloris* Mallard Creek, N. C.	*B. cupuloris* Lake Ponchartrain, La.	*H. armatum*	*H. pallidum*
Excretory bladder	tubular, short, barely reaching ends of ceca	tubular, extending from posterior excretory pore almost to posterior testis	sac-shaped, opening at posterior tip of body	
Cuticle	densely spined anteriorly, becoming less so posteriorly	densely spined anteriorly; spines extending to posterior tip, becoming less dense	anterior 2/3 spinose; entirely so in some cases	minutely spinose
Hosts	*Lepomis gibbosus*	*Lepomis microlophus*, *L. punctatus*	*Lepomis humilis*, *L. microlophus*, *L. gibbosus*	*Fundulus heteroclitus*, *Roccus americanus*

APPENDIX C

N = 6 months

$\bar{X}$ number of parasites per female fish per month = 55
$s_1 = 14.3$, $s_1^2 = 206$

$\bar{X}$ number of parasites per male fish per month = 45
$s_2 = 29.3$, $s_2^2 = 859$

$$t = \frac{(\bar{x}_1 - \bar{x}_2)\sqrt{\dfrac{N_1 N_2}{N_1 + N_2}}}{\sqrt{\dfrac{(N_1 - 1)(s_1^2) + (N_2 - 1)(S_2^2)}{N_1 + N_2 - 2}}}$$

$$t = \frac{(55 - 45)\sqrt{\dfrac{6(6)}{6 + 6}}}{\sqrt{\dfrac{(6 - 1)(14.3)^2 + (6-1)(29.3)^2}{6 + 6 - 2}}}$$

$$t = \frac{10\ (1.7)}{\sqrt{532.4}} = \frac{17}{23.1}$$

t = 0.730

Accept null hypothesis: No significant difference between samples (see Table IV).

Statistical Data Sheet 1: Student's "t" test: The relationship of Host Sex to Parasite Load.