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ABSTRACT

PRODUCTION AND TROPHIC ECOLOGY OF TWO CRAYFISH SPECIES COHABITING AN INDIANA CAVE

By

David Lawrence Weingartner

Two species of crayfish inhabit the stream of Shiloh Cave located in Lawrence County, Indiana. One species, *Orconectes inermis* Cope, is classified as a troglobite and, as such, is an obligate cavernicole highly adapted to the cave environment. The other crayfish, *Cambarus laevis* Faxon, maintains a population not only in the cave, but also in the surface stream issuing from the cave; it is considered a facultative cavernicole and is ecologically classified as a troglophile.

Major aims of the study, which was conducted primarily during 1969, were the delineations of the life histories, population parameters, productivities and ecological strategies of both species. The availability, procurement and utilization of energy were important themes of the investigation. Comparisons were drawn not only between the troglophile and the troglobite, but also between the epigean and hypogean crayfish.

The two crayfish species competed for highly restricted food resources, which were composed primarily of vegetative detritus and benthos. Particulate organic matter, which was mostly wood and leaf fragments and associated microflora, was quite limited quantitatively and was of a highly seasonal nature. The organic drift was in a highly decomposed state with the refractive compound lignin constituting almost half of its content. The benthic standing crop, which was primarily

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composed of oligochaetes, isopods and amphipods, was quite meager in comparison to that of the surface stream.

Crayfish ingestion was determined by chemical analysis of fecal matter. A trophic trend was observed in comparing the epigean *C. laevis*, the hypogean *C. laevis*, and the *O. inermis* crayfish; there was a sequential decrease in both the ingestion rate and the utilization of animal material as a dietary component.

Crayfish were individually tagged by a new technique. Both cave species were found to display home range behavior. Crayfish usually resided in a home pool, but wandered into adjacent riffle areas on occasion. Wandering was more frequent and more extensive in *C. laevis* crayfish. In addition, there was evidence that this species migrated between surface and cave habitats.

Various population attributes were studied. In comparison to the epigean crayfish, the hypogean pattern for both species was a small population with low growth, mortality, reproductive and production rates, and with extended longevities. Basal metabolism differed in that it was much lower in the *O. inermis* crayfish than in the equivalent rates found for the surface and cave *C. laevis* populations.

Energy budgets were constructed, and changes in energy partitioning associated with epigeal-hypogeal and troglophilic-troglobitic transformations were discussed.

PRODUCTION AND TROPHIC ECOLOGY OF TWO CRAYFISH
SPECIES COHABITING AN INDIANA CAVE

By

David Lawrence Weingartner

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Zoology

1977

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ACKNOWLEDGMENTS

In conducting this study, I spent many solitary hours in Shiloh Cave, but it would be misleading to give the impression that I alone should receive credit for the results; many people, through their contributions of time, effort, knowledge or interest, have made this study possible. In particular, I would like to express grateful acknowledgment to the following: Dr. William E. Cooper, my major professor, who has surpassed all requirements of his position to guide this project to a successful conclusion; the members of my guidance committee, Drs. T. Wayne Porter, Niles R. Kevern, Richard Merritt and Kenneth W. Cummins, who offered valuable suggestions; Dr. Thomas L. Poulson of the University of Illinois at Chicago Circle, who shared his expertise in cave ecology; Dr. Charles M. Stine of the Food Science Department, who provided essential research facilities; CDR John M. Hoffmann, Chairman of the Chemistry Department of the U. S. Naval Academy, who has been actively supportive of my efforts to complete the manuscript; Mrs. Linda Zelinski, also of the Naval Academy, who gave technical assistance; Mr. John Day, the owner of Shiloh Cave, who gave me permission to carry out this project; Miss Mona Pitman of Bedford, Indiana, who gave me shelter and friendship during the period of field work; my wife, Carol, who has given active support and assistance in all phases of this study; and Dr. E. C. Williams of Wabash College, who instilled in me an interest in cave ecology.

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CHAPTER III

CHAPTER IV

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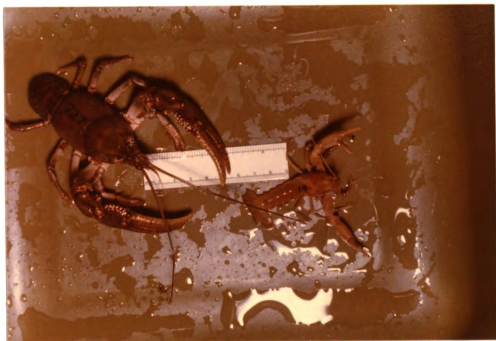
INTRODUCTION

Two species of crayfish, *Cambarus laevis* Faxon and *Orconectes inermis* Cope, co-inhabit the subterranean stream of Shiloh Cave (Figure 1). The association of these two species commonly occurs in many caves of southern Indiana (Faxon, 1914). The occurrence of similar associations involving other crayfish species has been reported frequently in epigean populations. In many cases, however, the species occupy different reaches of the stream (Rhoades, 1962a) or different habitats (Smart, 1962). The ecological isolation of two species studied by Bovbjerg (1970) is apparently maintained by competitive exclusion. Prins (1965) studied a complex association in which two crayfish species primarily inhabited different sections of the stream and, in those reaches where they were sympatric, they occupied different habitats. Cooper (1975) studied the lentic habitat of Shelta Cave, Alabama, and found a complex association of crayfish, in which three troglobitic species that share the same habitat are separated to some extent by different activity patterns. In Shiloh Cave, which has been chosen for the present study, the two crayfish populations are intermingled, both preferring the pool habitat, but also frequenting riffles (Figure 2).

There must be some degree of niche overlap between the two crayfish species occupying Shiloh Cave. This is suggested not only by their physical proximity, but also because of the nature of the food

Figure 1. *Cambarus laevis* (left) and *Orconectes inermis* (right); these individuals, both male, were the largest found.

Figure 2. Typical habitat of hypogean crayfish; Shiloh Cave at 400 meters.



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resources. The food base is quite limited in quantity, quality and variety. Fragments of leaves and twigs, which are washed into the cave by floods, are decomposed by bacteria and fungi. These decomposer populations are grazed by most of the benthic fauna, including the isopods and amphipods, which are among the most important components. Food resources available to the crayfish populations are thus restricted to the detritus-decomposer complex and the limited benthic fauna. Crayfish are typically omnivorous (Bovbjerg, 1952; Tack, 1941; and Vannote, 1963), with food being principally dictated by availability, and, although *C. laevis* grows to a larger size, all available food items are of particle sizes that can be preyed on by both species; therefore, it seems doubtful that the limited food resources would be partitioned between the two species. It is probable that food is the limiting factor for both species, and that they are in direct trophic competition. This conclusion was also drawn by Eberly (1960).

The evolutionary history of these two crayfish is also relevant to understanding their ecological positions in the hypogean community. Hobbs and Barr (1972) believe the caves of this region were invaded by a now extinct Orconectoid surface stock in the late Tertiary. A study by Rhoades (1962b) is in general agreement with this estimate, and states that a specific population of troglobitic crayfish (*O. pellucidus* was the pioneering species) was established and widely distributed in the caves of this region by the beginning of the Pleistocene. The present day *Orconectes inermis* is ecologically classified as a troglobite, and as such is an obligate cavernicole that is highly adapted to the cave environment. Much of this evolutionary modification seems directed toward conservation of energy. This is reflected in the small size of individuals and in their reduced locomotive activity.

Other modifications, such as highly developed sensory organ systems, including longer antennae and increased setation, maximize the ability to detect available food in a lightless environment. The other major type of evolutionary modification has been the loss of features, such as pigment and eyes, which have no function in the hypogean habitat. Barr (1960) believes that regressive evolution of this type can be explained in some troglobites as resulting from mutation pressure unopposed by selection pressure. However, because of the energy cost of developing and maintaining eyes and pigmentation, selection pressure may actually aid in the accumulation of mutated genes. Although the above-mentioned evolutionary mechanisms are widely accepted, the evolution of troglobites has often been explained by alternative theories, such as orthogenesis (Vandel, 1965) and changes in allometric growth rates stemming from reduced metabolism (Heuts, 1953). Because of the extremely small sizes of interbreeding populations, genetic drift must also play a significant role.

Cambarus laevis, on the other hand, is considered a facultative cavernicole, and is ecologically classified as a troglophile. Populations occur in both epigean and hypogean habitats, and any differences between them, such as a slight reduction of pigmentation in the cave-inhabiting form, is probably an ecophenotypic expression; Hobbs and Barr (1960) have described a similar situation for *Cambarus bartonii bartonii*. Eberly (1960) cites both the habitation of epigean and hypogean streams, and the dispersal patterns of ancestral *Cambarus* and *Orconectes* crayfish as two reasons to assume *C. laevis* to be a more recent introduction into caves of the region than *O. inermis*. *Cambarus laevis* is a larger, more robust crayfish than *O. inermis* and is also more active. Its evolutionary history has been primarily

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This ecological confrontation between troglobitic and troglophilic crayfish is not unique to the caves of the Mitchell Plain, but has also been reported from Hell Creek Cave in Arkansas (Hobbs and Bedinger, 1964). Contrary to the situation in Shiloh Cave, however, the troglobite is a species of *Cambarus* and the troglophile is an *Orconectes* species.

CHAPTER II

STUDY AIMS

The purpose of this study was to investigate the ecological roles of the two species of crayfish which co-inhabit the stream of Shiloh Cave, but which possess differing degrees of adaptation to the hypogean habitat. Major aims of the study were the delineations of the life histories, population parameters, productivities and ecological strategies for both the troglobite, *Orconectes inermis*, and the troglophile, *Cambarus laevis*, and the clarification of the relationship that exists between them. Because energy is such an over-ridingly important aspect of cave ecology, the ascertainment of its availability, procurement and utilization was the central theme of this investigation of the crayfish populations.

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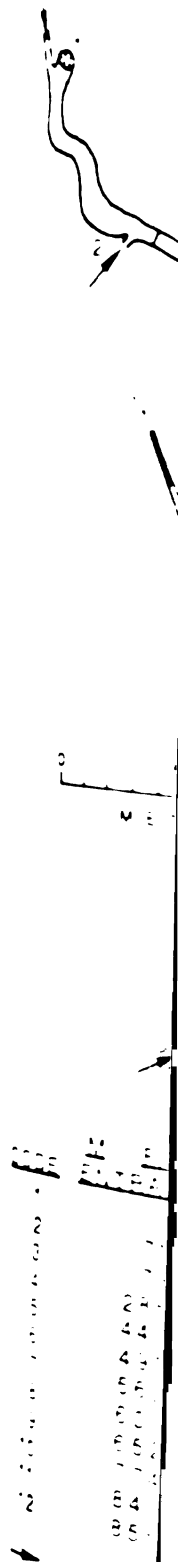
CHAPTER III

STUDY SITE

Large caves of southern Indiana are characterized by extensive subsurface drainage. This is due to solutional ground-water systems developed in a sequence of limestones 180 meters thick. The surface terrain exhibits karst features, such as sinkholes and sinking streams, and the subsurface contains numerous caves with active streams. This drainage system reaches its highest development in the Mitchell Plain of south-central Indiana. The Mitchell Plain is an area of low relief underlain by limestones more susceptible to solution than the other limestones of Indiana (Powell, 1961). Underground drainage is so extensive that few streams flow on the surface. Typically, rain water enters grikes and sinkholes to form subterranean headwater streams. These cave waters emerge as springs to form surface streams of usually short length.

Shiloh Cave, which is located 11 kilometers northwest of the city of Bedford in Lawrence County (section NW1/4 SE1/4 NW1/4 18, T5N, R1W, Bedford West quadrangle), was chosen as the primary study site (Figure 3). Its stream is fairly typical of the subterranean streams of the Mitchell Plain and was selected primarily because of accessibility. Its watershed of approximately 8 square kilometers is 20% forested and the remainder is principally agricultural (Figure 4). Water enters the subsurface system mainly by infiltration through numerous dolines, although the few collapse sinkholes also make a

Figure 3. Map of Shiloh Cave. An additional 400 meters of the Black Damp has been surveyed but is not shown.



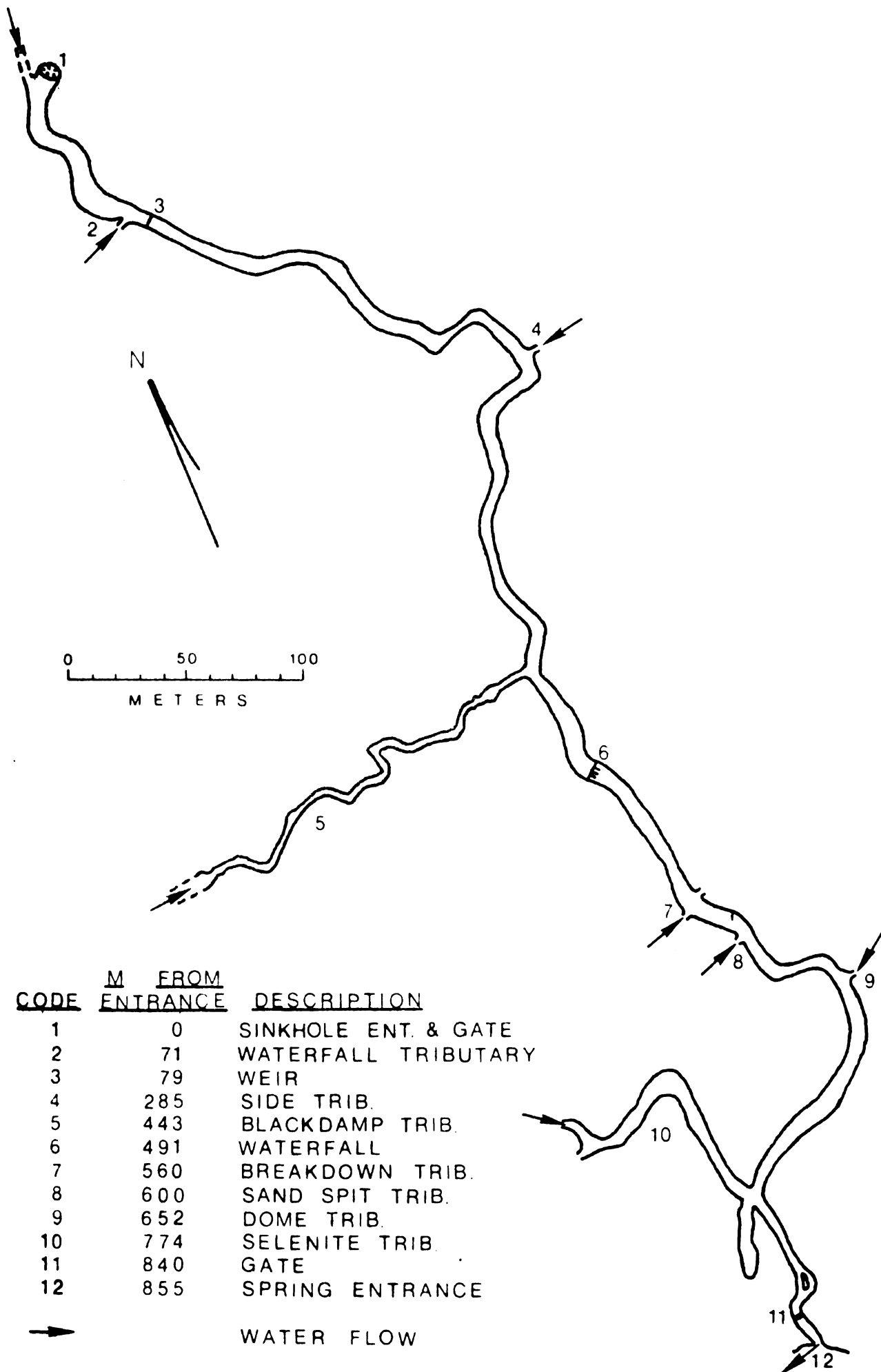


Figure 3

significant contribution (Figure 5). No permanent surface streams exist, although there are a few of intermittent nature. One of these, which is thought to influence the cave stream, emerges as a spring during heavy rainfall, flows on the surface for about 10 meters, then enters a swallow hole.

Human entrance into the cave is gained at two points. The main entrance is through a collapse sinkhole. Just inside the entrance the cave stream emerges from breakdown and flows southward through the main cave passage. The upstream nature of the cave stream is uncertain, but it is probable that it is principally of a diffuse input. The passage, which lies within the St. Louis Limestone formation, is from 5 to 10 meters high and about the same width for almost the entire length of the cave (Figure 6). Palmer (1969) determined the mean gradient of the passage to be 7.6 meters/kilometer. The stream, with a mean discharge of about 24 liters per second (0.85 cfs), flows in a riffle-pool sequence, meandering from one side of the passage to the other. These meanders usually undercut the passage walls and form partially inaccessible pools with depths up to one meter. The pool substrate usually consists of a thin layer of a silt-sand mixture overlying limestone bedrock. Riffles, which make up about 90% of the stream, have typically consolidated, but quite heterogeneous substrates, with various mixtures of rocks, pebbles, sand, silt, flowstone, and bedrock. The stream averages 1.5 meters in width and the remainder of the passage floor consists of mudbanks, which are water-laid deposits consisting primarily of silt.

The temperature of the water is quite stable with an average value of 12.5° C and an annual range of only about 11° C. Most of this temperature variation occurs during flooding, which can increase stream

flow from a median of 9 to over 450 liters per second (0.32 - 15.91 cfs). Flooding, which is responsible for most of the variations in the stream environment, such as changes in temperature, turbidity, and water chemistry, is also the primary agent for introducing allochthonous food material into the cave. This food, which is present in only very low levels, consists primarily of decomposing leaf and twig fragments.

The cave stream of the main passage is 855 meters in length, but several, usually inaccessible, tributaries enter at various points. Many of these are vertical in nature, entering as seeps, waterfalls or cascades through domes or higher level horizontal solution channels. The waters of this type of tributary are probably of local surface origin. The largest tributary of this type, the Graveyard Waterfall Tributary, flows through a horizontal channel 3 meters above the main stream and joins it as a waterfall 71 meters downstream from the entrance. The major tributary, however, is horizontal in nature and is accessible. It flows through the Black Damp passage made infamous by Blatchley (1897), who reportedly encountered dangerous gases during an early exploration. Contrary to the general southwestern tilt of the limestone strata of southern Indiana, this stream flows eastward and joins the main stream 443 meters downstream from the entrance. Whereas the Black Damp Tributary has several waterfalls and cascades, the main stream has only one waterfall (at 492 meters), which, however, may be a significant barrier to the upstream movement of aquatic fauna. The cave stream exits in a quarry as a spring, which also serves as the second entrance accessible to humans. The spring was the former site of a mill, and the dam, which is still intact, backs up water in the cave for a distance of 135 meters, resulting in the heavy silting of this lower portion of the cave stream.



Figure 4. Surface terrain overlying the study area of Shiloh Cave.

Figure 5. A sinkhole which feeds water into the Graveyard Waterfall Tributary. The entrance sinkhole is located in the woods in background.



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Tributaries of the main stream issuing from the cave by-pass the dam and emerge as a series of springs along the hillside. The tributaries, along with the main stream, form a broad cascade which descends 30 meters to the valley floor. At the foot of the cascade the stream abruptly changes character, following a lesser gradient with a riffle-pool series and a substrate of clay and decomposing organic matter. The stream flows through woodland, intersecting limestone quarry tailings at several points, and then flows into Salt Creek 0.6 kilometers from the cave.

Modifications of the cave were required in order to carry out the study. One major problem was the popularity of the cave with spelunkers. In order to control access into the cave, both entrances were gated. Bars were installed across the spring entrance, which allowed for faunal movement and stream flow, but obstructed human entry. The main entrance was quite large and required construction of a concrete block wall with a gated opening (Figure 7). Certain steps were taken to prevent interference with naturally occurring exchanges between the epigean and hypogean environments; open ports were placed in the wall to permit movement of aerial and terrestrial fauna, and a concrete gutter with a drain passing through the base of the wall was constructed to allow water-borne vegetative matter to pass into the cave. Another major modification was the construction of a weir across the cave stream at a point 79 meters downstream from the emergence of the cave stream from the entrance breakdown.

In the study, stream locations are identified by their distance in meters downstream from the collapse sinkhole. This procedure was aided by placement of location markings on the cave wall at 5 meter intervals. Crayfish were censused in a 500 meter stretch of cave stream that was

Figure 6. Typical section of study area; junction of the Main Stream and Black Damp Tributary.

Figure 7. Closure constructed at main entrance.



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designated the "census area". This area comprised that reach of the main stream from 0 meters to the waterfall at 492 meters, and also included a short downstream section of the Black Damp Tributary. A sub-area of 364 meters, designated the "quantitative study area", terminated upstream at the weir and downstream at the Black Damp Tributary. This reach of the main stream was relatively free of feeder streams and was used for the study of energy budgets. In the evaluation of food resources the entire main stream and most of its tributaries were studied.

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CHAPTER IV

MATERIALS AND METHODS

Hydrology

To measure the discharge of the cave stream, a permanent 90° V-notch weir, meeting specifications of the Water Measurement Manual (1967) published by the U. S. Bureau of Reclamation, was constructed; the single modification consisted of building the base of the notch flush with the substrate, in order to minimize pooling and interference with the normal movement of drift (Figure 8). This type of weir is an accurate flow measuring device particularly suited for small flows. A float-actuated pen continuously recorded the head on a kymograph. After an initial calibration against actual flow, the head was thereafter converted to discharge by the formula, discharge (l/sec) = $0.0147 (\text{head in cm})^{2.48}$; this formula was derived from a formula in the Water Measurement Manual by conversion to the metric scale.

The drainage areas of the main cave stream and each of its tributaries were estimated from levels of base flow and surface drainage patterns. The subterranean watersheds so determined are judged to be fairly accurate, but do not have the absolute accuracy of surface watersheds.

Precipitation records were obtained from the Oolitic Weather Bureau Station, which is located 2.3 kilometers northeast of the cave entrance, but only an estimated 0.5 kilometers from the watershed of

the cave. Precipitation measurements were converted to the metric scale.

Non-organic Water and Substrate Analyses

Alkalinity

The alkalinity of water samples was determined by the titration method described by Welch (1948). Samples were taken quarterly at 7 tributaries, 5 sites in the main cave stream, and 2 sites in the surface stream issuing from the cave. Duplicate samples were taken and tested in all cases.

Temperature

A maximum-minimum thermometer was placed in the cave stream at the weir. A temperature record was also kept on other mainstream sites and tributaries in conjunction with microseston and alkalinity studies.

Carbonate

The carbonate content of the mud was determined by the method of Kozlovskii, as described by Il'kovskaya (1965). This method is based on the decomposition of carbonates with HCl solution, followed by absorption of the evolved CO₂ with NaOH and subsequent titration of the excess alkali against acid in the presence of BaCl₂.

Organic Analyses

All samples to be chemically analyzed were first oven-dried at 60° C. Chemical analyses were performed in duplicate in all cases.

Ashing

Ashing was carried out in a muffle furnace at 500° C. Macroseston, leaves, mud, feces, and detritus from rocky substrates were ashed, and the results were expressed as ash-free dry weight or percentage ignition loss; these expressions are considered to be closely equivalent to the absolute and relative organic content.

Energy

Energy contents were determined for macroseston, microseston, benthos, leaves, mud, and feces. These determinations were based on quantitative dichromate oxidations as described by Maciolek (1962). A reagent strength of 0.25 N potassium dichromate was used in the analysis of microseston which had been filtered through filter pads, whereas 0.50 N reagent was used for all other determinations. The results, expressed as mg of oxygen consumed, were calibrated against a cellulose standard, adjusted for the incomplete oxidation of certain proximate groups in natural organic matter, and transformed to gram calories by the 3.4 conversion factor suggested by Maciolek (1962).

Cellulose

Cellulose determinations were made on feces, mud, leaves, and macroseston. The method of Crampton and Maynard (1938), which removes non-cellulolytic organic constituents by digestion with an acetic acid-nitric acid reagent, was employed.

Lignin

Lignin content was determined in leaves and macroseston by the method of Crampton and Maynard (1938); however, this method, which is based on the use of sulfuric acid, was modified by correcting for protein content (determined by the Kjeldahl method), instead of digesting protein with pepsin.

Crude Protein

Organic nitrogen was determined by the semi-micro Kjeldahl method. Results were expressed as crude protein by multiplying the nitrogen content by the conversion factor, 6.25. Macroseston, leaves, microseston, feces, and mud were analyzed for crude protein content.

Food Resources

Macroseston

Except for the small tributary entering the cave stream at 284 meters, the weir intercepted all water-borne food input into the 364 meter section of stream between the weir and the Black Damp Tributary; this section, designated the "quantitative study area", was utilized for the measurement of energy flow.

A drift net was employed in conjunction with the weir to sample macroseston entering the quantitative study area (Figure 8). The net was made of Nitex with 0.23 mm apertures, and, because of the fine mesh, was two meters in length to prevent back-flow. The mouth of the net formed a 22.6° arc, which, when positioned in the middle of the 90° V-notch, filtered 20% of the water at all flow rates. Tests employing

marked drift material confirmed that 20% of the drift was also intercepted. The drift net collected 24-hour samples and was used for three consecutive days in each 20 day period throughout the year.

A temporary weir and drift net were placed just upstream of the Black Damp Tributary at 440 meters. They were employed during a period of low flow in August to measure the macroseston leaving the quantitative study area. The drift collected at this drift station was adjusted by correction factors to compensate for the removal of 20% of the drift at the 79 meter station. The values of the correction factors are based on certain assumptions. Vegetative drift was assumed to entirely originate upstream of the 79 meter station, and a correction factor of 1.25 was applied to the results from the 440 meter station. Although it may not have been true of the few planktonic forms, such as the cyclopoids, animals of aquatic origin were generally assumed to have undergone only short range displacement and no correction was considered necessary. Terrestrial and aerial fauna and exuviae, on the other hand, underwent passive transport in the stream and could have been displaced great distances. Unlike the vegetative drift, however, their point of origin could have been either upstream or downstream of the 79 meter station, and an intermediate correction factor of 1.12 was applied to these drift categories.

Collected drift was preserved with 4% formaldehyde and sorted into the components, leaves, wood, animals, exuviae, and debris; after drying in a 60° C oven, their weights were determined. The wood category included everything of a "woody" nature — even leaf petioles. January and July drift samples, which were collected over four day intervals, were sorted, dried and analyzed for energy, protein, cellulose, lignin, and ash content.

Leaves, which made up a major portion of the drift, were studied further to determine the seasonal species ratios entering the cave and to monitor the changes in their composition (energy, protein, cellulose, lignin and ash) in passing from the living state to trypton in the cave stream.

Microseston

Microseston was considered to be that portion of the particulate matter that was not retained by the drift net. Water samples, usually of a one liter volume, were vacuum filtered through pre-fired glass fiber pads (Reeve Angel 934-AH, Arthur H. Thomas Co.); these pads are reported to retain at least 95% of the particles retained by membrane filters of 0.45 micron pore size. After filtration the pads were oven-dried at 60° C and were later analyzed for energy or protein content. The filtered water was tested for alkalinity as described by Welch (1948).

A series of 8 water samples was taken at the weir during the month of February in conjunction with the ebb of the largest flood, in order to relate microseston content to discharge. Other samples were taken quarterly at 7 tributaries, 5 sites in the main cave stream, and 2 sites in the surface stream issuing from the cave. Duplicate samples were taken and tested in all cases.

Substrate

Mud, consisting of sand, silt, clay, and organic fractions, was collected with a core sampler with a cross-sectional area of 11.6 sq cm and a depth of 1.0 cm. These shallow cores permitted measuring the organic material in the mud layer most accessible to benthic foraging. Mud samples were taken in January and July from 10 pools in the cave

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stream and from 2 pools in the surface stream; two cores were taken from each pool site, and these were arbitrarily assigned to one of two longitudinal sample series (designated series "A" or "B"). These samples were oven-dried at 60° C and energy contents determined. In addition, energy, ash, cellulose, protein and carbonate analyses were performed on the composite of cave mud from each longitudinal sample series.

Benthos

Quantitative benthic samples were taken in June and December. Sampling followed a stratified random design with 15 sample sites, of which 13 were located within the quantitative study area; each site consisted of a 15 meter longitudinal stream section. The sample sites were restricted to rocky substrates, as mud contained an extreme paucity of fauna.

A 0.05 m² cylinder sampler with a saw-tooth edge was employed to delimit the substrate to be sampled (Figure 9). The contained substrate was removed to a depth of approximately 3.6 cm and washed through a sieve series with a minimum aperture of 0.18 mm. The water remaining within the cylinder was agitated and strained through a net of similar aperture size. The samples were preserved in 4% formaldehyde, and later sorted into taxon and detrital components and oven-dried at 60° C.

Trophic Studies

Studies were carried out to determine the trophic ecologies of the crayfish populations. These studies focused on determining the relative importance of mud, plant and animal sources in the field diet, but also examined feeding patterns and predator-prey relationships under laboratory conditions.

Figure 8. Weir constructed 79 meters downstream from collapse sinkhole. Visible are the drift net, which captured macroseston, and, at the right, the automatic head recorder.

Figure 9. Equipment used for sampling benthos, with a typical pebble-cobble riffle substrate also shown.

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Diet Analysis

The traditional gut analysis technique is ineffective for quantitative measurement of dietary components, on account of their varying assimilation rates. Because of the highly macerated condition of their gut contents, this technique is especially unsuited to the study of crayfish, although it has been employed by many investigators.

In the present study a method was developed to determine the quantitative and qualitative nature of ingestion by chemical analysis of feces; this involved comparison of the chemical composition of field-collected feces with that of crayfish maintained on controlled diets of mud, animal or plant material. Feces were collected in the field by placing a rubber bladder over the anus and tail of a freshly captured crayfish (Figure 10). The opening of the bladder was sealed against the abdomen with a rubber band. This arrangement interfered with escape behavior, but not with normal feeding activity. The experimental crayfish was then placed in an undisturbed 40 m² portion of the stream that was enclosed by quarter inch hardware cloth. Two such foraging pens were set up, one in the surface stream for studying epigeal *C. laevis*, and another in the cave stream for studying both *O. inermis* and hypogean *C. laevis*. No more than three crayfish were placed in a pen at any one time, and they were removed after feeding for a period of two days. Neither the bladder nor the pen was observed to interfere significantly with normal behavior or food availability and, because of the delay of approximately 15 hours between ingestion and egestion, a large portion of the feces collected was derived from food eaten before the crayfish was captured.

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In the laboratory *O. inermis* and *C. laevis* were maintained in individual containers under cave conditions of temperature and darkness, and fed various quantitative diets. One group of crayfish was fed a complete diet of filtered mud, isopods, amphipods and three kinds of leaves. The other group was fed a ration consisting of one of the following: (1) mud, composed of sand, silt, clay and organic fractions, that was obtained from the cave stream and filtered through a 0.5 mm aperture sieve; (2) live amphipods; (3) one of three species of shed leaves conditioned for several weeks in water from the cave stream. The three kinds of leaves, *Fagus grandifolia*, *Acer saccharum*, and *Quercus velutina*, are the ones most commonly found in the cave stream. Leaf rations were punched from intact leaves in species-specific shapes.

The feces resulting from the various diets were collected in a manner similar to that of the field studies. Feces from both the field and the laboratory studies were weighed and chemically analyzed. The fecal constituents quantitatively determined were ash, crude protein, cellulose and energy. The ratio in the field feces of the dietary sources, mud, animal and plant, was determined from the following simultaneous linear equations with three unknowns:

$$D_1 = a_1x + b_1y + c_1z$$

$$D_2 = a_2x + b_2y + c_2z$$

$$D_3 = a_3x + b_3y + c_3z$$

$$D_4 = a_4x + b_4y + c_4z$$

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1 = ash

2 = crude protein

3 = cellulose

4 = energy.

Solving for three unknowns required simultaneous solving of three or four of the above equations; the last equation, which expresses the relationship that the summation of x , y and z equals unity, was always included. The ten possible combinations gave up to ten estimates of the dietary nature of the feces, although some combinations were not solvable because of inconsistencies of the data.

The proportions of the mud, animal and plant components of the feces were converted, by assimilation efficiencies determined in the laboratory studies, to the ingestion proportions of these dietary components. The assimilation of the mud diet was too low to be determined; based on an organic content in mud of 4%, the assimilation efficiency was assumed to be approximately 1%. The field egestion rate and the assimilation data also provided an estimate of the ingestion rate in the field. The accuracy of this method of trophic

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determination was checked by applying it to the complete laboratory diet, in which the rate of ingestion of the various dietary components was known.

Predator-Prey Relationships

A study was undertaken to investigate three aspects of predator-prey interactions: (1) the effect of bare versus rocky substrate on the predation rate; (2) species-specific predation rates of the two predators, *O. inermis* and *C. laevis*; and (3) species-specific predation rates on two of the most common cave stream prey taxa, *Asellus* sp. (an isopod) and *Crangonyx* sp. (an amphipod).

This study was carried out in the cave in order to best simulate natural conditions. Predator (25-30 mm carapace length) and prey (adult-sized) subjects were taken fresh from the cave stream, with the crayfish confined with mud food for a period of one day in order to adjust them to confinement. The crayfish were then tested individually over a period of 24 hours. The rocky substrate consisted of 6 pebbles that were grouped in the center of the tank and occupied approximately 10% of the total substrate; the purpose of the rocks was to serve as a refuge for the prey species. The experimental design attempted to simulate a low prey density situation typical of cave ecosystems; only 3 *Asellus* or 3 *Crangonyx* were introduced into a 0.1 m^2 tank containing a single crayfish — thus exposing the predator to an initial density of 30 prey per square meter. The experimental prey density was lower than those that existed in the cave stream; means of 268 isopods/ m^2 and 138 amphipods/ m^2 were obtained in benthic samples (see Table 9). This low prey density was used because it was thought that higher numbers of prey might have a tendency to test satiation levels instead of predation efficiencies.

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Crayfish Census

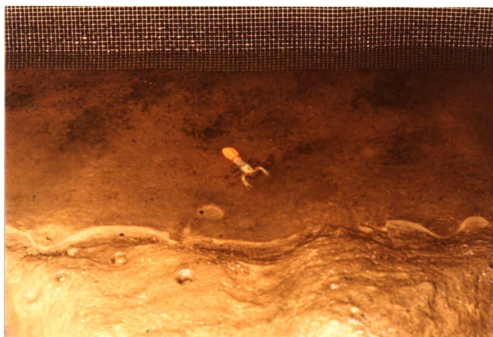
The census of crayfish was accomplished by visual and trap capture. Traps were constructed of quart jars with plastic funnels inserted in the jar mouth and held in place with a rubber band (Figure 11). The inner surface of the funnel was scored to allow additional purchase for entering crayfish. The traps were of two types: in one type the funnel opening was enlarged to a diameter of 16 mm, and the trap was intended to capture *O. inermis* and small *C. laevis*; in the other type, which was intended to capture larger *C. laevis*, the funnel opening was enlarged to a diameter of 32 mm, and the opening was covered by a hardware cloth gate, which opened only in the inward direction. The traps were baited with bologna, which was wrapped in bolting silt to prevent the crayfish from eating the meat. Alternating the two types, traps were placed every 5 meters, beginning at the upstream entrance to the cave at 0 meters and continuing to the waterfall at 490 meters. Traps were also placed in the first 10 meters of the Black Damp passage. This census area included not only the quantitative study section, which was located between the weir and the Black Damp Tributary, but also both upstream and downstream buffer zones. The visual captures were carried out using a gasoline lantern and electric head lamp for illumination, and a long-handled dip net for capture.

A survey consisted of a two way transit of the delineated area and required an average time of 3.3 hours, although individual surveys varied from 1.8 to 7.3 hours. A census consisted of 25 surveys conducted in a period of approximately 25 consecutive days. The actual length of time to complete a census depended upon weather conditions, since intensive rainfall increased the turbidity of the water and decreased

Figure 10. *O. inermis* rigged for diet study inside stream enclosure.

Figure 11. Trap used in crayfish surveys.

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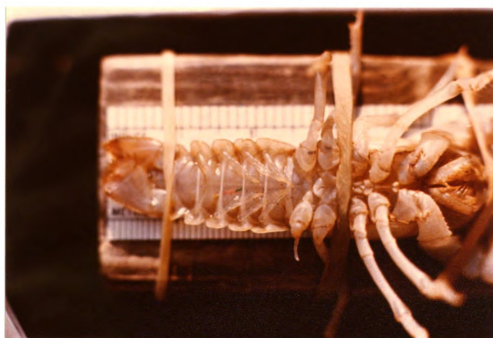
the efficiency of visual capture. A census was conducted every four months during the period from March, 1969, to March, 1970.

If a crayfish was captured for the first time, it was individually tagged. Tagging consisted of injecting a color-coded flexible rod into the space between the abdominal muscles and the sternum (Figures 12 and 13). Injection was accomplished by pushing the rod through a hypodermic needle with a wire plunger. To prevent interference with the ventral nerve cord, the rod was positioned parallel to, but to one side of, the midline. The rod consisted of flexible nylon and was made as short as possible to prevent interference with flexure of the abdomen. For the larger crayfish with a carapace length of 18 mm or larger, the rod consisted of a 3.5 mm section of monofilament fishing line with a diameter small enough to be injected with a 23 gauge hypodermic needle. For the smaller crayfish nylon sewing thread, 1.5 mm in length and fine enough to be injected with a 26 gauge hypodermic needle, was used for the rod. Crayfish with a carapace length of as little as 7.5 mm were successfully coded and recaptured. The rods were coated with three bands of color, using *Testor's pla* enamel paint (commonly used by hobbyists for model construction). Because the implanted color-coded rods maintained a stable position, it was possible to read the code unidirectionally. By employing a total of seven colors in a triplicate code, 210 permutations were possible, and, by repeating the same color in non-adjacent positions, 245 individual codes were available. This coding system was expanded by using duplicate codes for different species, sexes, and rod sizes. The code was visible through the transparent sternites and was retained after molting.

This new tagging technique is a refinement of a liquid injection method developed by Slack (1955a) for group-tagging crayfish, and of a

Figure 12. *O. inermis* with identification tag. The code was injected while the crayfish was secured to the mounting board.

Figure 13. *C. laevis* with identification tag.



ferromagnetic group-tagging technique described by Stewart and Squires (1968) that is employed in lobster studies. Hobbs (1973) has also developed a marking technique for the identification of individual crayfish, but the presently employed method appears to cause less trauma and to result in a higher certainty of long-term identification.

Each coded crayfish was assigned a number which was marked on its carapace with waterproof ink. In most cases this allowed noting recaptures without handling the crayfish. A paper punch was used to clip the margin of the outer ramus of a uropod. The clipped margin partially repaired itself with the next molt; thus, the appearance of the margin provided evidence as to whether molting had occurred during the inter-census period. A crayfish was clipped every time it was captured in a different census, and the particular uropod clipped depended on the census. The length of the cephalothorax was measured with dividers to the nearest 0.5 mm. The species and sex were recorded, and the sexual form of the male and breeding condition of the female were noted. The linear location of the crayfish in the stream and the type of capture were also recorded. For recaptured crayfish, the location and type of capture were recorded; if they had molted, they were remeasured, examined for sexual condition, and their carapace number replaced. After collection of data, all crayfish were returned to the stream at the point of capture.

Crayfish living in surface streams were also censused for comparative purposes. One site was a stream issuing from Sullivan's Cave that was sampled with modified (enlarged entrances) minnow traps. The primary surface site, however, was at the base of the cascade in the stream issuing from Shiloh Cave, and it was sampled with modified minnow traps and jar traps. The crayfish population of a 50 meter

section of this stream was isolated by placing hardware cloth barriers across the stream. The barrier edges, at both the top and sides, were recurved on both sides to guide crayfish back to their original section of stream.

Based on census data, the standing crops of the various crayfish populations were determined by the Schumacher and Eschmeyer (1943) mark-recapture method, which is based on a weighted least squares fitting. The formula for estimating the population size is

$$N = \frac{\sum_{k=1}^k [n^2(m+u)]}{\sum_{k=1}^k (nm)}$$

where n = number of marked individuals in the population,

m = number of marked individuals captured during a given survey,

u = number of unmarked individuals captured during a given survey,

s = summation over all surveys (k).

DeLury (1958) gives arguments for preferring this method over the Schnabel method, which is based on maximum likelihood. Momot (1967) compared both techniques in his crayfish study and found the Schumacher method gave more consistent results.

Cave populations were permitted free movement, with corrections made for the interference of such movements with population estimates. Sex, size, season, and species-specific movement rates were calculated from the displacements between initial and final captures of crayfish encountered at least twice during a census. The applicable value, expressed as displacement per day, was divided into the average distance to the census area boundaries for each crayfish captured during a survey. In this manner the expected length of time for a particular marked crayfish to remain in the study area was calculated.

This "estimated sampling life span" was reassigned after each recapture. If a marked individual was not recaptured within this time interval, it was assumed to have left the study area and was removed from the tally of marked crayfish. If population densities are similar in various longitudinal sections of a stream, then this method should be valid whether movement is random or directed at the population level. Even if population densities do vary in different reaches of a stream, non-directed population movements would not invalidate this technique.

The more sophisticated mark-recapture method described by Jolly (1965) was also applied to the data. This stochastic model, which accounts for migration, failed to produce reliable population estimates. This conclusion was based on results indicating that the estimated total population was often less than the number of crayfish encountered during a single survey. The failure apparently resulted from the extremely small crayfish populations being dealt with in this study.

Length-Weight Relationships

After completion of the field study, specimens of both crayfish species were removed from the cave for measurement. Carapace length, which is referred to by the abbreviation CL, was measured with dividers. Wet weight was determined after lightly blotting formaldehyde-preserved crayfish; dry weight was determined after drying to constant weight at 60° C.

Molted skins were either found in the cave stream or obtained from laboratory-held animals. Skins were measured by the same methods employed for crayfish bodies.

Population Attributes

Tissue Growth

Marked crayfish recaptured at annual intervals (± 15 days) were measured for growth of carapace length. Growth measurements were made on crayfish captured during censuses and other sampling occasions from November 1968 to July 1970. Subsequent recaptures monitored growth of individuals for periods of up to 3.5 years. Recapture of marked crayfish and size frequency analysis were employed to determine growth of epigeal *C. laevis*.

Based on the relationship between the annual increase in carapace length and the initial carapace length, a growth curve was constructed. This curve showed the relationship between mean carapace length and age. An individual was assigned to a year group by comparing its length to the growth curve; for example, if the length of a crayfish indicated that it was between 3 and 4 years old, then it was assigned to the fourth (3-4) age group. Growth in length was converted to dry weight increment by means of the length-weight relationship. From this the relative annual rate of growth (h) and the instantaneous growth rate (g) were calculated using the formulae:

$$h = \frac{w_t - w_o}{w_o}$$

$$g = \log_e (h+1)$$

where w_o = weight of crayfish at the beginning of the time interval,

w_t = weight of crayfish at time t ,

t = time interval of one year.

These formulae, derived from Ricker (1958), were used to calculate growth for each year group.

An experiment was carried out to compare the growth rates of young-of-the-year *C. laevis* kept under epigean and hypogean conditions. Crayfish from a single brood were used; they had just detached from the pleopods of the mother and were 5.0 mm in carapace length. Each experimental crayfish was placed singly in a glass quart jar containing some substrate and organic detritus from either the epigean or hypogean stream. The jar was sealed with a plastic screen having 2 mm apertures. Fifteen jars each were placed in representative riffle areas in the cave and surface streams, with contained substrates matching the location. The jars were placed so that the substrates inside and outside the jar were flush and separated by the screen, allowing the exchange of some substrate, detritus, and fauna. These crayfish were sampled periodically for a year, with five jars selected at random from each habitat on each sampling occasion. The dry weight and carapace length of sampled crayfish were determined.

Molting

Data on molting was gathered as follows: the annual molting pattern was determined by observing molting and growth of individual crayfish during the three 4-month intervals between the four censuses; molt increment was determined by the above method and also from newly molted individuals found associated with their old skins. Because of its aberrant nature, molting information from laboratory animals and regenerating crayfish in the field was not included.

Reproduction

Ovarian egg counts were not made because of the disruption this would have caused to the crayfish populations. The populations of these cave-inhabiting crayfish were so small that the removal of even a few individuals would have had a significant effect. The reproductive cycle and production of fertilized eggs were estimated from counts of eggs carried on the pleopods of captured females.

Mortality

The annual total mortality rate (a) and the instantaneous rate of mortality (i) were calculated using the formulae:

$$a = \frac{N_o - N_t}{N_o}$$

$$i = -\log_e (1-a)$$

where N_o = the number of crayfish present at the beginning of the time interval,

N_t = the number of crayfish surviving to time t ,

t = time interval of one year, except for 0.5 year interval in calculation of pleopod egg mortality.

The above formulae, derived from Ricker (1958), were used to calculate mortality for both sexes, and for each year group. Mortality rates, derived from a modified type of horizontal sampling, were based on the observed annual mortality of year class cohorts. In addition, the mean age composition, as determined from four censuses, was used to establish "mortality" rates based on vertical sampling.

Population Size and Structure

Crayfish of both species were censused by the mark-recapture technique of Schumacher and Eschmeyer (1943), with recapture rates computed for stratified groupings based on species, sex, age and season. For *O. inermis* differences between the sexes and seasons were found to be only random variations, and the recapture data were reblocked into age categories only. The census area consisted of 500 linear meters (750 square meters), and population estimates were obtained at 4-month intervals during the year of study. The populations inhabiting the five sequential 100 meter sections of the census area were also calculated. Estimations were based on the proportions of initial captures in the various sections. The population of *C. laevis* inhabiting two surface streams, one issuing from Shiloh Cave and the other from Sullivan's Cave, were also censused for comparative purposes.

Biomass and Productivity

Known growth rates were used to follow the various age classes through the four censuses. Annual body tissue production was computed for each class by summing production computed for the three 4-month intervals between censuses. Production was calculated by the formula,

$$\log_e P = \frac{\log_e N_t + \log_e N_o}{2} + \log_e (\bar{w}_t - \bar{w}_o).$$

Thus the production for the period was equal to the average number of

specimens, $e^{\frac{(\log_e N_t + \log_e N_o)}{2}}$, multiplied by the weight increment of a specimen, $\bar{w}_t - \bar{w}_o$. Computation of the average number is

based on the exponential nature of mortality. Production of young-of-the-year was derived for the annual interval with N_0 and $\bar{w}_0$ referring to pleopod eggs. Egg production was based on pleopod egg counts and data on egg weights.

Annual production of molted skins for each age class is based on the formula, $\bar{N} (\sum Bd)$, where $\bar{N}$ is the mean number of the cohort from the four censuses and Bd represents the total weight of skins shed annually by a member of this age group. Information on molted skins was derived from growth rate and molt increment data.

Production was computed for the census area of 500 meters and the quantitative study sub-area of 364 meters. The cave stream had a mean width of 1.5 meters; thus the census and quantitative study areas contained 750 and 546 square meters, respectively.

Respiration

The oxygen consumption of *O. inermis* and *C. laevis* from both habitats was studied by measuring oxygen depletion in sealed chambers. Crayfish were collected and individually placed in plastic chambers with sufficient natural food for a 24-hour period of adjustment to experimental conditions. They were then transferred to a clean chamber of similar dimensions that contained cave water with a known oxygen content. A small amount of calcium carbonate was added to buffer against change of pH, and the chamber was sealed. The buffering precaution was taken, despite the findings of Fox and Johnson (1933) that ventilation in *Astacus fluviatilis* was unaffected by a rise in the carbon dioxide tension and of Helff (1928) that changes in pH and carbon dioxide content had no marked effect on the oxygen consumption of *Orconectes immunis*. A wide size range of crayfish was studied, and

the size of the chamber was chosen so that its volume was approximately 300 times the volume of the crayfish. In order to maintain an accurate temperature, these chambers, together with control chambers, were kept in the cave stream for the duration of the experiment, which was terminated after 24 hours when oxygen levels had decreased to approximately half of the saturation value. Studies of the epigean crayfish population were carried out in the surface stream, which maintained the same temperature as the cave stream.

This oxygen consumption study, which was conducted in the field during May, was followed by a similar laboratory study in November. Because the rate of respiration in crayfish is often correlated with oxygen tension, studies were carried out to ascertain this relationship, and to allow computation of a correction factor for the change in respiratory rate incurred during the experiment. These rate-tension experiments were carried out in Erlenmeyer flasks that were sealed with a layer of mineral oil; this arrangement permitted periodic sampling by withdrawing a water sample with a syringe.

Because of accumulation of nitrite nitrogen as a waste product during the course of the experiment (less than 1 ppm), the azide modification of the Winkler method, as described in *Standard Methods for the Examination of Water and Wastewater* (twelfth edition, 1965), was used for determination of dissolved oxygen content. A micro-Winkler technique, described by Fox and Wingfield (1938), was employed to analyze the 10 ml samples obtained in the rate-tension study. Lawton and Richards (1970) concluded that the various respiration-measuring methods — cartesian diver, Gilson, Warburg and Winkler — were equally suitable for ecological work, provided they were used with a sound knowledge of the animal under investigation.

Time Frame

The study commenced in July, 1968, with construction of the weir and the entrance closures. By October the weir was operational and data collection commenced on stream flow and drift. In November, 1968, a preliminary crayfish census was carried out in which the coding procedure was tested. The study was in full operation during the 1969 calendar year, with data collection on food inputs and crayfish populations. Trophic and respiration studies, which disrupted the crayfish populations, were carried out during the summer of 1970 after other phases of the study had been terminated. Follow-up crayfish surveys were made through 1975 to collect long-term growth data.

CHAPTER V

THE ENVIRONMENT

Results

The Stream

Hydrology

The mean rate of flow at the weir for the study year of 1969 was 2,077 kl/day; the median daily discharge was 776 kl. There was a 186-fold variation in daily flow rate, from a low of 173 kl to a high of 32,300 kl. The two greatest floods occurred in January, but other floods took place in April, July, November and December. The lowest flow was recorded in October (Table 1 and Figure 14).

In general, flow rates throughout the year underwent a dual pattern. Flow rates were high from December to April, and discharge was highly correlated to precipitation; during the remainder of the year, however, flow rates were low, and discharge was not very responsive to precipitation (Figure 15). For example, similar 8 cm rainfalls resulted in a 22-fold increase in discharge rate in January, whereas there was only a 10-fold increase in July; in addition, the discharge rate of the January flood was 6 times as great. There was even a greater dichotomy at lower precipitation levels between the two annual phases.

TABLE 1.--PRECIPITATION, STREAM DISCHARGE, AND SESTON LEVELS

TIME PERIOD	PRECIPITATION* (cm)		TOTAL DAILY INPUT AT WEIR (MEAN ± SD)			
	ACTUAL	DEVIATION FROM RECORDS	FLOW (kl)	MICROSESTON ENERGY CONTENT (kcal)	VEGETATIVE MACROSESTON	
					DRY WEIGHT (g)	ENERGY CONTENT (kcal)
1968 Nov.	12.3	+3.1	1061 ± 1822	1349 ± 3124	2.80 ± 7.86	9.30
Dec.	11.6	+2.9	4018 ± 3891	6639 ± 9719	9.53 ± 18.95	31.64
Jan.	20.2	+9.2	8163 ± 8636	21609 ± 33959	28.87 ± 62.85	95.85
Feb.	2.5	-5.4	5567 ± 3671	9339 ± 8877	13.02 ± 15.40	43.23
Mar.	3.9	-7.6	999 ± 226	947 ± 240	1.99 ± 0.58	6.61
Apr.	11.6	+1.2	4790 ± 4468	8505 ± 12917	21.56 ± 45.09	71.58
May	6.5	-5.4	1040 ± 380	1000 ± 416	2.13 ± 0.85	7.07
Jun.	10.7	-1.6	495 ± 121	441 ± 117	1.52 ± 0.98	5.05
Jul.	15.5	+5.9	703 ± 798	706 ± 994	2.15 ± 3.75	7.14
Aug.	4.6	-3.6	340 ± 85	297 ± 78	0.70 ± 0.27	2.32
Sep.	4.7	-4.5	228 ± 20	196 ± 18	0.54 ± 0.11	1.79
Oct.	11.2	+4.6	329 ± 197	291 ± 186	0.77 ± 0.44	2.56
Nov.	11.2	+2.0	1438 ± 2402	2017 ± 4506	4.18 ± 11.70	13.88
Dec.	7.4	-1.3	1124 ± 641	1117 ± 781	2.30 ± 1.44	7.64
1970 Jan.	3.1	-7.9	2246 ± 2985	3333 ± 7076	5.48 ± 15.35	18.19
Feb.	3.9	-4.1	3453 ± 1725	4463 ± 3263	6.62 ± 8.25	21.98
1969 DAILY INPUT (MEAN ± SD)	..	..	2077 ± 3933	3839 ± 12421	6.59 ± 24.49	21.87
1969 TOTAL INPUT (Annual Rate)	110.0	-6.5	..	1,401,239	2405	7984

*obtained from Oolitic Weather Station

Figure 14. Daily precipitation and stream discharge records for the year of study.



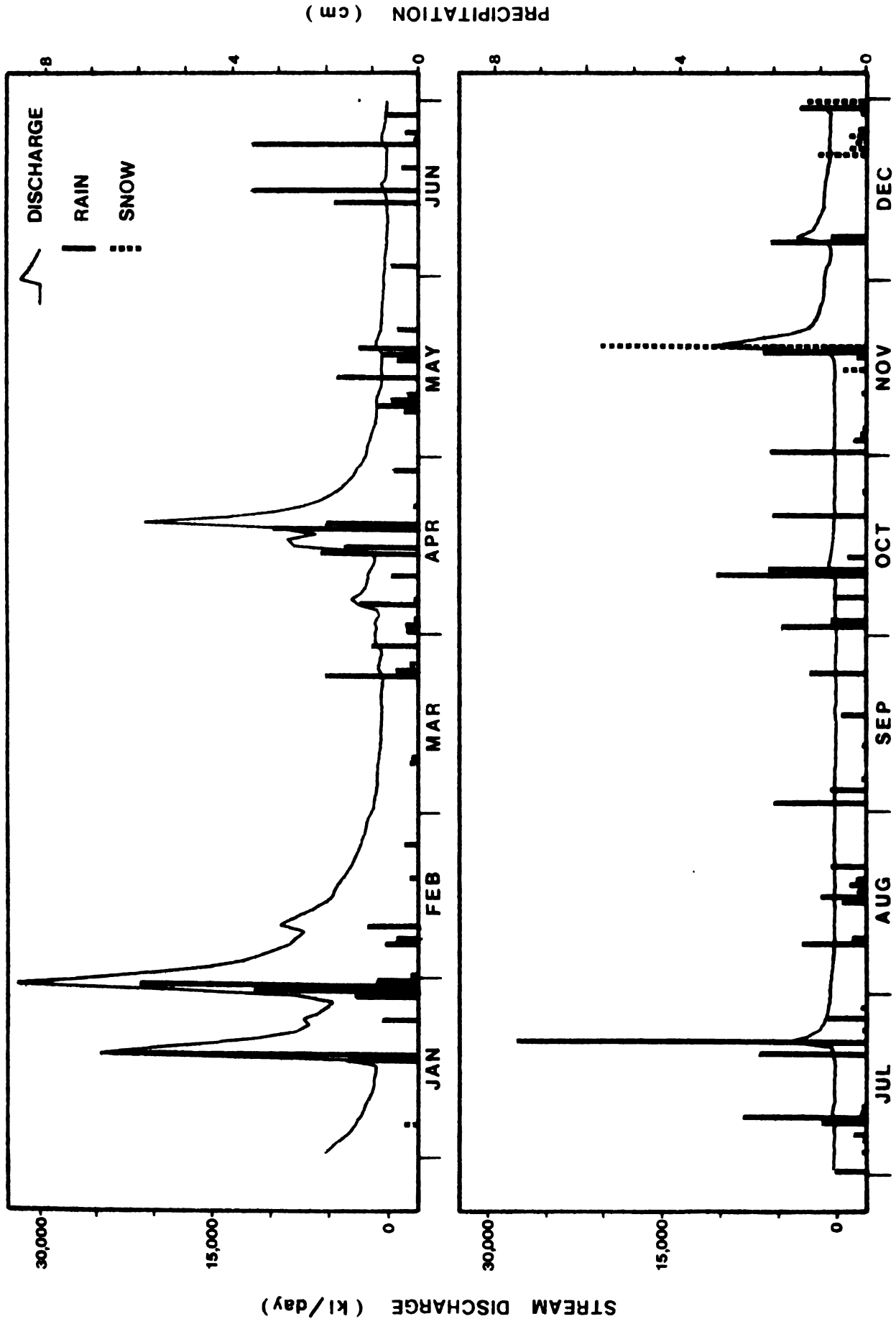


Figure 14

Figure 15. Relationship between precipitation and discharge of the cave stream.

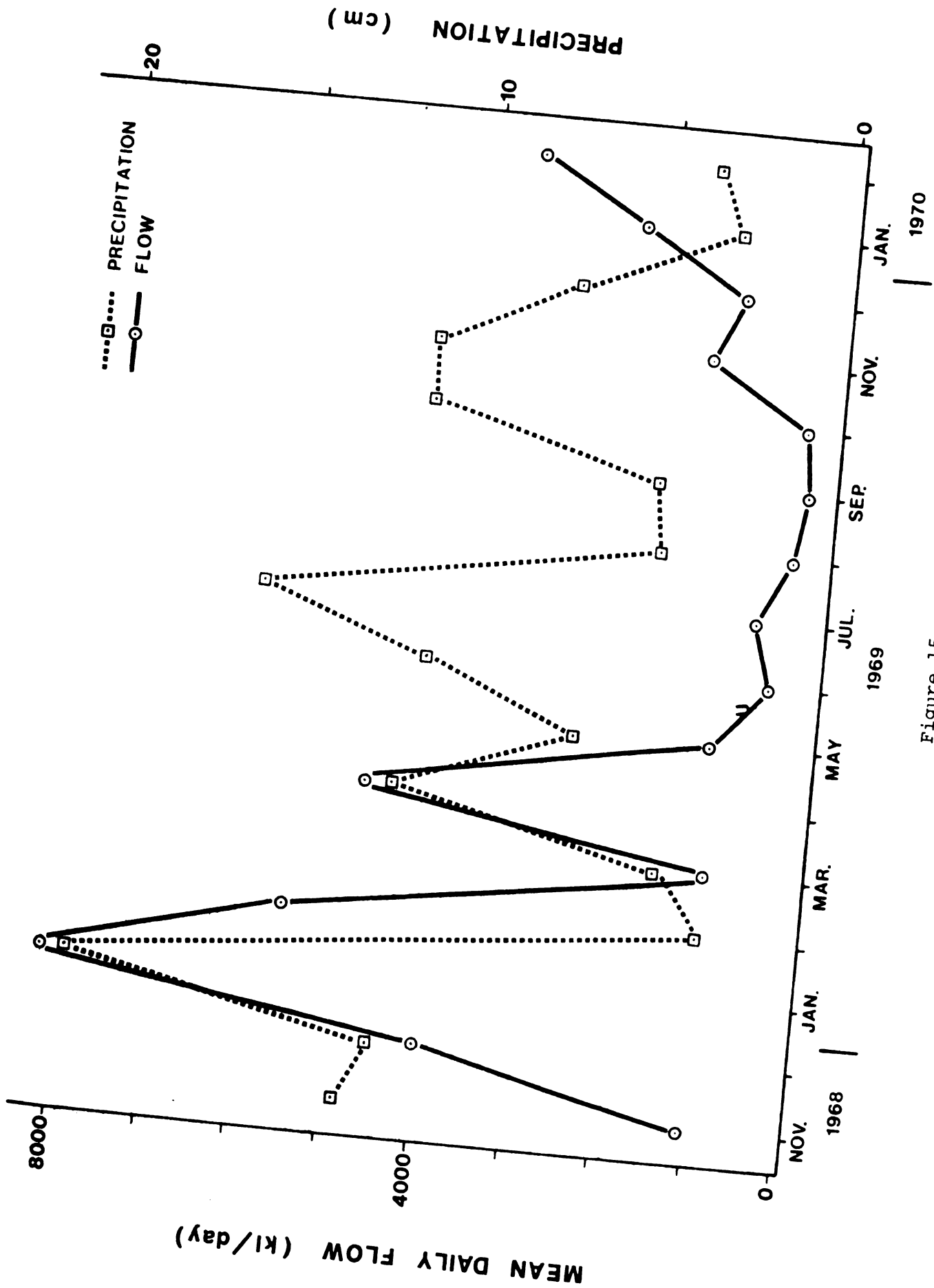


Figure 15

Discharge records extended over a 16-month period, and that portion of the annual cycle from November to February, when flooding was maximal, was repeated. It was quite evident that the extent of flooding varied considerably from year to year; the mean daily discharge of the repeated 4-month period underwent a 57% decrease.

The observed inter- and intra- annual variations in stream flow are largely the result of precipitation patterns. The average annual precipitation at the Oolitic Weather Station was 116.8 cm. Precipitation for 1968 was 4.0 cm above the norm, but 1969, the study year, was 6.6 cm below. The precipitation of the last two months of 1968 and the first month of 1969 was consistently above the norm, especially in January when precipitation was 83% above the average level. The 44.1 cm of precipitation during this 3-month period, which was 38% of the average annual precipitation, resulted in the extensive flooding of the cave stream observed during this same period.

The stream of Shiloh Cave has an estimated watershed of 7.8 km^2 , of which 20% is forest and the remainder is primarily farmland (Table 2). This watershed can be subdivided into smaller drainage areas associated with the various tributaries of the cave stream, although the accuracy of these determinations is increasingly uncertain the smaller the area. The three major tributaries — the Black Damp, the Graveyard Waterfall, and the Main Stream — are all in fairly open communication with the surface by either collapse sinkholes or swallow holes. The minor tributaries do not have obvious points of communication with the surface and probably form from water percolating through dolines or intermittent stream beds. The watersheds of these minor tributaries possess the extremes of surface features, with the 285 Meter Tributary draining flat, cleared land with a high sinkhole density, and all others draining forested hills that lack sinkholes.

TABLE 2.--NATURE OF SURFACE FEATURES AND WATER OF ESTIMATED WATERSHEDS

SHILOH CAVE TRIBUTARY	ESTIMATED WATERSHED					JANUARY 13 WATER SAMPLES	
	DRAINAGE AREA (km ²)	% FOREST	DENSITY/m ²		EPIGEAN- HYPOGEAN COMMUNICATION	ALKA- LINITY (ppm)	ENERGY CONTENT (cal/l)
			SINK- HOLES	SEPTIC TANKS			
Main Stream (0 meters)	2.8	16	16	10	Collapse sinkhole	197	0.65
Graveyard Waterfall	2.4	30	24	3	Swallow hole	200	1.52
284 Meter Branch	0.10	0	54	54	Percolation	226	1.17
Black Damp	2.3	11	30	14	Swallow hole	188	0.50
Break Down	0.09	48	0	23	Percolation	176	1.98
Sand Spit	0.02	100	0	0	Percolation	165	1.18
Half Dome	0.02	100	0	0	Percolation	131	1.75
Selinite	0.05	79	0	20	Percolation	242	2.22
Total	7.78	20	23	10	..	..	..

CORRELATION (WITH t-TEST) BETWEEN WATERSHED SURFACE FEATURES AND WATER CONTENT
(Selinite tributary not included)

STAT	ALKALINITY			ENERGY		
	FOREST	SINKHOLES	SEPTIC TANKS	FOREST	SINKHOLES	SEPTIC TANKS
r	-0.89	+0.84	+0.69	+0.50	-0.45	+0.05
d f	5	5	5	5	5	5
t	-4.30	+3.48	+2.14	+1.30	-1.13	+0.11
P	.01>P>.001	.02>P>.01	.10>P>.05	.30>P>.20	.40>P>.30	P>.50
SIGNIFICANCE						
HS = highly sign.						
S = significant	HS	S	ns	ns	ns	ns
ns = not sign.						

Alkalinity

Methyl orange alkalinity ranged from a low of 131 ppm to a high of 280 ppm (Table 3). In the main stream, seasonal alkalinity changes were minimal (a mean range of 21 ppm), and those that did occur were probably highly influenced by stream flow. The highest alkalinities were found in October, when stream flow was near base level. The alkalinity level in the main stream was stable not only seasonally, but also among the different longitudinal sites; there was only a slight decrease in the downstream direction, from a seasonal mean of 203 ppm at 0 meters to 198 ppm at 800 meters (Figure 16). Alkalinity levels in the surface stream continued this downward trend at an accelerated rate.

There was considerable variation in alkalinity among the various tributaries, with the 284 Meter Branch and the Selinite having much higher levels, the Graveyard Waterfall, Breakdown and Black Damp having levels equivalent to the main stream, and the Sand Spit and Half Dome having lower levels. Part of the explanation is that the Graveyard Waterfall and Black Damp passage are major tributaries with large flows; the fact that their alkalinities were approximately equivalent to those of the main stream indicates that these waters had been within the cave system for a time sufficient to permit equilibration with the limestone substrate.

An analysis of January water samples (Table 2) also indicated that alkalinity was correlated with various surface characteristics, such as forest cover ($r = -0.89$), density of sinkholes ($r = 0.84$), and density of septic tanks ($r = 0.69$); the correlations were significant in the first two cases. These three surface aspects are inter-related,

TABLE 3.--ALKALINITY AND ENERGY CONTENT OF SEASONAL WATER SAMPLES

DATE		Jan. 13		April 11		July 29		Oct. 28		QUARTERLY SAMPLES COMBINED			
FLOW AT WEIR (1/sec)		15.9		17.6		9.6		2.0					
COMPONENT:	ALK (ppm) ENERGY (cal/l)	ALK.		ALK.		ALK.		ALK.		$\bar{x}$	s	$\bar{x}$	s
		Energy	Energy	Energy	Energy	Energy	Energy	Energy	Energy				
MAIN STREAM	0 meters	197	0.65	203	0.47	192	1.12	219	0.56	202.8	11.7	0.70	0.29
	200 meters	198	1.07	201	0.87	188	1.05	215	0.48	200.5	11.2	0.87	.27
	400 meters	197	0.76	200	0.71	194	0.80	208	0.47	199.8	6.0	0.69	.15
	600 meters	197	0.81	194	0.74	192	1.06	210	0.42	198.3	8.1	0.76	.26
	800 meters	199	1.51	194	0.66	191	0.81	209	0.70	198.3	7.9	0.92	.40
SAMPLE SITE	Graveyard waterfall	200	1.52	200	1.03	189	1.19	228	0.60	204.3	16.7	1.11	.33
	284 meter branch**	226	1.17	239	1.09	232	1.07	222	1.28	229.8	7.4	1.15	.10
	Black Dump	188	0.50	190	0.46	194	0.70	216	1.19	197.0	12.9	0.71	.34
	Breakdown**	176	1.98	194	0.58	223	0.90	191	1.05	196.0	19.6	1.13	.60
	Selinite	242	2.22	..	..	280	0.88	*	*				
	Sand Spit**	165	1.18	*	*	*	*	*	*				
	Half Dome**	131	1.75	*	*	*	*	*	*				
	CONSTANT FLOW	$\bar{x}$	197.6	1.11	201.7	0.73	199.8	0.97	213.1	0.75			
	SITES	s	13.1	0.49	14.6	0.23	16.1	0.17	10.5	0.33			

*no water flowing

**tributary entering from higher rock strata

Figure 16. Alkalinity of water sampled quarterly from the main stream and its tributaries.

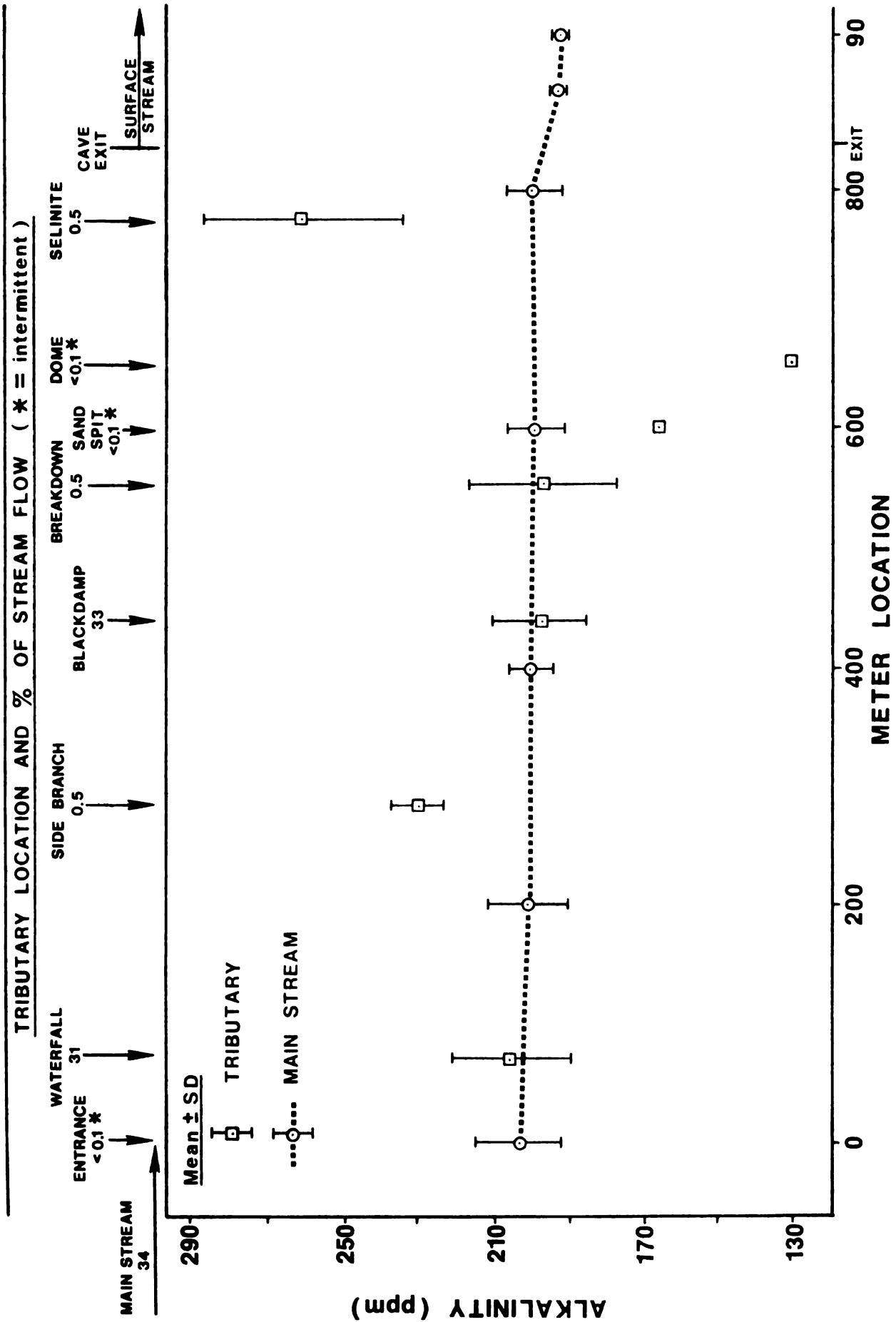


Figure 16

because sinkholes occur more commonly on flat land, and it was this land that was cleared and used for human habitation. The causal agent is uncertain, but lower alkalinities would logically arise from waters percolating through acidic woodland soils. Alkalinity values for the Selinite Passage were not included in the correlation analysis, because its high alkalinity level did not correspond to the pattern apparent in the other tributaries. Although this deviation may have been real, there was a strong possibility that the water samples had been contaminated with substrate, since it was physically very difficult to obtain a water sample from this particular site.

Some of the minor tributaries, such as the Sand Spit, Half Dome, and Selinite, were only found flowing during the winter months. Compared to the main stream, those tributaries that were active throughout the year showed fairly large fluctuations (a mean range of 33 ppm) in seasonal alkalinity levels. Although the mean alkalinity for the constant flow tributaries steadily increased from January through October, seasonal patterns varied widely among them. This was especially noticeable in the October samples that were taken when the stream was near its base level. The large horizontal tributaries resembled the main stream in that their alkalinities were at a yearly high. On the other hand, the minor tributaries, with water flowing from higher rock strata, had low alkalinity levels compared to samples taken at other times of the year.

Water dripping from a stalactite at 250 meters during October was found to have an extremely high alkalinity level of 328 ppm. Although there were many active stalactites over-hanging the stream, their rate of dripping was quite slow, and their combined effect on the cave stream was probably minimal.

Temperature

The water temperature of the main stream was quite stable throughout the year. The average temperature was 12.5° C, and variation between summer and winter, during periods of normal seasonal discharge, was only 1.5° C. The total annual variation, however, was 11.0° C. This indicates that large temperature fluctuations are of a transitory nature, apparently associated with flooding.

Food Resources

Food resources in the cave are essentially of an allochthonous nature, since photosynthesis can only occur in the twilight zone and chemosynthesis is thought to be of only minor significance. Although a small fraction of this imported organic matter may be brought into the cave by the movements of air currents, troglaphiles and troglaxenes, the great bulk of food enters the cave as vegetative detritus carried in by water. This study of food resources focuses on measuring the quantity and quality of this major source of allochthonous matter, but also analyzes the stream water and its substrate for organic content from all sources.

Leaf Input

Leaves randomly collected from litter at the cave entrance in January were by weight 51% sugar maple (*Acer saccharum*), 35% black oak (*Quercus velutina*), 13% beech (*Fagus grandifolia*) and 1% other species. In July the composition had changed, with 24% maple, 46% oak, 26% beech and 4% other species. The compositional change reflects the fact that the beech and oak trees do not shed their leaves until spring; these

1

leaves were not available for the January sample and by July had not undergone the decomposition experienced by the maple leaves, which had existed as moist litter throughout the winter.

The three most common leaf species were chemically analyzed from samples taken from the living foliage, newly-shed leaves, and January and July entrance litter (Table 4). In the foliage the lignin content was much lower in maple than in the other two species, whereas cellulose and ash were lowest in oak. Crude protein varied within narrow limits, with oak having the highest value and beech the lowest. Maple was highest and beech lowest in "other components"; the values of this category were obtained indirectly as the percentage of the leaf not accounted for by the other categories. Extensive decomposition had occurred in the leaves collected from the litter at the cave entrance. In both January and July leaves the lignin content had increased greatly in all species, but was still lowest in maple. The litter leaves had also increased in ash content, with the highest value found in maple and the lowest value still found in oak. The cellulose content from the litter samples was approximately the same as that from fresh leaves, and was still lowest in oak. Both protein and "other components" underwent a marked decrease during decomposition. The components of newly-shed leaves were generally of intermediate value, with ash content similar to that of fresh leaves and protein levels resembling those of the entrance litter. The energy content of the foliage was 5.06 cal/mg in oak, 4.60 cal/mg in beech and 4.56 cal/mg in maple, but in the entrance litter the values decreased to 4.92, 4.40 and 3.91, respectively. The species ranking probably reflects to a large extent the content of energy-rich lignin.

TABLE 4.--PERCENTAGE COMPOSITION AND DECOMPOSITION OF LEAVES IN VARIOUS STATES; composition data based on duplicate chemical tests and decomposition computed from the percentage change in the component/lignin ratio

ANALYSIS	COMPONENT	LIVING FOLIAGE				NEWLY SHED LEAVES				ENTRANCE LITTER			
		OAK	BEECH	BEECH	MAPLE	OAK	BEECH	BEECH	MAPLE	OAK	BEECH	MAPLE	JULY
COMPOSITION	Ash	5.4	8.1	8.1	8.0	5.7	8.1	9.4		7.6	8.2	15.1	7.3
	Lignin	16.5	17.5		7.5	31.7	24.9	12.0		36.4	35.2	23.4	36.0
	Cellulose	13.9	19.0		18.8	16.5	23.2	21.0		18.9	21.8	23.6	16.1
	Protein	17.7	13.2		14.9	6.7	5.4	5.7		6.3	6.2	7.1	7.0
	Other	46.5	42.2		50.8	39.4	38.4	51.9		30.9	28.5	30.8	33.6
DECOMPOSITION	Ash	..	..	..	..	45.0	29.8	26.5		36.1	49.7	39.5	37.9
	Cellulose	..	..	..	..	38.2	14.2	30.2		38.4	43.0	59.8	46.8
	Protein	..	..	..	..	80.3	71.3	76.1		83.9	76.7	84.7	81.9
	Other	..	..	..	..	55.9	36.0	36.1		69.9	66.4	80.6	66.9

Percentage composition analysis does not indicate the rate of decomposition of the various components between foliage, shed and litter leaves; this was measured by selecting lignin, an extremely refractory substance, as a marker to measure the change in the other components. Lignin actually decomposes at a low, but unknown rate, and the decreases calculated for the other components must be regarded as underestimates. Absolute and relative changes of component content, measured as the percentage change of the component/lignin ratio, were computed for the three major species of foliage and July entrance litter leaves (Figure 17).

Relative to lignin, the ash content underwent the smallest decrease, ranging from 29 to 38%. The decrease in cellulose ranged from 41 to 73%, and in "other components" from 58 to 80%. Protein decomposition was the greatest, with decreases that ranged from 79 to 87%.

The relative component decomposition was consistently the lowest in beech and greatest in maple. At least part of the greater decomposition in maple leaves can be accounted for by the fact that they exist as moist litter throughout the winter, whereas beech and oak leaves are not shed until spring. Warm, moist air flowing out of the cave during the colder months maintains a microclimate in the entrance litter that is favorable for decomposition.

Some seasonal differences in the decompositional state of entrance litter were noted, although these differences were generally of small magnitude and often not consistent; mixing of the current crop with previous litter, especially in the slowly decomposing oak and beech, probably minimized seasonal differences between samples. In maple seasonal differences in the percentage decrease from the fresh state

Figure 17. Decompositional changes between foliage and July entrance litter; based on component/lignin ratios and assuming that lignin is not decomposed.

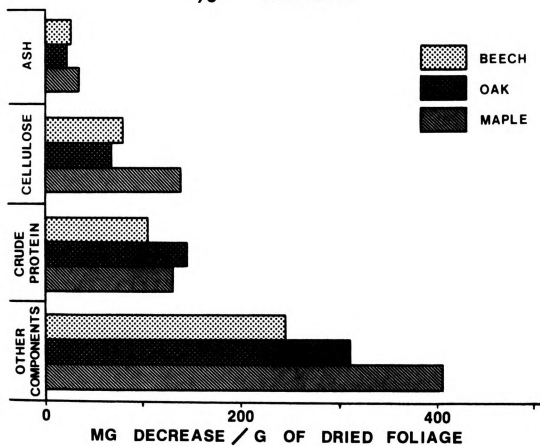
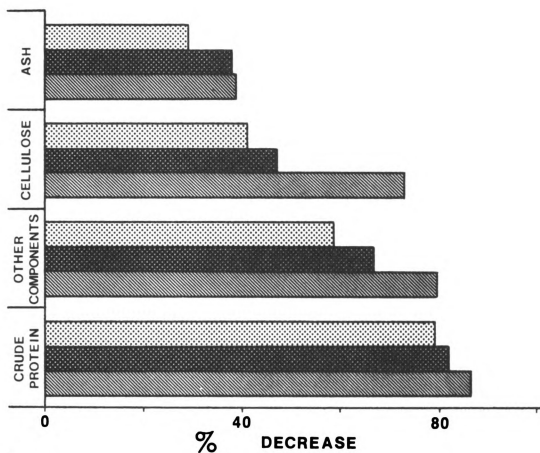


Figure 17

were minimal for all components, except for cellulose, which was not so extensively decomposed in January (59.8%) as it was in July (73.1%). In beech litter, cellulose, "other components" and especially ash were more highly decomposed in January; beech leaves collected from the litter in January were probably heavily weighted by leaves that had been dead for 15 months, whereas those in the July sample were mostly shed only 9 months previously. Although oak has a pattern of shedding similar to beech, it showed no consistent or extensive seasonal differences, and cellulose, which varied the most, was actually more highly decomposed in July. The overall seasonal decompositional state for the three species could be simply deduced from the percentages of lignin in January and July litter. Lignin was relatively more abundant in the more highly decomposed leaves; therefore, oak and beech were more decomposed in January, and maple was more decomposed in July — a pattern which reflected the length of time spent as litter. These seasonal differences, however, were very minor, and the decompositional state of litter entering the cave was approximately the same throughout the year. A more important seasonal difference was the species mix, which was probably highly weighted with maple leaves during the winter, and with oak and beech leaves during the spring and summer.

The decompositional process could be followed in leaves, from foliage through shed leaves and litter to cave stream macroseston, by analyzing species mixtures based on the proportions found at the entrance in either January or July (Figure 18). Relative to lignin, the amounts of cellulose and "other components" decreased at a near-constant rate as the leaves proceeded from foliage to macroseston, with "other components" decreasing at a faster rate. The ash content was decreased in newly-shed leaves, but this level was maintained and even slightly

Figure 18. Component changes in decomposing leaves, with January and July samples of entrance litter and macroseston. Leaves are a composite of oak, beech and maple, weighted according to the species ratio of the entrance litter in January (35, 15 and 50%) or in July (50, 25 and 25%), respectively. The percentage change of the components, ash, cellulose, protein and "other components", is based on changes of the component/lignin ratio.

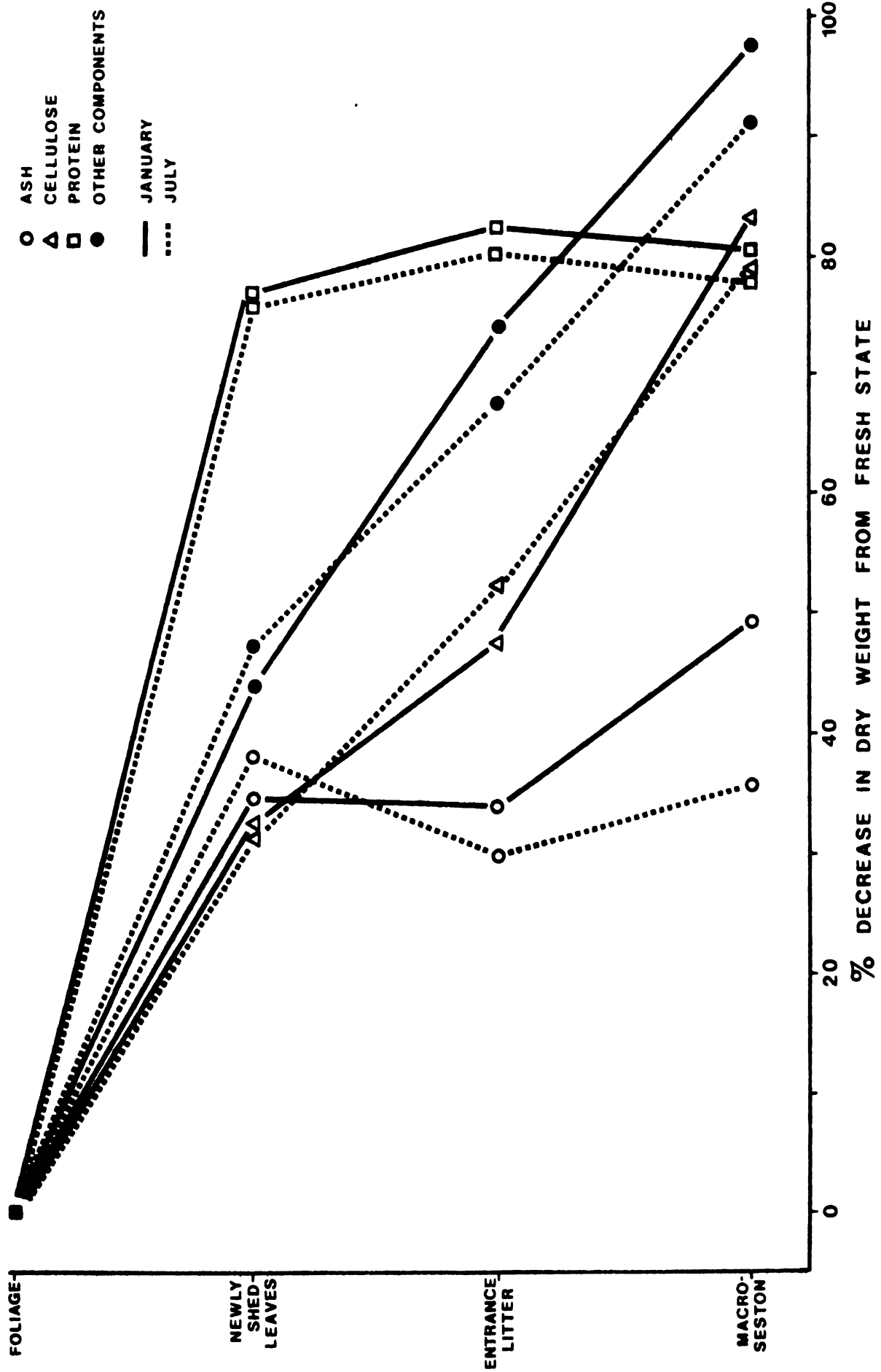


Figure 18

increased in the entrance litter; perhaps this resulted from substrate contamination or a rate of decomposition even less than that of lignin. Ash again decreased as the entrance litter was converted to macroseston, but the net decomposition from foliage to macroseston was much less than that of the other components. Crude protein underwent the most extensive decomposition of any component during the conversion from foliage to newly-shed leaves. The state of protein decomposition in the entrance litter, however, was only slightly greater, and, by the time the leaves had been transformed into macroseston, the protein level, relative to that in the litter, had actually increased by 18%. This increase could have been an artifact resulting from lignin decomposition, although the increased microfloral populations attacking leaf substrates in an aquatic environment were probably responsible.

The leaves of the macroseston contained only a fraction of their original decomposable components; only 57% of the ash, 21% of the protein, 19% of the cellulose and 6% of "other components" remained. Lignin increased in importance as the most abundant leaf component. All decomposable macroseston components — ash, cellulose, protein and "other components" — were in a more decomposed state in January than in July (Figure 18).

Macroseston

Several factors, such as the time interval between floods, the relative magnitude of flooding, and the time of year, seemed to have some influence upon the amount of vegetal macroseston, although the relationships were not well defined. The daily amount of macroseston passing through the weir was best described by a log-log relationship with daily discharge, with different regression formulae for decreasing

and increasing flows (Figure 19). The macroseston load for discharge rates above 650 kl/day was greater during periods of increasing flow. The load increased with discharge during periods of increasing flow, but declined slightly for periods of decreasing flow.

In calculating the annual macroseston input, the preliminary daily input for those days not sampled was computed by applying the proper regression formula to the known discharge rate. This computed daily input was corrected by a factor that was derived by calculating the ratio between actual and calculated inputs of the bracketing sample periods. This correction adjusted for a complex pattern of temporal variation, and also for an underestimation of macroseston inherent to the log transformation. The calculated annual input of vegetal macroseston into the quantitative study area was 2405 dry grams (Table 1). Macroseston entering the cave during the months of January and April, when the two greatest floods occurred, accounted for 64% of the total annual input. By contrast, the three-month period from August through October, which experienced the lowest mean flow rates, contributed less than 3% of the annual input.

The mean composition of the vegetal macroseston was 12.6% leaves, 36.8% wood and 50.6% detritus (Table 5). Chemical analysis of the detritus indicated that it was composed of about 53% plant material, 6% animal material, and 41% inorganic matter. There were seasonal differences in the ratios of the macroseston components. Relative to wood, leaves were less abundant in the summer samples, but the difference was not significant. Similarly, detritus comprised consistently greater percentages in the winter samples, but again the difference was not significant (Table 5).



Figure 19. Relationship between vegetal macroseston and discharge for both increasing and decreasing flows.

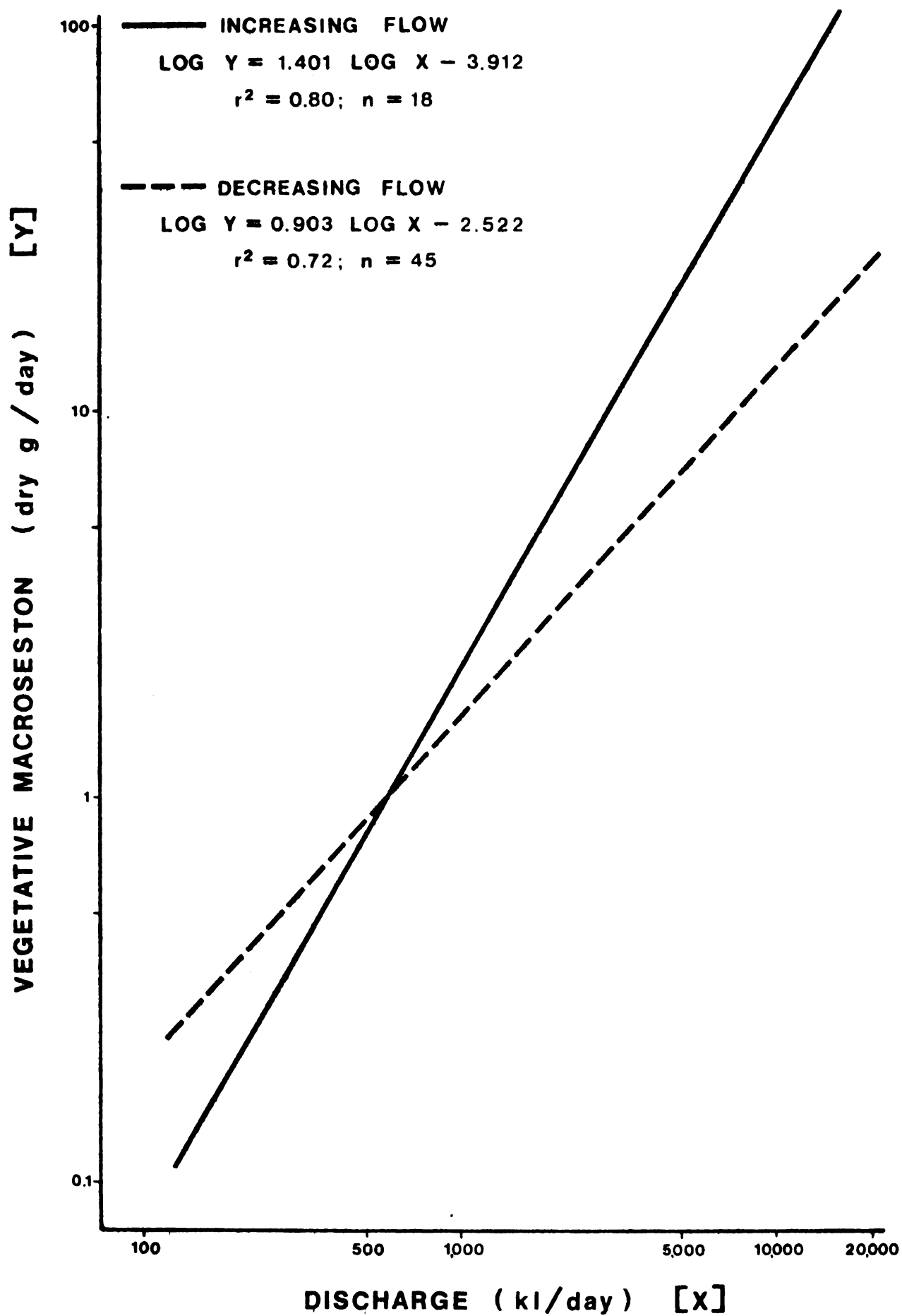


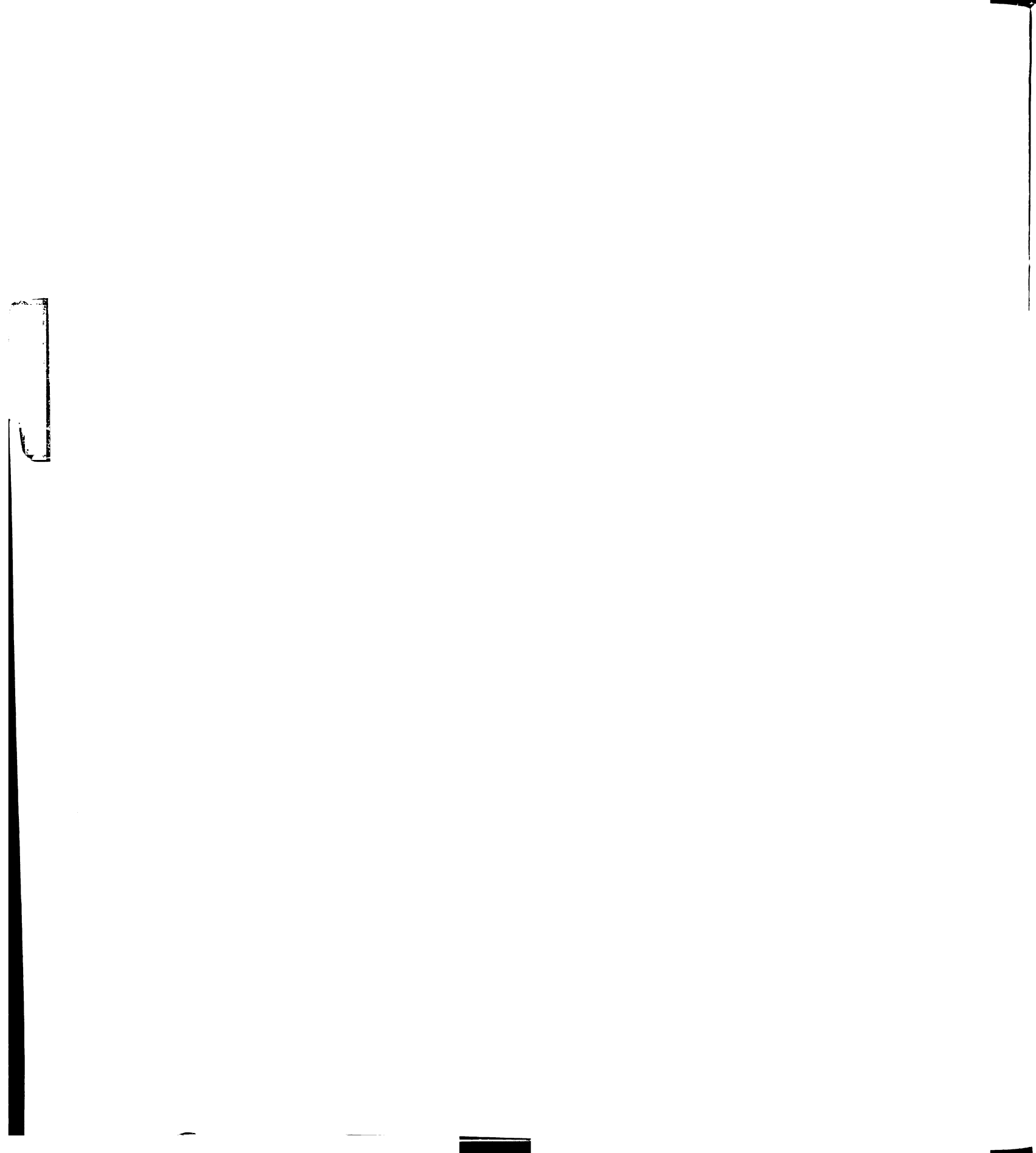
Figure 19

TABLE 5.--ANALYSIS OF VEGETATIVE MACROSESTON ENTERING THE QUANTITATIVE STUDY AREA FROM FOUR WINTER AND FOUR SUMMER 24-HOUR DRIFT SAMPLES

COMPONENT		LEAVES		WOOD		DETRITUS	
SAMPLE PERIOD		1/5-9	7/25-29	1/5-9	7/25-29	1/5-9	7/25-29
% OF VEGETATIVE DRIFT ($\bar{x} \pm$ daily s)		14.4 ± 4.8	10.8 ± 1.4	27.5 ± 9.0	46.0 ± 9.1	58.0 ± 6.6	43.2 ± 7.7
ANALYSIS OF SEASONAL COMPOSITES ($\bar{x} \pm s$ of duplicate tests)	ENERGY CONTENT (kcal/g)	4.05 ± 0.02	4.11 ± 0.02	4.13 ± 0.01	4.32 ± 0.03	2.39 ± 0	2.52 ± 0.02
	% CRUDE PROTEIN (N x 6.25)	13.4 ± 0.5	11.4 ± 0.2	10.6 ± 0.2	10.0 ± 0.6	8.0 ± 0.3	8.5 ± 0.7
	% CELLULOSE	12.5 ± 0.5	11.5 ± 0.7	16.0 ± 0.4	16.8 ± 0.7	8.6 ± 0.1	7.9 ± 0
	% LIGNIN	53.0 ± 1.3	48.7 ± 2.6	35.8 ± 1.8	40.8 ± 1.4	18.0 ± 1.7	25.2 ± 0.6
	% ASH	15.6 ± 0.2	14.4 ± 0.4	14.1 ± 0.3	11.3 ± 0.3	43.7 ± 0.5	39.7 ± 0.4

t-TEST OF SEASONAL DIFFERENCES IN THE RATIOS OF MACROSESTON COMPONENTS

COMPONENT RATIO TESTED	WINTER		SUMMER		d f	t	P	SIGN ns=not sign.
	$\bar{x}$	s	$\bar{x}$	s				
<u>LEAF</u> <u>WOOD</u>	0.60	0.34	0.24	0.07	6	2.06	P>.05	ns
<u>LEAF + WOOD</u> <u>DETRITUS</u>	0.74	0.19	1.38	0.51	6	-2.35	P>.05	ns



Based on component/lignin ratios, which showed component changes relative to the extremely refractory substance, lignin, seasonal differences were observed in the state of decomposition of the macroseston (Figure 20). Ash, cellulose, protein and "other components" occurred at reduced levels in July wood and detritus. Leaves underwent more complicated seasonal changes, with a slightly reduced protein content and an increased content of "other components" in July. The greater proportion of lignin in the January sample indicated that leaves were more highly decomposed in the winter; this agreed with data derived from the study of the decomposition of leaf input, but did not match the seasonal pattern evident in wood and detritus. The seasonal differences in the lignin content of leaf macroseston did not appear to result from seasonal differences in the species mix of litter entering the cave, which would have had an effect just opposite of that observed. A consistent increase in energy content for the summer samples of all macroseston categories was correlated with a concomitant decrease in ash content.

Information from Table 5 was combined with published (Maciolek, 1962) energy values for lignin and cellulose to obtain the annual energy and crude protein budgets for the various categories of the macroseston (Table 6). Leaves, wood and detritus contributed 15%, 47% and 38% of the annual energy input, respectively. The total annual energy input was 7980 kilocalories; however, 46% of this amount was contained in lignin, which is highly resistant to decomposition, and 11% was contained in cellulose, which can only be directly utilized by a few organisms. The annual amount of crude protein entering the quantitative study area was 230 dry grams; this was 9.6% of the vegetal macroseston. Leaves, wood and detritus contributed 16%, 40% and 44% of the crude protein, respectively.

Figure 20. Percentage change of component/lignin ratios of vegetal macroseston components between four January and four July 24-hour drift samples; based on the assumption that lignin is not decomposed.

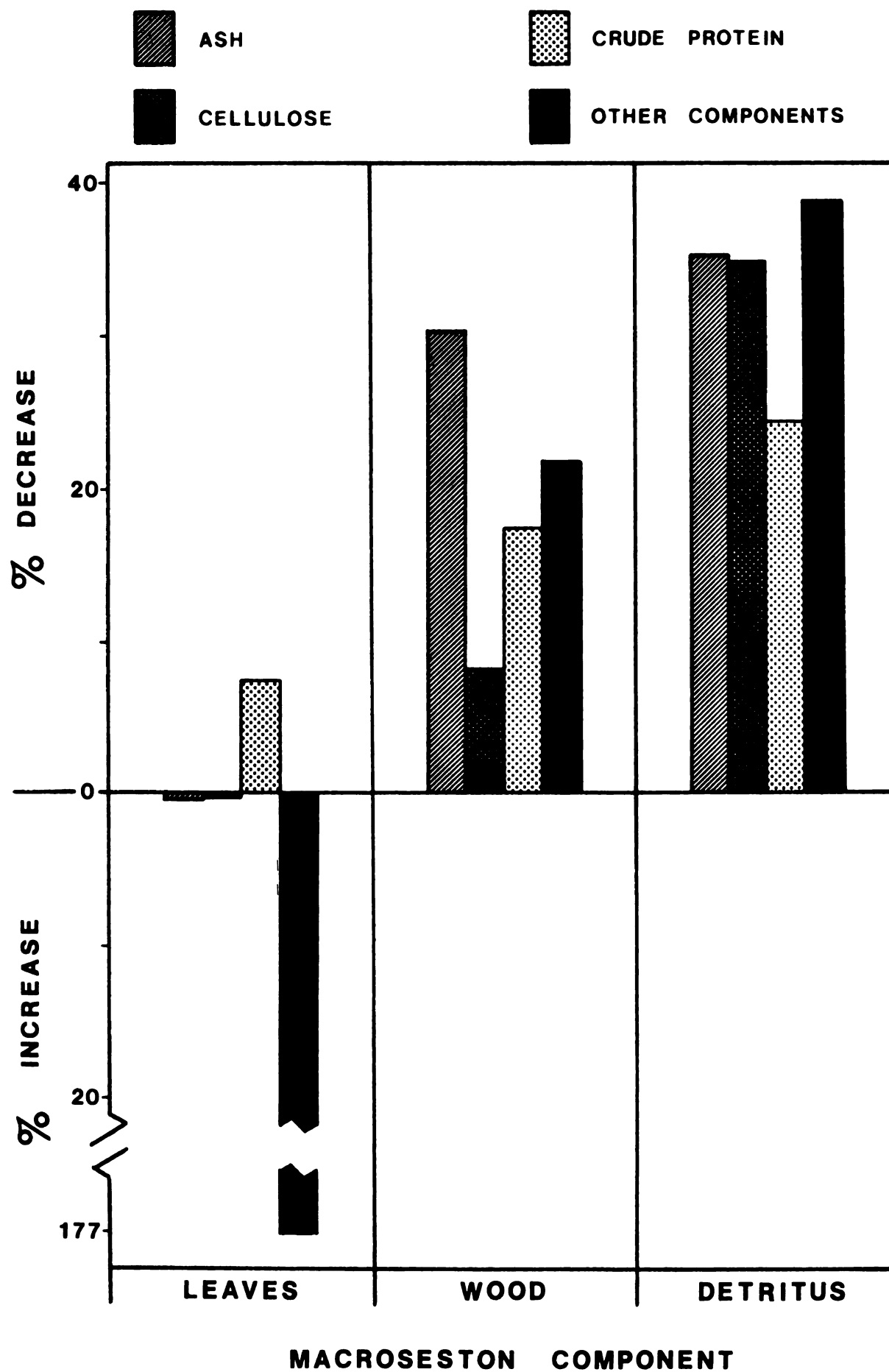


Figure 20

TABLE 6.--PROTEIN AND ENERGY CONTENT OF SESTON COMPONENTS ANNUALLY ENTERING OR BEING RETAINED*
IN THE QUANTITATIVE STUDY AREA

SESTON COMPONENT	ANNUAL LEVELS									
	TOTAL AND (PROTEIN) DRY GRAMS		ENERGY CONTENT (kcal)							
			LIGNIN		CELLULOSE		OTHER COMPONENTS		TOTAL	
	INPUT	RETAINED	INPUT	RETAINED	INPUT	RETAINED	INPUT	RETAINED	INPUT	RETAINED
VEGETATIVE MACROSESTON	303	48	992	156	146	23	99	15	1237	194
	(38)	(6)								
	885	99	2180	243	582	65	982	110	3744	418
	(91)	(10)								
DETRITUS	1217	287	496	117	118	28	2385	563	2999	708
	(101)	(24)								
TOTAL	2405	434	3668	516	846	116	3466	688	7980	1320
	(230)	(40)								
MICRO- SESTON	449,620	464							1,401,239	1445
	(144,328)	(149)								

*retained values derived from data obtained during a period of low stream flow and extrapolated as an absolute level throughout the year; crayfish ingestion rates indicated that the computed retention was an underestimate of the true rate.

By employing two drift nets, one to monitor macroseston entering the quantitative study area and the other to measure output, quantitative and qualitative changes in the macroseston flowing through the quantitative study area were observed (Table 7). The components of the plant macroseston — leaves, wood and detritus (chemical analysis indicated the detritus to be about 53% plant material) — underwent proportional changes in passing through the quantitative study area. Wood increased from 24.4% to 34.1% of the vegetative drift; on the other hand, leaves decreased from 10.2% to 3.3%, and detritus declined slightly from 65.5% to 62.5%. These changes probably resulted from the higher resistance of woody tissue to biotic processing.

The animal drift also showed differences in input and output composition. Animals of terrestrial origin increased from 2.1% to 12.9% of the animal drift, and aerial animals increased from 15.1% to 22.9%. The aquatic components declined, however, with animals decreasing from 74.3% to 60.4%, and exuviae from 8.5% to 3.9%. This shift in component proportions resulted from both an increase in the amount of terrestrial and aerial animals, and a decrease in the aquatic animals and exuviae. The lesser amount of terrestrial and aerial drift intercepted at the 79-meter station probably indicates a restricted non-aquatic habitat associated with the Graveyard Waterfall Tributary and the main cave stream above the entrance breakdown. The greater amount of aquatic animals and exuviae caught at the 79-meter drift station is thought to be a result of the more populous benthos associated with the area immediately downstream of the collapse sink-hole at 0 meters.

The percentage of macroseston of animal origin was only 3.0% at the 79-meter station; however, the proportion increased to 18.7%

TABLE 7.--COMPARISON OF MACROSESTON COMPONENTS ENTERING AND LEAVING THE QUANTITATIVE STUDY AREA; macroseston was estimated for the total daily water flow from three 24-hour samples taken in August during a period of low discharge; the 440 meter station values were adjusted to compensate for drift intercepted at the 79 meter station

MACROSESTON COMPONENT	MACROSESTON COLLECTION STATION				CHANGE IN INPUT IN PASSING THROUGH STUDY AREA			
	INPUT AT 79 METERS		OUTPUT AT 440 METERS					
	Mg Dry Wt ($\bar{x} \pm s$)	% by Wt ($\bar{x} \pm s$)	Mg Dry Wt ($\bar{x} \pm s$)	% by Wt ($\bar{x} \pm s$)	Mg Dry Wt	% by Wt		
Aerial	6.6 $\pm$	6.3	0.54 $\pm$ 0.57	7.6 $\pm$	2.3	4.18 $\pm$ 1.73	+1.0	15.2 inc
Terrestrial	1.0 $\pm$	1.4	0.09 $\pm$ 0.12	6.4 $\pm$	7.0	2.48 $\pm$ 1.63	+5.4	540.0 inc
Aquatic	29.5 $\pm$	2.7	2.15 $\pm$ 0.31	25.2 $\pm$	19.7	11.34 $\pm$ 1.74	-4.3	14.6 dec
Exuviae	3.6 $\pm$	1.9	0.28 $\pm$ 0.21	1.6 $\pm$	1.2	0.73 $\pm$ 0.09	-2.0	55.6 dec
Animal Total	40.7 $\pm$	7.0	3.05 $\pm$ 1.21	40.7 $\pm$	30.2	18.72 $\pm$ 1.73	0	0
Detritus	889.4 $\pm$	221.3	63.46 $\pm$ 0.97	102.0 $\pm$	56.0	50.90 $\pm$ 7.62	-787.4	88.5 dec
Leaves	136.9 $\pm$	22.8	9.86 $\pm$ 0.68	6.6 $\pm$	6.5	2.70 $\pm$ 1.27	-130.3	95.2 dec
Wood	332.3 $\pm$	90.2	23.64 $\pm$ 0.92	61.7 $\pm$	49.0	27.69 $\pm$ 4.62	-270.6	81.4 dec
Plant Total	1358.6 $\pm$	334.4	96.96 $\pm$ 1.21	170.3 $\pm$	111.5	81.29 $\pm$ 1.73	-1188.3	87.5 dec
GRAND TOTAL	1399.3 $\pm$	327.4		211.0 $\pm$	141.7		-1188.3	84.9 dec

at the point where it left the quantitative study area. The absolute amount of animal drift in the input and output was remarkably balanced (less than a 0.2% increase), and the change in proportion between the animal and plant components was instead due to an 88.1% decrease in the amount of vegetative drift in passing through the quantitative study area. This represented a daily consumption of 1188 ± 223 mg of vegetative macroseston by the biota of the quantitative study area. The equality in the levels of animal drift entering and leaving the study area indicated that animal drift had no net effect on energy flow and should not be included as an energy input component.

Microseston

Analysis of the microseston load carried by the receding waters of the largest observed flood showed a definite relationship ($r^2 = .89$) between load and rate of discharge. This relationship is described by the regression formula,

$$\text{microseston load in cal/l} = 0.00922 (\text{discharge in l/sec}) \pm 0.836.$$

This regression indicates the increased load capacity of higher flow rates that apparently results as a function of scouring.

The flow of microseston into the quantitative study area was quantified by applying the regression formula to daily discharge rates. The annual input of microseston, which included particles in the 0.5 - 230 micron size range, was calculated to be 1,401,239 kilocalories (Table 1). Because of the variation in flow rates, the seasonal distribution of microseston underwent extreme fluctuations. For instance, 48% was carried into the study area during January, while the five-month period from June through October only accounted for 4%.

The decline in microseston levels between water samples collected quarterly from the 200 and 400 meter stations indicated that only 0.10% of the microseston input was retained in the quantitative study area.

Samples taken quarterly from five stations positioned every 200 meters along the main stream indicated that the microseston level remained fairly stable throughout the entire length of the cave stream; it averaged 0.79 cal/l and had a range of 0.42 - 1.51 cal/l for all stations and all seasons. Microseston of the tributaries flowing from higher rock strata was consistently greater (mean = 1.22 cal/l; range = 0.58 - 2.22 cal/l) than that of the main stream, and it seemed evident that the tendency for microseston of the main stream to decrease as it flowed downstream was counterbalanced by the input from the tributaries (Figure 21). There were seasonal variations in microseston levels, especially at the tributary stations (Table 3). Most of the variation, however, was caused by differences in discharge rates, as A.O.V. testing of seasonal microseston levels adjusted for discharge indicated that the variation was not significant ($.10 > P > .05$).

There was not a significant correlation between microseston and any measured surface feature of the various watersheds (Table 2). Another phase of the study, in which microseston and benthos of several nearby caves were compared to various surface features, indicated a strong correlation with septic tanks. That this same correlation did not occur in considering the various watersheds of Shiloh Cave, itself, may have resulted from inaccuracies in delineating the watersheds of the extremely small tributaries, or from rapid processing of the microseston-poor water entering the cave system.

figure 21. Energy of microseston sampled quarterly from the main stream and its tributaries.

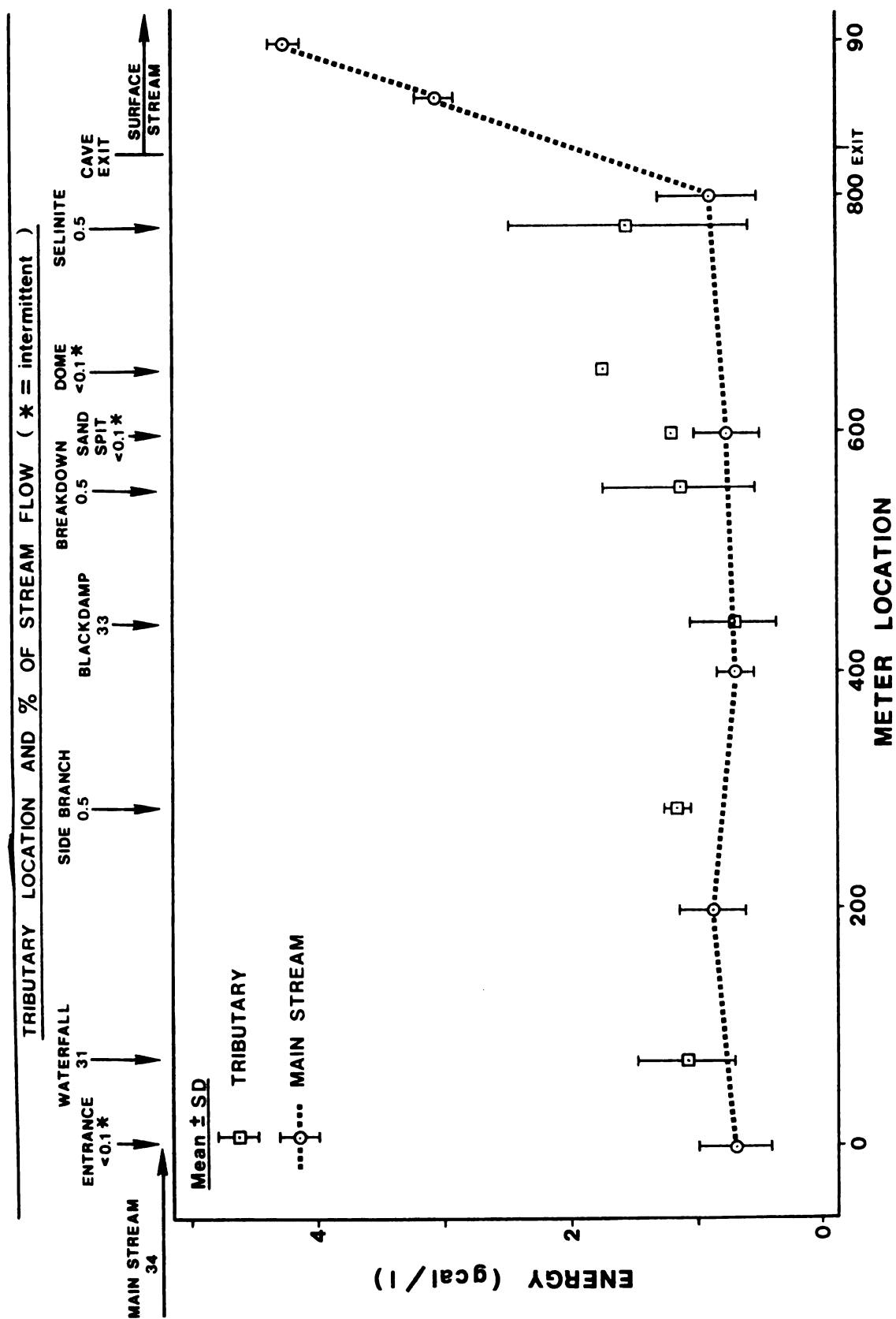


Figure 21

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Besides all the named tributaries that were studied, the cave stream received minute inputs of water dripping from the many active stalactites that overhung the stream. In October water was collected from a typical stalactite, located at 250 meters, and it was found to contain only 0.41 cal/l; thus, based on this low energy content and the small fraction of total stream flow that the stalactite seepage must contribute, it is concluded that the microseston input from this source was insignificant.

The microseston levels of the surface stream issuing from the cave were much higher than those of the cave stream, itself (Figure 21). At a point 45 meters from the cave exit the microseston level had increased to 3.10 cal/l, and at 90 meters the level of 4.29 cal/l indicated a continuation of the upward trend. It was not determined at what distance downstream the microseston leveled off, but the 4.7-fold increase in a distance of only 90 meters illustrated the fundamental differences between the trophic characteristics of epigean and hypogean streams.

Water was sampled at the weir in both January and July to determine the energy, crude protein and ash-free dry weight relationships in the microseston. Crude protein was estimated to compose a mean of 32.1% of the ash-free dry weight of the microseston. From the relationship between crude protein and energy content (mg crude protein = calories/9.71) it was possible to calculate that approximately 144,328 grams of crude protein entered the study area annually as a component of the microseston. Measurement of organic material leaving the quantitative study area, however, indicated that probably only 149 grams became incorporated into the substrate or benthos (Table 6).

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Substrate

The mud from two sites in each of 10 pools dispersed along the entire length of the cave stream was sampled both in January and July; the mean energy content was found to be 89.3 cal/gm. Longitudinal variation among pools was apparent (Figure 22). The pool at 50 meters had a very high energy content, which apparently resulted from large amounts of organic material entering the cave through the collapse sinkhole at 0 meters. The next three pools downstream showed a general decline in the energy content of the mud, and the remaining pools were fairly well stabilized at the lowest level. This pattern seemed to be related to the location of tributaries, and suggested that organic material entering by way of the tributaries was counterbalancing a tendency of pool mud to decrease in energy content in the downstream direction; large seasonal variations in energy content, however, make this assumption tentative. The energy content of the mud of the surface stream increased in the downstream direction, but at 90 meters was still not as great as that of the mud of the cave stream at 50 meters.

A more extensive seasonal analysis of pool composites (Table 8) showed the mud to be composed of about 8.0% carbonates. Ash-free organic content averaged 3.6% of the mud, with the organic fraction being composed of 59.7% cellulose and 12.9% crude protein. These values for ash-free organic content and its composition, because of the difficulty in selectively ashing only the organic fraction of the mud, must be considered only approximations. There were no seasonal differences for crude protein and ash-free organic content. The carbonate component was slightly increased in July. July samples were also

Figure 22. Energy (mean $\pm$ standard deviation) of mud substrate sampled semi-annually from two sites at each pool station.

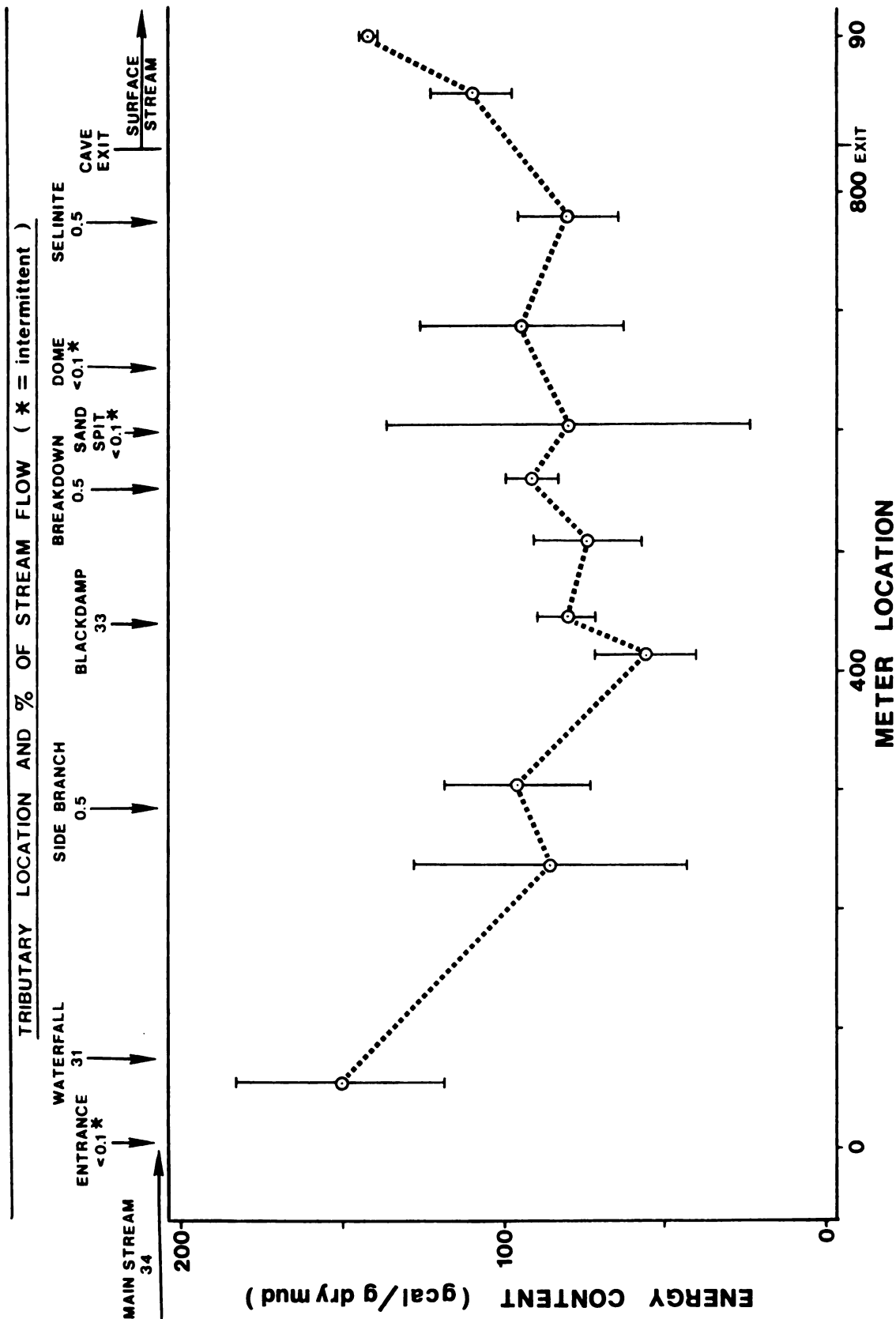


Figure 22

TABLE 8.--SEASONAL COMPONENT ANALYSIS OF THE COMPOSITES OF MUD TAKEN FROM 10 POOLS; two samples, arbitrarily designated "A" or "B", were taken from each pool

DATE	MUD SAMPLES COMPOSITE	ENERGY CONTENT (cal/g)	CRUDE PROTEIN (mg/g)	% IGNITION LOSS (ash-free organics)	ORGANIC FRACTION		% CARBONATE
					% CELLULOSE	% PROTEIN	
JANUARY	A	86.29	5.16	3.65	63.4	12.6	6.92
	B	79.39	5.12	3.58	61.6	13.2	7.59
	MEAN	82.84	5.14	3.62	62.5	12.9	7.26
	S E	3.45	0.02	0.04	0.9	0.3	0.34
JULY	A	95.98	5.05	3.54	56.6	12.7	9.15
	B	95.62	5.27	3.77	57.4	13.0	8.52
	MEAN	95.80	5.16	3.66	57.0	12.8	8.84
	S E	0.18	0.11	0.12	0.4	0.1	0.32
MEAN		89.32	5.15	3.64	59.7	12.9	8.05
S D		8.00	0.09	0.10	3.3	0.3	0.99

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higher in energy content, although cellulose was lower; these differences may have arisen from an increased lignin content in the July samples. Although there were seasonal differences in the energy content of mud between pool composites, with higher values in July, seasonal fluctuations in individual pools showed a very inconsistent pattern, with only 60% of the pools having higher energy values in July. The seasonal fluctuation in an individual pool was consistently greater than the difference between the two samples taken from the same pool on a particular date.

The substrate detritus in riffle areas was sampled in conjunction with the benthos. The mean of 26 samples taken in the quantitative study area was 11.5 ash-free grams detritus/m²; this was only 16.3% of the quantity of substrate detritus found in samples taken in the surface stream (Table 9).

Benthos

Quantitative sampling of the benthos in the quantitative study area provided evidence for a very meager benthic community, with a total biomass of only 77 dry mg/m² (Table 9). Oligochaetes were the dominant element, making up 59.5% of the biomass. This figure, however, may have over-estimated their importance in crayfish diets, since a large portion of the oligochaete biomass was inorganic gut contents. Isopods were more numerous than oligochaetes, even though their percentage of the biomass was only second highest. The dominant isopod species, *Asellus stygius*, was a troglobite. A second species, *Lirceus* sp., was a surface form that was represented by only a few individuals. Their presence indicated a heretofore unsuspected surface stream located upstream of the collection site.

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TABLE 9.--COMPARISON OF SURFACE AND CAVE STREAM BENTHOS AND SUBSTRATE DETRITUS

SITE	ANALYSIS		BENTHIC COMPONENT							TOTAL
			ASH-FREE SUBSTRATE	OLIGO- CHAETES	ISOPODS	AMPHIPODS	DIPTERA LARVA	PLANARIA	OTHER	
CAVE STREAM	MEAN $\pm$ SD OF 26 0.05 m ² SAMPLES	NUMBER	..	11.4 ± 13.1	13.4 ± 12.3	6.9 ± 7.3	2.2 ± 2.4	0.077 ± 0.277	0.077 ± 0.277	..
		mg DRY WEIGHT	580 ± 620	2.27 ± 2.64	0.84 $\pm .67$	0.63 ± 1.16	0.058 ± 0.062	0.030 ± 0.107	0.000077 ± 0.000277	..
	MEAN EXPRESSED FOR m ² AREA	NUMBER	..	228	268	138	44	2	2	682
		mg DRY WEIGHT	11530	45.35	16.78	12.64	1.16	0.59	0.0015	77
	% OF BIOMASS		..	59.3	21.9	16.5	1.5	0.8	0.002	100
SURFACE STREAM	MEAN $\pm$ SD OF 5 0.05 m ² SAMPLES	NUMBER	..	7.0 ± 9.3	107.6 ± 54.3	1128.0 ± 926.0	17.2 ± 15.8	24.8 ± 21.4	20.8 ± 44.3	..
		mg DRY WEIGHT	3550 ± 2800	2.46 ± 3.90	9.22 ± 7.39	221.36 ± 126.41	0.75 ± 1.24	6.00 ± 5.43	4.41 ± 8.98	..
	MEAN EXPRESSED FOR m ² AREA	NUMBER	..	140	2152	22,560	344	496	416	26,108
		mg DRY WEIGHT	70,910	49.27	184.42	4427.29	15.10	119.98	88.29	4884
	% OF BIOMASS		..	1.0	3.8	90.6	0.3	2.5	1.8	100

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Close inspection revealed an intermittent stream that issued from a spring and, after flowing only a few meters, entered a swallow hole. This was the probable source of the surface isopods, which apparently entered the cave as drift by way of the Graveyard Waterfall. Troglobitic amphipods were the third major component of the benthos and composed 16.6% of its biomass. There appeared to be two very similar species that differed in adult size and habitat. The smaller-sized amphipod, *Crangonyx gracilis packardii*, was restricted to riffle areas, whereas the larger species, probably *Crangonyx obliquus*, was rarely encountered, except for those found in crayfish traps located in pools.

Diptera larvae, planaria and stonefly nymphs were numerically insignificant members of the benthos. The planarian was a rare troglobite; it was probably *Sphalloplana weingartneri*, described by Kenk (1970a) from nearby Bronson's Cave as the first troglobitic planarian known to occur in Indiana caves. The numerous species of diptera larvae and the stonefly nymph appeared to be troglaphiles. Several troglaphilic species of caddis larvae and mayfly nymphs occurred in drift samples and in other benthic samples, but were not represented in the 26 samples under consideration; they were apparently regular, but uncommon, members of the benthos. Various nematodes, copepods and ostracods also occurred in the samples, but, being approximately the same size as the apertures of the sieve used in the collection of the samples, were quantitatively under-represented and were not included in the tabulated data.

Quantitative benthic samples from the surface stream, which were taken 30-90 meters downstream of the cave exit, produced dramatically different results. The total biomass was 4860 dry mg/m^2 — a level 64 times greater than that in the cave. Epigean *Gammarus* amphipods

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were the dominant component, comprising 90% of the total biomass. A few hypogean amphipods were found which evidently had been washed out of the cave.

As in the cave benthos, isopods were the second most abundant component, but they made up only 3.8% of the total biomass of the surface stream, as compared to 22% in the cave. The isopods were predominantly an epigean form, *Lirceus* sp., but 16% of the isopod biomass was contributed by *Asellus stygius*, which had apparently left the cave as drift. These hypogean isopods seemed to be established in certain portions of the stream, especially in those samples taken closest to the cave exit, and they had grown to a size not attained in the cave.

The planarian, *Phagocata gracilis*, was the third most abundant member of the stream community. This species is basically an epigean form, but has often been reported to invade caves, where it is often found with reduced pigment and eyes (Kenk, 1970b). In the present study *Phagocata* was found to only inhabit the surface stream. The fourth most abundant organism (classified as "other" in Table 9) was a fingernail clam, which was also restricted to the epigean habitat.

Oligochaetes were the fifth most common component of the biomass of the surface stream benthos. Their weight density of 49 dry mg/m² was almost equivalent to the level of cave oligochaetes, which were the dominant component of the cave stream benthos. Other minor members of the benthos were stonefly nymphs and caddis larvae, which also occurred at levels similar to those found in the hypogean habitat.

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Discussion

The cave stream ecosystem is almost totally dependent upon the importation of organic matter. Photosynthetic activity is lacking, and this may be responsible, by causing a lack of utilizable carotenoids, for the lack of pigment in many cave organisms (Maguire, 1961). Also, in a study of Mammoth Cave, Barr and Kuehne (1971) suggested that, while chemosynthetic bacteria could be potentially significant, their quantitative contribution is actually relatively small. Autotrophic activity, therefore, is thought to play only a minor role, and food resources in the cave stream are based on allochthonous plant debris.

However, even surface streams depend heavily on food importation. Minshall (1967) emphasized the importance of allochthonous detritus in the ecology of a springbrook. Nelson and Scott (1962) found that primary consumers of a Piedmont stream derived 66% of their energy from allochthonous organic matter consisting largely of leaf fragments, and Chapman (1964) found that in Coho salmon only 9% of the known energy sources had an aquatic origin. Hynes (1963) stressed the importance of the terrestrial habitat to the production of streams; he stated that, in this respect, running water differs from other lighted environments and resembles dark areas, like the bottoms of lakes and deeper parts of the sea. Perhaps caves would also fit into his concept. Consequently, from the standpoint of food quality there seems little difference in epigean and hypogean streams, although the living vegetation of epigean streams may play a role disproportionate to their abundance.

An important difference between surface and subterranean streams is that allochthonous material has unrestricted access along the entire length of surface streams, whereas in cave streams imported organic

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matter can enter the stream at only those few points where surface and subterranean waters interface. Thus, not only are hypogean streams lacking in autochthonous food production, but the importation of organic material is highly restricted.

Debris was carried into the cave by water entering collapse sink-holes or swallow holes. Organic matter of small particle size could also enter the cave system by percolation through dolines and the beds of surface streams. Although precipitation was fairly evenly distributed throughout the year, flooding generally occurred from late November through April when vegetation was dormant and the soil saturated. This seasonal pattern of discharge was not inherently different from that of surface streams, such as Walker Branch, which was studied by Elwood and Henderson (1975). This variation in discharge imposed an extremely uneven temporal distribution of food input into the cave.

Plant debris entering the cave was primarily leaves in various stages of decomposition. Autumn-shed sugar maple leaves were the dominant species throughout the winter months, whereas beech and black oak leaves were shed in the spring and dominated the leaf input during the rest of the year. Maple leaves entered the cave in the most highly decomposed state. Kaushik and Hynes (1971) have also observed that maple leaves decompose much faster than oak and beech, and they have associated this increased rate with an increased fungal population. Beech and oak are high in lignin content, and this may be responsible for their slow rate of decay. The occurrence of lignin in cellulosic materials greatly hinders enzymatic breakdown of the cellulose, and this has been attributed to a barrier effect. Mandels and Reese (1963) have isolated natural cellulase inhibitors, which appear to be polyphenols, related to leucoanthocyanins, that form physical complexes with

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cellulase; the variable occurrence of these natural inhibitors may have been the cause of the differences in decompositional rates observed in the different species of leaves. In comparison to other leaf types, however, a study by Triska (1970) indicated that all three species should be considered to be in a class most highly resistant to decomposition.

Upon entering the cave stream the leaves served as a substrate for increased microfloral populations, as measured by an increase in the absolute protein content of the leaves. Thomas (1970) has also noted that weight losses from maple and oak leaves that had partially decomposed on the land sharply increased when they were placed in a stream. The increase in protein content was associated with the growth of fungi, since bacteria are not associated with the increase in protein (Kaushik and Hynes, 1968). Johnston (1962) stated that common soil fungi, such as many species of *Aspergillus* and *Penicillium*, are the primary attackers of cellulose.

It was apparently this microfloral component, which processed the remaining cellulose, that was assimilated by the benthos. Kaushik and Hynes (1971) found that amphipods and isopods preferred leaves with microbial growth to those without such growth, and that leaf species were preferred in the order, maple, oak, beech — which corresponded to their rate of decay. This microflora was rich in protein and was a high quality foodstuff. However, there are indications that the regrowth of grazed microflora proceeded at a relatively slow rate in the cave habitat. Information presented by Halliwell (1963) indicates that, at least for the fungus, *Myrothecium verrucaria*, the enzymatic activity of cellulase at cave temperatures is only about 10% of its optimal activity at 50° C. Crossley and Witkamp (1964)

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have shown that arthropods, apparently by fragmenting the leaves, also increase the rate of weight loss in the leaf litter, itself. The fact that the benthic population of the cave stream was quite small might have tended to inhibit leaf decomposition.

Despite the possibility of relatively low decomposition rates, leaf fragments of the macroseston collected in the cave stream just 79 meters from their probable source, the collapse sinkhole, were already highly degraded; only a small amount of cellulose remained, and lignin was the principal substrate, comprising 46% of the incoming energy. Lignin appears to play a potentially important role, not only in cave stream ecology, but also in other aquatic ecosystems. For example, Kleerkoper and Grenier (1952) estimated that lignin accounted for half of the organic matter of the surface bottom deposits of a lake. Lignin is extremely refractory and the ability to break it down is possessed primarily by aerobic fungi, which only occasionally occur in water (Ruttner, 1963). Temperature is known to have a marked effect on the rate of decomposition of lignin (Waksman and Gerretsen, 1931), and the constant, low temperature of the cave must greatly retard its utilization. If lignin is significantly processed in the stream, then it must be a major source of energy input into the cave community, not only because of its high content in inwashed debris, but also because of its high energy content on a per unit weight basis. Lignin is one of the most highly reduced carbohydrates, and its oxygen equivalent (weight of oxygen required for complete oxidation of a unit weight of organic matter) is 1.89, as compared to 1.18 for cellulose and 1.06 for glucose (Maciolek, 1962).

Wood constituted a larger fraction of the macroseston than did leaves. This was quite surprising, considering the fact that leaves

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were obviously the principal input into the cave. The leaves were apparently more rapidly processed than wood, and leaf petioles made up a large portion of the wood component. The highly decomposed state of the leaves in the cave stream was indicated by the fact that their lignin content was greater than that of the wood. From information on the lignin contents of living oak and beech wood tabulated by Doree (1947), it appears that decomposition had increased the lignin content about 3.0 times in leaves, but only approximately 1.4 times in wood. Either the physical relationship between lignin and the other components or the lower surface/volume ratio apparently prevented the rapid decay of wood. Cowling (1963) discussed the susceptibility of cellulose to hydrolysis by cellulases and stated that it was determined largely by the accessibility of cellulose to extracellular enzymes secreted by, or bound on, the surface of cellulolytic microorganisms.

The other component of the macroseston was the animal fraction, which included animals from the aquatic, terrestrial and aerial habitats. Compared to the results of Bailey (1966), who studied the drift of an alkaline surface river and its tributary, the terrestrial fraction of the animal drift of the cave stream was quite small. This result stemmed from the fact that the net flow of energy, from terrestrial to aquatic habitat, that commonly occurs in the epigeal situation is reversed in most caves, including Shiloh. Instead, water-borne organic matter enters the hypogean environment, and a small portion of it is deposited on the mud banks by receding floods. This results in a terrestrial fauna even more depauperized than either the aquatic fauna or the aerial fauna which develops from aquatic stages.

The organic input was arbitrarily separated into two study categories — macroseston, which included particles larger than 230

microns, and microseston, which included particles ranging from 0.5 to 230 microns. The microseston fraction was substantially more abundant, and accounted for 99.4% of particulate matter. It is probable that the macroseston particles, with their microfloral component, were processed by all detritivores, whereas the microseston particles were too small to be utilized by the crayfish, except for that portion which became mixed into the mud. It is also possible that young-of-the-year crayfish may have directly consumed some of the larger food particles classified as microseston.

Dissolved organics were not measured, but, although probably not utilized directly by the benthos, may have been a significant energy input into the cave stream community by having become incorporated into the microflora. In the study of a forest stream, dissolved organic matter accounted for 47% of the total energy input (Fisher and Likens, 1972). Nelson and Scott (1962) found the dissolved and colloidal organic load to be two to ten times greater than the particulate at low to moderate flows; on the other hand, the particulate organic matter was at times double the dissolved and colloidal at high flows. These data, which were gathered from a soft-water, surface stream, probably are much higher than the dissolved organic levels in the hard water of the Shiloh Cave stream, because organic matter is precipitated by dissolved salts (Hynes, 1970). A study by Cummins *et al.* (1972) indicated that dissolved organic matter was processed primarily by bacteria in transport, rather than by organisms inhabiting organic substrates, such as leaf litter. This suggests that in the cave stream, where filter feeders were of minor importance, dissolved organics passed through the system and did not play a major role.

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The particulate organic matter (microseston) in the cave stream occurred in very low concentrations; less than 1 gcal/liter was the normal level, and the highest concentration, occurring during the peak of the greatest flood, was less than 3 gcal/liter. This is comparable to levels occurring in a Kentucky springbrook (Minshall, 1967), and to values of less than 2 gcal/l found in headwater and seepage sources of a mountain stream (Maciolek and Tunzi, 1968). Particulate organic matter in the Shiloh surface stream at a point just 90 meters from the cave exit attained a level 4.7 times that of the cave stream, attesting to the profound effect of the cave environment on the trophic ecology of the stream. The lack of primary production and the depressed level of allochthonous input were undoubtedly responsible for the trophic deficiencies of the cave stream.

The energy content of the seston entering the cave cannot be regarded as the energy input into the cave community, because much of it was not incorporated and was carried out of the cave. It is estimated from drift collections taken from water flowing out of the quantitative study area that only 0.1% of the microseston and 16.5% of the macroseston were retained (incorporated). Although probably not directly comparable, Fisher and Likens (1972) found a 34% energy retention in a study area of a forest brook. A portion of the retained organic material may have become stored in the substrate; such "organic lenses" have been suggested by Poulson (personal communication). The remainder of the retained organics were processed by the biota, and either left the study area as CO₂ or dissolved particles.

The estimated annual retention of the macroseston was based on measurements made when the stream was near basal flow, with the assumption that the measured absolute retention rate was maintained

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on a daily basis throughout the year. The resulting estimate apparently did not accurately reflect the fate of macroseston during periods of flood. It is thought that macroseston was retained at higher levels during floods, and that this stored food base, along with drifting macroseston, was incorporated into the biota during periods of low discharge; this would mean that incorporated energy was underestimated. Indeed, budget analysis (see section on energy and protein budgets below) seems to indicate that this is the case.

The retention of the various components of the macroseston differed. In the case of drifting animals there was a balance between input and output. This led to the conclusion that animal drift should not be considered as an energy input; accordingly, this component was separated from the vegetal macroseston and was not included in the tabulated data. This does not imply that members of the benthos that commonly occurred in the drift were not important energy sources for the crayfish populations, but that energy flow was from vegetal macroseston entering the cave, through the benthos standing crop, to the crayfish. In the vegetal macroseston, although wood was the dominant component, leaves were retained in the quantitative study area at a proportionally higher rate. Despite the fact that a higher proportion of wood drifted through the study area than did leaves, wood was retained at an absolute level 2.2 times that of leaves and appeared to be the major food resource. It must be pointed out, however, that leaf petioles were categorized as wood.

Although the input of microseston was much higher than that of macroseston, the amounts retained (as measured) were practically equal. This might have been partly the result of the fact that larger particles had a greater tendency to "hang up" in transiting a stream

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section, especially in areas of loose substrate. Although some of the sampled macroseston floated, most was waterlogged and was probably transported by being rolled along the bottom. A portion of this waterlogged macroseston probably formed organic lenses. This process probably occurred to the greatest extent during periods of flood; if this was the case, then, contrary to their measured equality of retention, macroseston must have been the seston component predominantly incorporated. From the above discussion it is obvious that the seston retained in the study area cannot be regarded as strictly equivalent to the amount of food incorporated into the benthos, because a portion of this seston removed from the drift may have been stored instead of utilized. Another factor in the differential retention of the two seston categories was that larger particles were probably processed by benthos of a wider size range than were smaller particles, which could probably only be utilized by smaller-sized organisms.

The study of the organic content in the substrate emphasizes the standing crop aspect of food resources, as opposed to the input rates previously discussed. It is also of importance because it represents a balance between input and utilization. Organic content would be expected to be of large value in situations where input was high and utilization was low; conversely, organic content would be of small value under the opposite conditions. In stable ecosystems, where the biota are quantitatively adjusted to the food base, the standing crop of food, no matter what its input rate, would be expected to fall somewhere in the moderate range.

These conclusions are supported by the observation that, despite the fact that the benthic standing crop of the surface stream was 64 times that of the cave, the organic content of the mud from the two

habitats was practically the same, and the organic detritus collected with the benthos was only 6.1 times greater in the surface habitat. Wilhm (1970), in a study of a spring, found a detrital level that was intermediate between the levels of the cave and surface stream habitats of the present study; the organic content was of a moderate level even though the benthic biomass of the spring was approximately 3 times greater than that of the surface stream. In the Palouse River, Buscemi (1964) found organic sediments, in relation to the Shiloh study, to be slightly higher in stream sections flowing through coniferous forest and lower in reaches passing through meadowland. Lellak (1965) found no correlation between benthic biomass and organic content of the substrate, but did find a dependence of the bottom fauna on the immediate supply of fresh food. It is interesting that lake sediments studied by Steiner and Meloche (1935), who found 15.4 - 62.1% organic material, and Roelofs (1944), who found a 11.0 - 72.0% organic content, were much higher (3.0 to 19.5 times) than that of the mud of the surface stream. This difference may have been due to under-utilization and storage of the food base contained in lake muds; this situation could have resulted from anaerobic conditions, which are often encountered in lake substrates.

The mean organic content of 3.6% for the mud of the cave stream was higher than the applicable value of 0.5 - 1% that was tabulated by Poulson and White (1969) as typical for cave streams with a diffuse (sinkhole) input; the organic content fell, instead, within the 2 - 6% range stated to be typical of cave streams that are underground courses of surface streams. The organic material contained in the mud had a high cellulose content, and the cellulose/protein ratio was 4.6 as compared to the 1.2 ratio characteristic of the vegetal macroseston.

Assuming microorganisms to have been the source of the protein, this high ratio indicated a reduced level of cellulolytic activity. The explanation may be found in the study of Johnston (1962), who stated that clay reduced the digestion of cellulose by the cellulolytic fungus, *Penicillium chloron*.

The organic content of the stream substrate was 385 g/m^2 for the mud of pools, but only 3.2 g/m^2 for the rocky substrate of riffle areas. These values cannot be directly compared, however, because of the different sampling methods used in the two cases. The organic content of mud was probably over-estimated due to errors associated with ashing mud; the organic content of rocky substrates was underestimated because of the loss during sieving of particles smaller than 0.18 mm.

The benthos of the surface stream seemed quite typical for this type of habitat. In comparison to a Kentucky springbrook studied by Minshall (1968), not only were the three most abundant taxa present in the same descending order, amphipods, isopods and planaria, but their numerical percentages of the total biota did not differ by more than 4 percentage points; the total density of the benthos was also similar to levels found by Minshall at collecting stations close to a spring. In both streams the benthos was characterized by high biomass and low species diversity.

The biomass of the cave benthos was only 1.6% of that of the surface stream. Crayfish trophic studies (see section on trophic ecology below) indicated that the diet of crayfish inhabiting the cave consisted of a high percentage of animals — 67% in *O. inermis* and 81% in *C. laevis*. This dietary dependence on such a limited food resource imposed severe restraints on the populations of the two crayfish.



A comparative study of the benthos of several neighboring caves indicated that Shiloh was relatively poor. However, the few other quantitative studies that have been published suggest subterranean benthic levels that are even more meager. Poulson (1963) studied nearby Bronson's Cave, which he characterized as having more than the usual amount of benthos. In microhabitats with the largest benthic populations he reported densities of isopods that were only 22% of the Shiloh level and amphipod populations only 11% as large; only the planaria of Bronson's Cave were more numerous. In a study of West Virginia caves Culver (1970) collected benthos with a Surber sampler; in a cave where an isopod, *Asellus holsingeri*, was dominant, the density of isopods in their most frequented microhabitat was only 11% of the isopod density of Shiloh Cave. In addition, the mean biomass of 77 mg/m^2 for Shiloh Cave is much greater than the 10 mg/m^2 value that Poulson and White (1969) say is typical of a cave stream with a diffuse input. Despite these findings, it is concluded that Shiloh was a relatively poor cave in terms of its food base and biomass; inefficient collection techniques, especially in regard to individuals of smaller size, probably led to an under-estimation of the benthic populations in the studies cited above.

The structure of the benthic community was decidedly skewed toward detritivores, especially those that specialized in grazing on the microfloral film associated with detritus substrates, and those that specialized in processing large particles, the shredders. Fine particle detritivores, the collectors, were not a significant component and, consequently, dissolved organic matter was probably not incorporated to any great extent into the benthos. The smaller-sized benthos, unlike the crayfish, probably fed extensively on the microseston.

In this way the benthos, as dietary components of the crayfish, functioned to funnel microseston-derived energy to the crayfish populations. Some members of the benthos were limivores, either in whole (oligochaetes) or in part (isopods, amphipods and crayfish). Culver (1970) tested several cave species of amphipods and isopods and found that all gained weight on both leaf and mud diets. Some members of the benthos, such as amphipods, crayfish and planaria, had carnivorous habits to various degrees. Both species of crayfish could best be described as omnivores.

The benthos were for the most part restricted to riffle areas. A few collections made in mud pools revealed oligochaetes to be the only significant component. Amphipods frequently were found in crayfish traps set in the pools, but their populations were too low to sample by standard quantitative methods. Culver (1970) observed a similar benthic distribution and concluded that several factors, such as flooding, food supply and prevalent occurrence, resulted in riffle areas being the optimal habitat. In Shiloh Cave predation might also have been a factor, since the crayfish spent a majority of the time in the pools.

In summary, the crayfish populations depended upon food resources that were found to be wanting both quantitatively and qualitatively. Food resources were narrowly based on decomposing vegetal detritus. The amount of this allochthonous matter entering the system was highly restricted by the largely diffuse nature of communication between the epigeal and hypogean ecosystems. Flooding was the agent primarily responsible for transporting food into the cave, and for this reason the energy input was highly seasonal. Of the food entering the cave stream, only a small fraction was retained in the cave habitat; the

bulk of the food was exported as flow-through and returned to the epigeal ecosystem. Much of the food retained in the cave was in the form of lignin and lignin-cellulose associations that were highly refractory to biological processing. Much of the energy contained in these components may have entered storage compartments and, therefore, may not have been incorporated into the community. The incorporated energy, in the form of detritus and the microfloral component that it sustained, was either consumed directly by the crayfish or consumed indirectly by predation on detritivores; however, the detritivores, by degrading a large portion of the biologically-utilizable energy to heat, reduced the amount of energy available for crayfish consumption. Despite these quantitative and qualitative restrictions of the food base, the cave habitat supported not one, but two crayfish populations.

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CHAPTER VI

THE CRAYFISH

Initial Findings

Results and Discussion

The census

Population estimates

Assumptions - Southwood (1966) stated the basic assumptions underlying the mark-recapture technique; these assumptions, which are essential for unbiased population estimates, are listed below, and each is accompanied by a judgment as to the degree of fulfillment achieved in this study:

(1) *The marked animals are not affected by being marked and the marks will not be lost.*

To ascertain whether the marking procedure affected the crayfish, 5 *C. laevis* and 15 *O. inermis* were injected with the marking code in the fall of 1968. These marked crayfish included 5 *O. inermis* that had carapace lengths less than 12.0 mm, since it was assumed that these extremely small crayfish would be most affected by the marking procedure. All the marked crayfish were placed in cages which were submerged in the cave stream. The crayfish were monitored for a month in order to ascertain whether trauma or infection would be significant factors; no mortalities were recorded during this observation period.

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There is also the question of whether the tags might have been lost. The marking procedure included, not only injecting the code, but also clipping a notch in the outer ramus of a uropod. Any recaptured crayfish that had a clipped uropod, but that did not carry the injected code, would thus be evidence of tag loss. During the course of the study three such cases were found. This indicated that the injected codes sometimes migrated to unobservable positions or were lost during molting. However, when it is considered that a total of well over 300 crayfish that had molted were subsequently recaptured and identified, the identification loss is seen to be less than 1%. In marked crayfish that were recaptured after a period of several years, it was often more difficult to identify the color code of the tag. This was caused by a gradual process of encapsulation, which obscured the tag. In most cases the color code could still be read, but, in a few instances, the code had to be removed from the crayfish for proper identification.

(2) *The marked animals become completely mixed in the population.*

Crayfish captured visually were released at the point of capture, and crayfish captured by trap were released within a meter of the trap position. Because the traps were spaced 5 meters apart, the trapping procedure probably did not displace crayfish more than 2.5 meters from their normal locations. Consequently, sampling did not segregate marked specimens, but instead returned them to their normal location, where they mixed into the population.

(3) *The population is sampled randomly with respect to its mark status; this assumption has two aspects: firstly, that all individuals of the different age groups and of both sexes are sampled in the proportion in which they occur; secondly, that all the individuals*

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are equally available for capture irrespective of their position in the habitat.

The first aspect of this assumption was found to be violated by non-random recapture rates; recapture rates were correlated with carapace length. This difficulty was overcome by stratifying mark-recapture data into several carapace length categories, which corresponded to the estimated age classes.

The second aspect was not violated, because the entire reach of the study area was surveyed, both by visual search and by traps spaced every 5 meters. Since stream width varied between the very narrow limits of 1 to 3 meters, all individuals were equally available for capture, irrespective of their position in the habitat. The only exception to this was the occurrence of certain reaches of the stream where overhanging ledges interfered with visual search; capture by trap was proportionally more important in such areas.

Although problems of non-random sampling based on sex, age and habitat either did not exist or were alleviated, some problems were encountered with certain individuals whose behavior made them unusually susceptible to capture. There were two particular *O. inermis* individuals that habitually positioned themselves in extremely exposed locations, resulting in numerous recaptures by visual search procedures. In a few other cases particular *O. inermis* individuals entered traps at a disproportionately high rate. These instances of non-random recapture, which were easily identified, occurred in only a few cases, and adjustments were made to counteract their biasing effects. All data from such individuals were disregarded in the calculation of the mark-recapture formula, but the number of these individuals was added to the formulated results to obtain the estimated population.

(4) *Sampling must be at discrete time intervals and the actual time involved in taking the samples must be small in relation to the total time.*

A census consisted of 25 discrete surveys carried out in a period of approximately 25 days. With each survey requiring approximately 3 hours, the actual time involved in taking the samples was about 13% of the total census period. It is unknown whether this fulfills the requirements of this loosely defined assumption.

(5) *The population is a closed one or, if not, immigration and emigration can be measured and calculated.*

In the case of *C. laevis* the population was definitely not closed, but the capability to identify individual crayfish permitted the calculation of movement rates that were used to correct for migration errors.

(6) *There are no births or deaths in the period between sampling or, if there are, allowance must be made for them.*

This assumption was not met in the strictest sense. A mark-recapture technique that accounted for, not only natality and mortality, but also migration, was found to be unusable in this particular study. The mark-recapture method actually employed made no allowance for these factors. Nevertheless, errors stemming from natality and mortality probably had very little effect. The census interval was only 25 days, and the population changes transpiring during that short interval must have been of a minor nature, especially when the extremely low turnover rates of these cave populations are considered. The 25-day census interval, which gave the maximal — not the mean — error of the survey series, represented only 0.7% of the calculated life-spans of these crayfish.

Accuracy - Basic assumptions underlying the mark-recapture technique that are essential for unbiased population estimates were, in general, fulfilled. In addition, the survey procedure did not interfere excessively with the crayfish populations, which had previously been subjected to extensive spelunker activity. Another important consideration is the recapture rate. These rates were very high; the mean values were 59.2% for *O. inermis* and 60.3% for *C. laevis*. With reasonable adherence to the inherent assumptions, lack of excessive investigative interference with the natural populations, and recapture rates of this magnitude, population estimates were judged to be quite accurate.

Correction for displacement - In the *O. inermis* crayfish the rate of displacement due to locomotive activity was insufficient to require corrections to the basic Schumacher procedures; this was true for all stratified groupings, which were based on season, sex and carapace length. In *C. laevis*, however, displacement rate played a more significant role. Its magnitude, which varied with carapace length, sex and season, was in many instances great enough to require application of a correction factor to the basic Schumacher procedure. The correction consisted of removing from the tally of marked crayfish those individuals which were not recaptured within their estimated sampling life span (see pages 39 and 40 above).

The population/capture ratio - For *O. inermis* the ratio of the Schumacher estimated population to the total number of individuals captured during a census, both visually and by trap, was tested by A.O.V. (Table 10). With data blocked into nine carapace length categories, the ratio was shown to have no relationship to sex

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TABLE 10.--A 4X2 FACTORIAL ANALYSIS OF THE INFLUENCE OF SEASONAL AND SEXUAL FACTORS ON THE RATIO OF THE POPULATION SIZE, AS ESTIMATED BY MARK AND RECAPTURE, TO THE NUMBER OF INDIVIDUALS CAPTURED DURING A CENSUS; the data were blocked into nine carapace length categories which corresponded to the various year classes.

<i>O. inermis</i>			
SOURCE	SEASON	SEX	INTERACTION
d f	3	1	3
MS	0.079	0.0076	0.020
F	2.27	0.22	0.58
PROBABILITY OF A LARGER F	.10>P>.05	P>.10	P>.10

<i>C. laevis</i>			
SOURCE	SEASON	SEX	INTERACTION
d f	3	1	3
MS	0.060	0.0088	0.048
F	1.12	0.16	0.90
PROBABILITY OF A LARGER F	P>.10	P>.10	P>.10

($P > .10$) or season ($.10 > P > .05$). On the other hand, the ratio was obviously dependent on carapace length, with a much higher ratio characteristic of the smaller crayfish; this was related to both their lessened tendency to enter traps and the decreased efficiency of their visual capture. The variation of the ratio with carapace length was not linear on either arithmetic or logarithmic scales, however, and required the use of a French curve to express the relationship (Figure 23). In making population estimates, the length-specific ratio, as determined from this smooth curve, replaced the values derived from the seasonal and sexual grouping of length-blocked data.

In *C. laevis*, A.O.V. testing of the population/capture ratio, which was blocked into nine carapace length categories (Table 10), showed no significant difference related to either season ($P > .10$) or sex ($P > .10$). In addition, the magnitude of the movement rates and their erratic variation with carapace length invalidated any linear or curvilinear relationship to carapace length alone, at least for the larger-sized crayfish (Figure 23). In several of the longer carapace length groups, movement was so extensive that, combined with the large proportion of the population captured, the ratio between population and number captured was less than one. In these cases extensive movement during the census created a large turnover in the individuals comprising the population of the census area; this resulted in the capture of more individuals during a census than existed in the population at any one time. The smallest value for the ratio, which occurred at the carapace length category centered at 40 mm, corresponded with maximum movement as measured by displacement rate and wandering (see Figure 30 below). Although neither sexual nor seasonal variations in the ratio were found to be significant, population estimates were made independently for

Figure 23. The ratio of the population, as estimated by mark and recapture, to the actual number of individuals captured during a census, and the relationship of this ratio to carapace length. Means and standard deviations are based on four censuses, and a smoothed curvilinear relationship is established for *O. inermis*, but not for *C. laevis*.

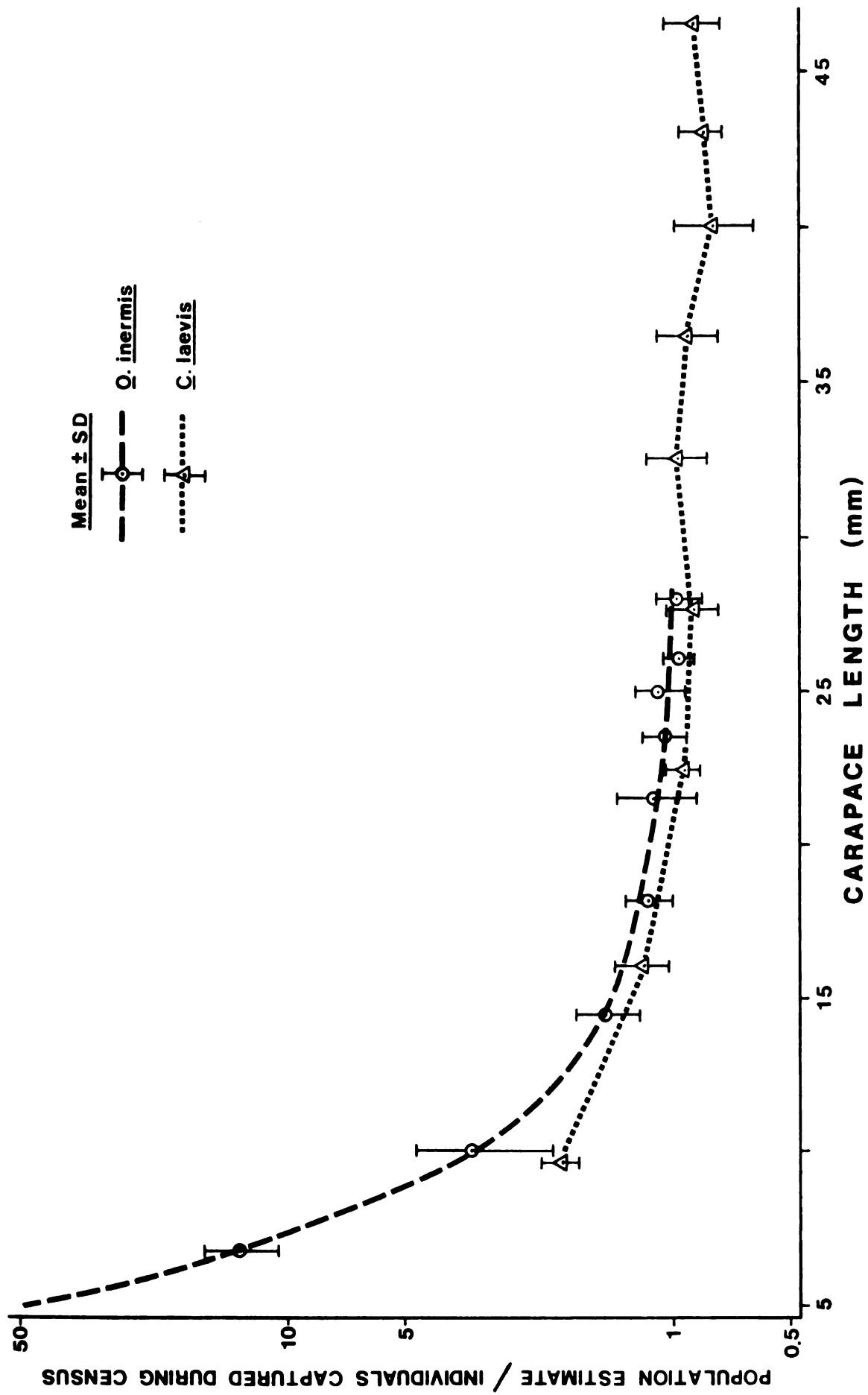


Figure 23

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groups that were stratified by sex, season and carapace length; carapace length categories corresponded to ascertained year-groups.

Capture rates

Trap versus visual - In crayfish surveys 12.6% of the captured *O. inermis* were caught by trap (Table 11). Both mature males and females were trap-captured at a slightly higher rate than immatures. In contrast, 31.3% of *C. laevis* were captured by trap, and immatures were captured at a much higher rate than mature crayfish (Table 11).

C. laevis had a higher percentage of trap capture, even though they seemed to more readily escape from traps, especially the large-mouthed type. The inefficiency of the large-mouthed traps was reflected by the fact that mature *C. laevis* were much less frequently trap-caught than immatures.

The higher percentage of trap capture for *C. laevis* was probably due to two causes: (1) *C. laevis* were not as highly visible as the white *O. inermis*, and many were probably visually overlooked. (2) *C. laevis* seemed to be more readily attracted into baited traps, since it was often observed after baiting traps that *C. laevis* would respond to the bait much more quickly than *O. inermis*. This was in agreement with the study of Cooper (1969), who found that *O. inermis* required more time to detect and locate odoriferous, inanimate food than did its epigean relative, *O. limosus*.

Temporal differences - Surveys were carried out during different times of the day, and censuses were performed during different seasons of the year. This provided information on temporal differences in visual capture rates, as related to diel and annual cycles (Table 12).

TABLE 11.--COMPARISON OF THE NUMBER OF CRAYFISH CAPTURED PER SURVEY BY VISUAL AND TRAP METHODS; data based on 45 surveys, with 15 from each of 3 annual periods and, within each annual period, 5 from each of 3 diel periods

SPECIES	GROUP	NUMBER CAPTURED PER SURVEY			% CAPTURED BY TRAP
		VISUAL ($\bar{x} \pm s$)	TRAP ($\bar{x} \pm s$)	TOTAL ($\bar{x} \pm s$)	
<i>O. inermis</i>	Immatures	4.8 $\pm$ 2.1	0.5 $\pm$ 0.8	5.3 $\pm$ 2.0	9.9
	Females	4.6 $\pm$ 2.7	0.8 $\pm$ 1.2	5.4 $\pm$ 2.9	14.8
	Males	5.3 $\pm$ 2.8	0.8 $\pm$ 1.3	6.1 $\pm$ 2.9	13.1
	Total	14.7 $\pm$ 4.7	2.1 $\pm$ 2.7	16.9 $\pm$ 4.9	12.6
<i>C. laevis</i>	Immatures	3.6 $\pm$ 1.8	2.2 $\pm$ 2.4	5.8 $\pm$ 3.2	37.6
	Females	0.8 $\pm$ 1.0	0.1 $\pm$ 0.2	0.9 $\pm$ 1.0	7.9
	Males	0.5 $\pm$ 0.6	0	0.5 $\pm$ 0.6	0
	Total	4.9 $\pm$ 2.4	2.3 $\pm$ 2.5	7.2 $\pm$ 3.6	31.3

TABLE 12.--TEMPORAL DIFFERENCES IN THE NUMBER OF VISUAL CRAYFISH CAPTURES PER SURVEY.
Data based on 45 surveys, with 15 from each of 3 annual census periods and, within each census, 5 from each of 3 diel periods

SPECIES	TIME PERIOD					
	DIEL			ANNUAL		
	9:00 A.M.	3:00 P.M.	9:00 P.M.	July '69	Nov. '69	Mar. '70
<i>O. inermis</i>	13.7±3.9	17.2±5.0	13.3±4.4	16.7±4.4	12.7±2.6	14.8±5.9
<i>C. laevis</i>	4.6±2.0	5.7±2.9	4.5±2.3	6.7±2.4	4.1±1.4	4.0±2.3

ANALYSIS OF VARIANCE TABLE

SOURCE	SPECIES	d f	SS	MS	F	P	SIGNIFICANCE
							HS = highly sign. s = sign. ns = not sign.
DIEL	<i>O. inermis</i>	2	138.53	69.27	3.90	.05 > P	S
	<i>C. laevis</i>	2	12.98	6.49	1.37	P > .10	ns
ANNUAL	<i>O. inermis</i>	2	116.13	58.07	3.27	P = .05	ns
	<i>C. laevis</i>	2	69.38	34.69	7.33	.005 > P	HS
INTERACTION	<i>O. inermis</i>	4	74.94	18.74	1.06	P > .10	ns
	<i>C. laevis</i>	4	2.88	0.72	0.15	P > .10	ns
ERROR	<i>O. inermis</i>	36	639.20	17.76			
	<i>C. laevis</i>	36	170.40	4.73			
TOTAL	<i>O. inermis</i>	44	.968.80				
	<i>C. laevis</i>	44	255.64				

On an annual basis, there were found to be no significant differences in the capture rates for *O. inermis*. The capture rates for *C. laevis*, on the other hand, were significantly different at the 0.5% level; the rate was higher in July than in the other two, nearly equivalent, census periods. Mark-recapture analysis (see page 112) suggested that the capture rate probably reflected, to a large extent, the population level; this indicated seasonal stability in the population of *O. inermis* and seasonal fluctuation in the population of *C. laevis*. Annual variation in catchability due to behavioral modification has not been demonstrated.

Diel variation of the capture rate was not significant in *C. laevis*, but was significant at the 5% level in *O. inermis*. The capture rate was higher for surveys conducted in the afternoon, and the variation probably resulted from a diel activity pattern. This topic will be more fully discussed in the section on activity.

Length-weight relationships

In all cases linear correlation was found between the log of length and the log of weight, and the regression formulae for these relationships were determined (Table 13). For any given length, the weights of both body and exuvia were less for *O. inermis* than for *C. laevis*. The skin/body weight relationship was also less in *O. inermis*, so that exuvia weight loss per molt was greater in *C. laevis*.

The ratio between wet and dry weights was not constant; increased weight loss on drying occurred in *O. inermis* and with decreasing length in both species. This variation apparently resulted from species-specific and size-specific differences in the degree of development of the

TABLE 13.--LENGTH-WEIGHT REGRESSIONS FOR CRAYFISH BODIES AND MOLTED SKINS

REGRESSED VARIABLES (Y on X)	SPECIES	REGRESSION FORMULA Y = weight in grams X = carapace length in mm	COEFFICIENT OF DETERMINATION (r^2)	OBSERVA- TIONS
Y = Wet Weight of body	<i>O. inermis</i>	$\log Y = 3.264 \log X - 4.05$	0.992	70
X = Carapace length	<i>C. laevis</i>	$\log Y = 3.281 \log X - 3.95$	0.997	91
Y = Dry weight of body	<i>O. inermis</i>	$\log Y = 3.490 \log X - 5.09$	0.962	35
X = Carapace length	<i>C. laevis</i>	$\log Y = 3.654 \log X - 5.08$	0.995	48
Y = Dry weight of molted skin	<i>O. inermis</i>	$\log Y = 3.268 \log X - 5.19$	0.945	6
X = Carapace length	<i>C. laevis</i>	$\log Y = 3.392 \log X - 5.11$	0.975	6

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exoskeleton. That is, the exoskeleton, which contained relatively little water, constituted a greater proportion of the total body weight in *C. laevis* and in larger crayfish, in general.

Length-weight regressions indicated that, in crayfish of equal carapace length, *O. inermis* had a lighter body than *C. laevis*. This weight reduction, which is typical of troglobites, probably developed in response to energy conservation, although Vandel (1965) regards the slender body of many cavernicoles to be the result of orthogenesis of a phyletic line. The reduced weight of the exoskeleton in *O. inermis* also functions in conservation of energy, and it apparently evolved upon the relaxed need of the exoskeleton's protective function in the cave environment. In this regard, exposure to both solar radiation and predation was drastically reduced as a result of the invasion of the cave habitat by the epigean ancestor of *O. inermis*. Subsequent to the evolutionary development of its troglobitic form, *O. inermis* was joined by the troglophilic crayfish, *C. laevis*. Its larger size, robustness, heavily armored exoskeleton with well-developed chelae, and aggressive behavior posed a predatory threat with which *O. inermis* was poorly equipped to cope, at least in an offensive sense. The low mortality rates, which were characteristic of the *O. inermis* population (see Table 27 below), indicated that the troglobite had successfully dealt with the problem by other means.

Activity

Introduction

Most epigean crayfish are regarded as being nocturnal. Penn (1943) found *C. clarki* to be most active at night, responding positively to

twilight conditions. The locomotor activity of *O. virilis* was investigated by Guyselman (1957), who found maximal activity between 7:00 and 10:00 P.M. Park, Roberts and Harris (1941) studied the troglobitic crayfish, *O. pellucidus*, under laboratory conditions and concluded that it had an arrhythmic diurnal activity pattern. However, Brown (1961), analyzing the same data, found a statistically significant circadian rhythm with minimum activity about 9:00 A.M. and maximum activity about 7:00 P.M. His conclusions, however, were based on a questionable procedure and on data from only one crayfish. Jegla and Poulson (1968) found evidence for a circadian rhythm in *O. pellucidus* and *O. inermis*, but emphasized its variable periodicity in different individuals.

To shed further light on this situation, crayfish surveys were conducted at different times during the day. Although trap captures were not relevant because of uncertainty of the time of entry, visual capture rates would seem to indicate activity patterns under natural conditions. Surveys were conducted in the morning (9:00 A.M.), afternoon (3:00 P.M.) and evening (9:00 P.M.). The three time periods are indicated by a time that fell within the surveys, although it must be understood that the surveys required a variable length of time of up to four hours. Besides diel activity patterns, also of interest was the longitudinal displacement of crayfish that resulted from locomotive activity. A few studies have undertaken to determine movement of stream-dwelling crayfish in terms of displacement rates and home range. Most of these studies were at the population level, employing mass tagging or weir trapping. A few crayfish studies, notably the cave studies by Cooper (1975) and Hobbs (1973), have had success with a tagging method that permits the recognition of several individuals. In the present study the crayfish were individually tagged by a different method that

permitted monitoring the movements of several hundred individual crayfish. The longitudinal position of a particular crayfish in the census area was noted at each capture and recapture during the four censuses. This procedure was aided by placement of location markings on the cave wall at every five meters.

Results

Circadian rhythm

As mentioned in the section on temporal differences of capture rates, analysis of variance indicated a significantly different ($.050 > P > .025$) diurnal rate of capture for *O. inermis*, although the highest rate at 3:00 P.M., instead of 9:00 P.M., does not correspond with the findings of Brown (1961). *C. laevis* also showed the highest rate of capture at 3:00 P.M., but it was not significantly different from the other two time periods (Table 12).

Longitudinal movement

Patterns in individuals

Troglobite:

The pattern of movement in *O. inermis* was fairly simple (see Figure 24 below, which displays movement during the July census when the crayfish were especially active). Basically, this species exhibited the phenomenon of home range. Most recaptures occurred within ten meters of the initial capture site, indicating the equivalency of home range, in its strictest sense, with the local pool. In many cases, however, especially in the males of the larger size categories,

Figure 24. Movement patterns of all *O. inermis* captured at least three times during the July census. Data from 56 individuals are shown, and the connecting lines in the capture sequences indicate the existence and extent of wandering.

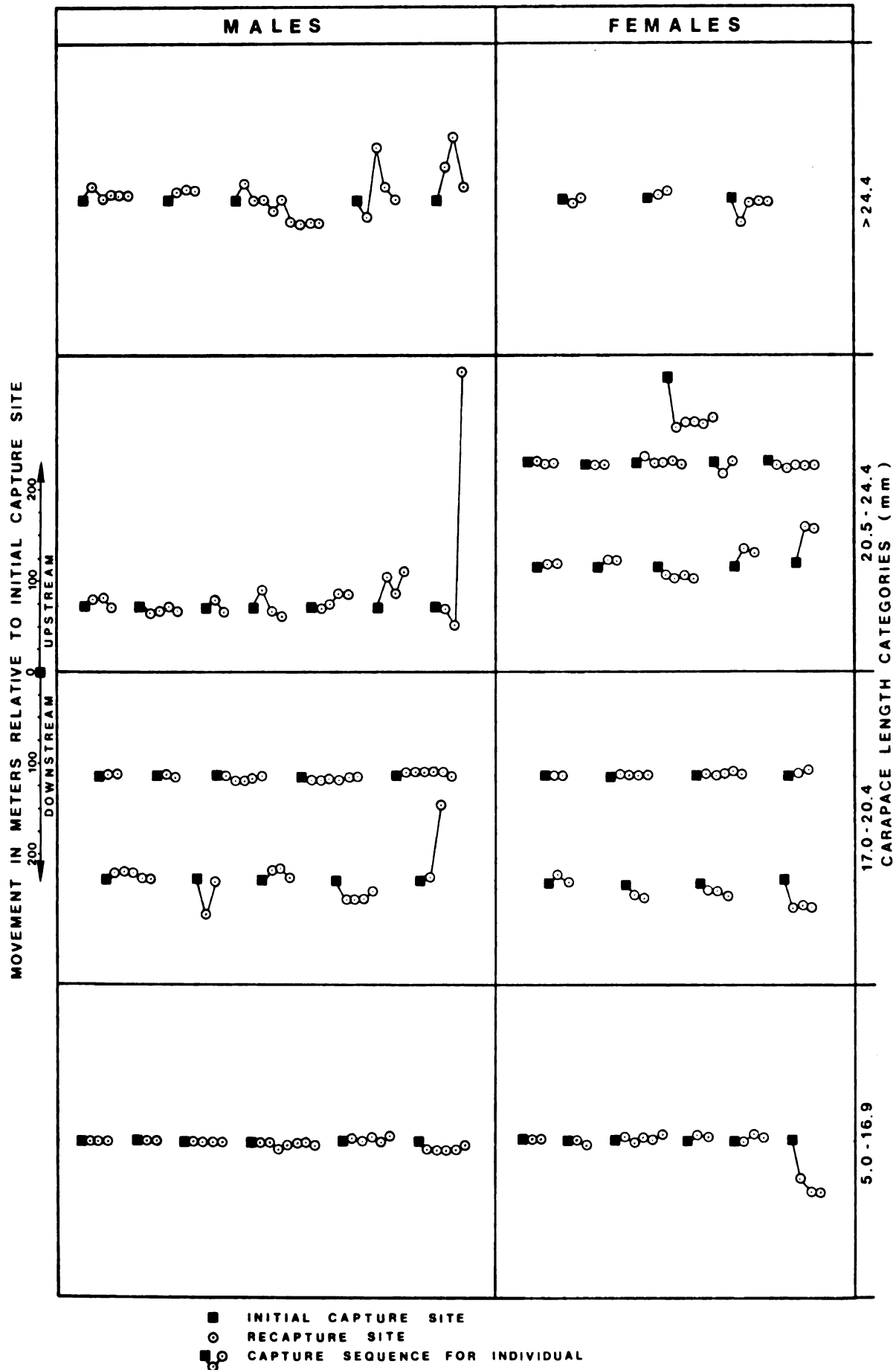


Figure 24

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crayfish wandered up to 60 meters from the home pool and returned. This extended area should be included as part of the home range, since analysis of movement over a year's time indicated these wanderings to be habitual; some male *O. inermis* normally traveled an area of up to 300 meters (Figure 25). There were a few instances in which crayfish underwent a net displacement during a census; at least some of these could be explained by the home range concept as cases in which the crayfish had not yet completed the return trip to its home pool. Possibly two or three examples of annual net displacement were observed (Figure 25), but these exceptions merely accentuated the positional stability and home range behavior of the majority.

The complex data on crayfish movement was condensed in order to permit further analysis. This was accomplished by dividing net displacement of a crayfish during a census by the elapsed time and expressing movement as a rate in meters per day (Table 14). The rate of movement was related to size with the highest rate occurring in the 17.0 - 20.4 mm size group, although larger adults in the next two size categories also showed a high rate (Figure 30a). There were also sexual and seasonal variations in movement. Males had a greater movement rate in all size categories (Figure 26a), and both March censuses had a generally lower rate of movement than the July and November censuses (Figure 26b). Movement rates ranged from a low of 0 meters per day for females over 26.5 mm C L during the March 1970 census, to a high of 6.1 meters per day for 17.0 - 20.4 mm C L males during the November census; the mean rate was 2.3 meters per day.

The percentage of crayfish with a net displacement of more than 10 meters during a census was also determined. This, in effect, gave an estimate of that fraction of the population which had entered a

Figure 25. Annual movement of five randomly selected individual *O. inermis* in different sex and size categories.

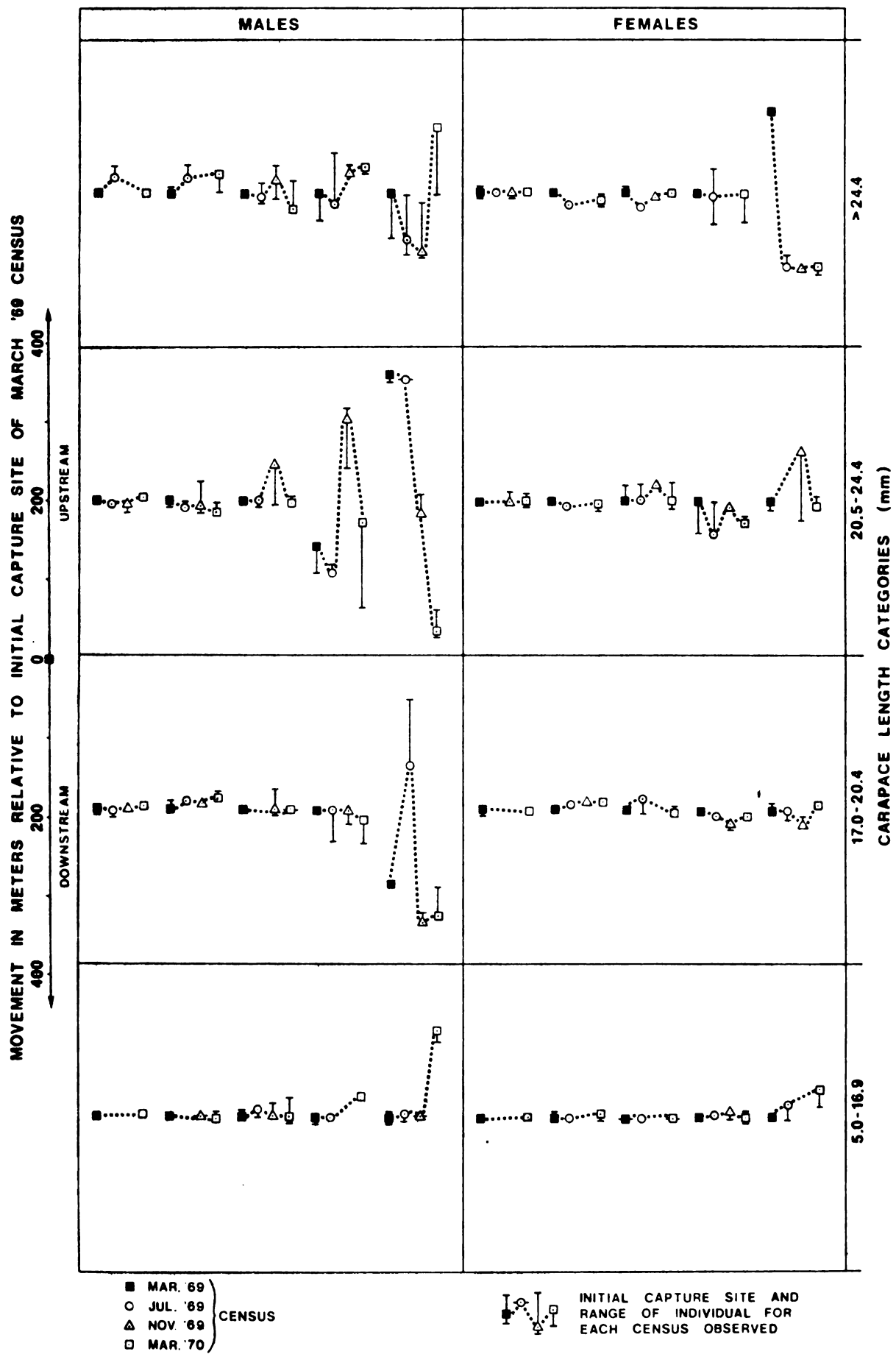


Figure 25

Figure 26. Daily displacement in *O. inermis*.

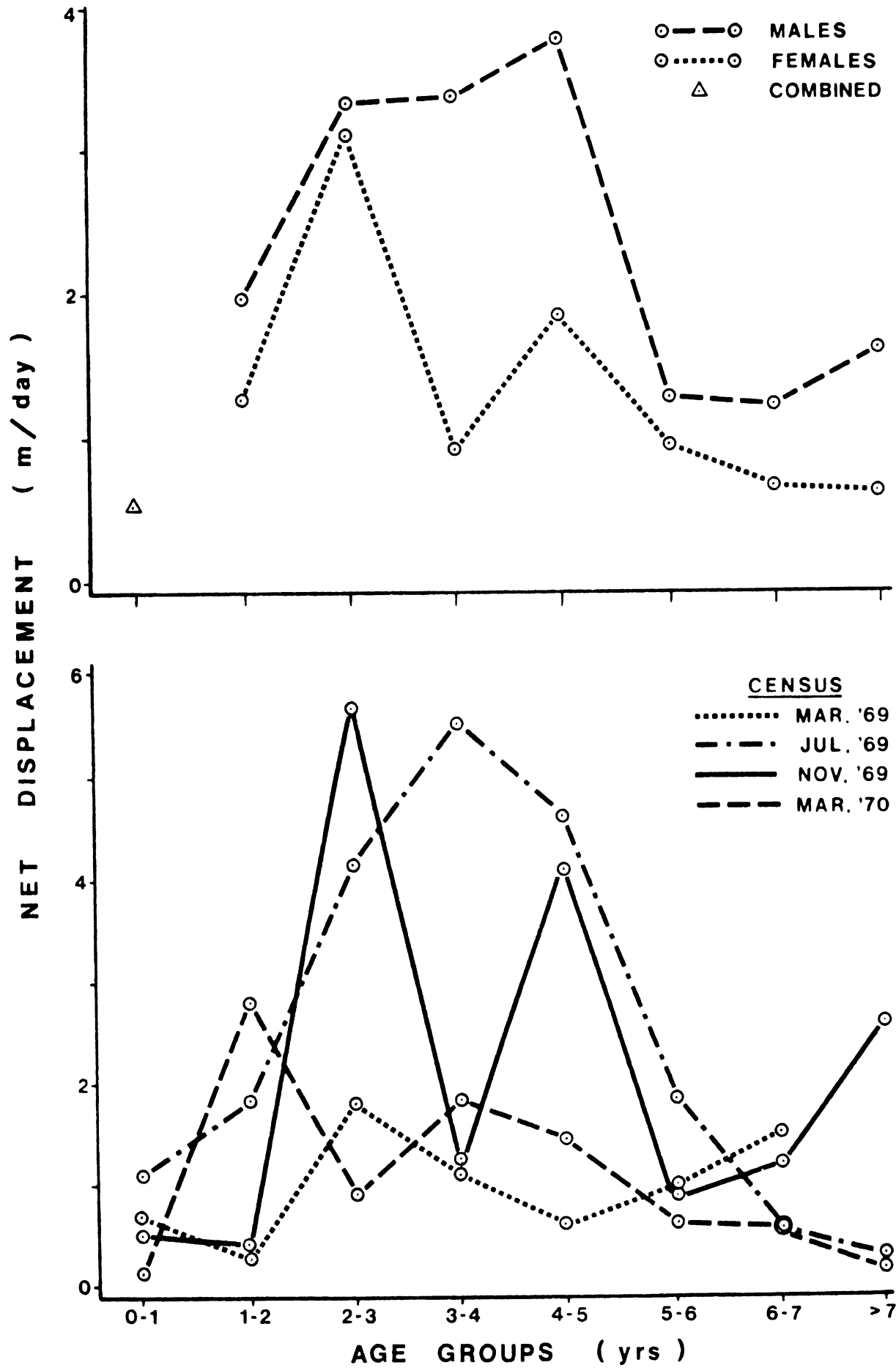


Figure 26

TABLE 14.--LONGITUDINAL MOVEMENTS OF *O. inermis* INDIVIDUALS
Based on net displacement of individual between first and
last sighting during census

CENSUS OR SEX GROUP		STAT OR ANAL *	CARAPACE LENGTH CATEGORIES (mm)								
			5.0- 11.9	12.0- 16.9	17.0- 20.4	20.5- 22.5	22.6 24.4	24.5 25.5	25.6 26.5	over 26.5	ALL LENGTHS
CENSUS	MARCH 1969	$\bar{x}$	0.7	0.3	1.8	1.1	0.6	0.9	1.5	..	1.0
		s	0.6	0.3	3.5	1.5	0.5	0.9	1.9	..	2.0
		n	4	12	19	17	12	8	4	0	76
		% ₁	0	0	36.8	29.4	50.0	50.0	50.0	..	31.6
		% ₂	..	..	71.4	40.0	66.7	75.0	100.0	..	66.7
	JULY 1969	$\bar{x}$	1.0	1.8	4.1	5.5	4.6	1.8	0.6	0.2	3.4
		s	1.4	4.1	10.3	20.0	6.0	1.8	0.6	0.1	10.9
		n	2	26	31	19	6	7	3	2	96
		% ₁	0	15.4	29.0	36.8	50.0	42.9	33.3	0	28.1
		% ₂	..	100.0	55.6	28.6	33.3	33.3	100.0	..	51.9
	NOV. 1969	$\bar{x}$	0.5	0.4	5.6	1.1	4.1	0.9	1.2	2.6	2.9
		s	0.8	0.5	13.6	2.4	4.9	0.7	1.6	2.5	8.1
		n	5	14	26	14	15	6	2	3	85
		% ₁	20.0	7.1	38.5	28.6	60.0	33.3	0	100.0	35.3
		% ₂	100.0	100.0	60.0	75.0	55.6	100.0	..	33.3	63.3
	MARCH 1970	$\bar{x}$	0.1	2.8	0.9	1.8	1.4	0.6	0.5	0.1	1.5
s		0.1	7.9	1.7	3.5	3.1	0.6	0.8	0.2	4.5	
n		2	23	27	17	11	6	3	2	91	
% ₁		0	21.7	40.7	58.8	27.3	50.0	33.3	0	36.3	
% ₂		..	80.0	27.3	40.0	66.7	66.7	100.0	..	48.5	
SEX	MALES	$\bar{x}$	..	2.0	3.3	3.4	3.8	1.3	1.2	1.6	2.9
		s	..	6.6	10.3	13.3	5.2	0.8	1.5	2.6	9.6
		n	..	33	54	44	15	10	6	4	166
		% ₁	..	9.1	31.5	34.1	66.7	60.0	50.0	50.0	33.7
		% ₂	..	100.0	52.9	40.0	60.0	66.7	100.0	0	55.4
	FEMALES	$\bar{x}$	..	1.3	3.1	0.9	1.9	1.0	0.7	0.6	1.8
		s	..	3.3	7.8	0.9	3.4	1.3	1.0	0.6	4.8
		n	..	42	49	23	29	17	6	3	169
		% ₁	..	16.7	40.8	47.8	37.9	35.3	16.7	33.3	33.7
		% ₂	..	85.7	50.0	45.5	54.5	66.7	100.0	100.0	57.9
ALL GROUPS		$\bar{x}$	0.6	1.6	3.2	2.5	2.5	1.1	1.0	1.2	2.3
		s	0.8	5.0	9.1	10.8	4.1	1.2	1.3	1.9	7.4
		n	13	75	103	67	44	27	12	7	348
		% ₁	7.7	13.3	35.9	38.8	47.7	44.4	33.3	42.9	32.8
		% ₂	100.0	90.0	51.4	42.3	57.1	66.7	100.0	33.3	57.0

*STATISTIC OR ANALYSIS

$\bar{x}$ = mean (m/day)

s = standard deviation (m/day)

n = number of individuals observed

%₁ = % moving more than 10 meters (% wandering)

%₂ = % of wandering individuals that were moving downstream

wandering phase and left their home pools. The overall wandering frequency was 32.8%. The wandering fraction was size dependent and peaked at 47.7% in 22.6 - 24.4 mm C L *O. inermis* (Figure 30b). The fraction of the population in a wandering phase did not show much seasonal variation. Likewise, there was no overall sexual difference, although a higher percentage of females wandered in the smaller size categories, whereas in crayfish larger than 22.5 mm C L, males wandered more often.

Of those crayfish moving more than 10 meters, 57% were observed to move downstream. If the home range phenomenon completely controlled all *O. inermis* movement, then the number of crayfish moving upstream should have balanced those moving downstream. Chi-square testing indicated that this slight discrepancy was not significantly different from the lack of populational net displacement expected.

Troglophile:

Positional changes occurring in the *C. laevis* population during the July census showed that movement was extensive and complex (Figure 27). In some individuals movement was less than 10 meters and was apparently confined to a single pool. In other instances, crayfish wandered up to 100 meters and returned to a home pool. Other crayfish underwent a net displacement during the limited observational period of the census. These movements superficially resembled either migration or kinesis, but observation over the course of a year (Figure 28) provided contrary evidence. These movements, in most cases, were probably fragments of home range behavior, in which the home range was extremely large and the completion of a circuit temporally protracted. The sizes of the home ranges varied greatly among individual *C. laevis* (Figure 28).

Figure 27. Movement patterns of all *C. laevis* captured at least three times during the July census. Data from 22 individuals are shown, and the connecting lines in the capture sequences indicate the existence and extent of wandering.

captured at least
Data from 22
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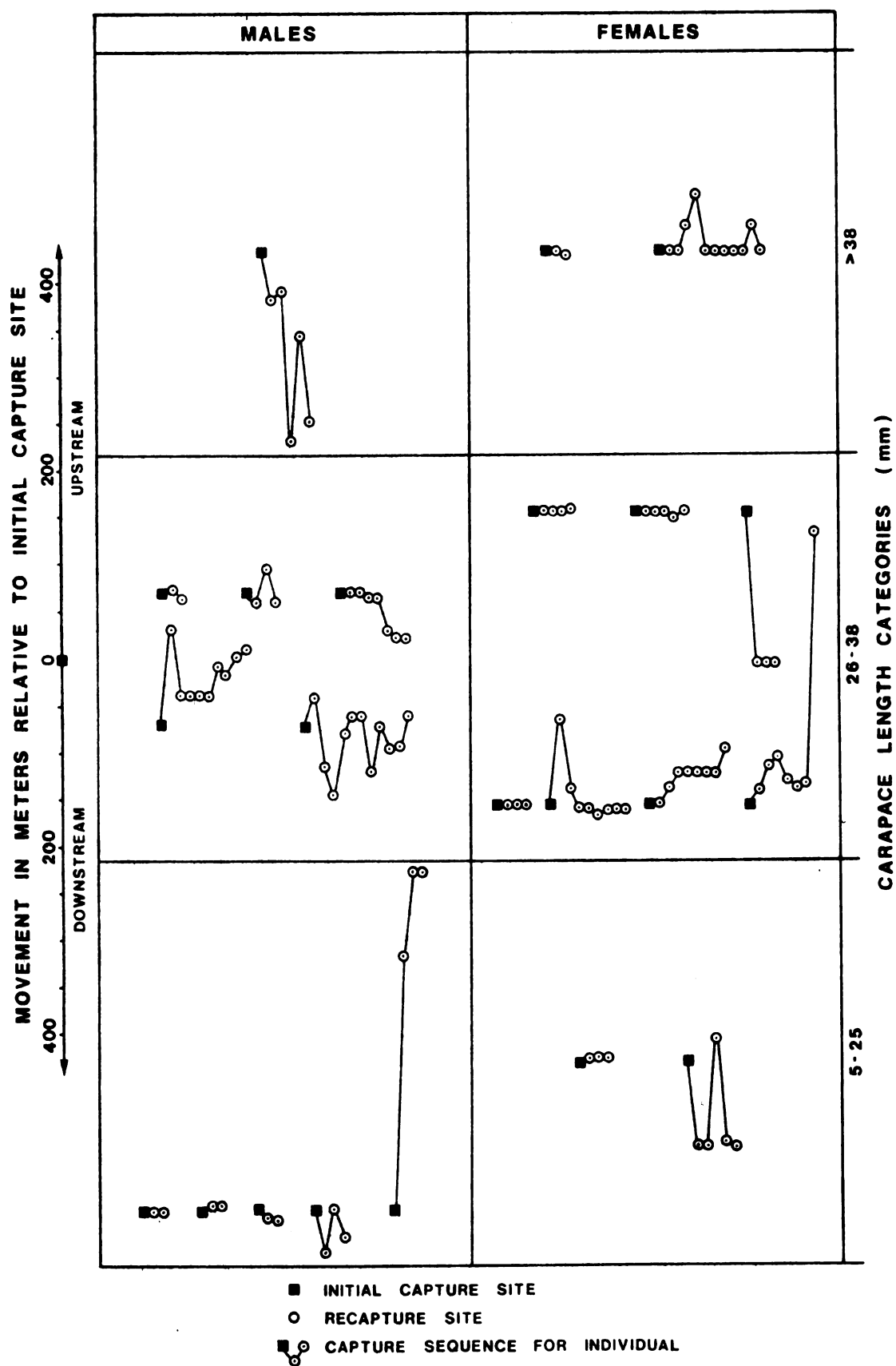


Figure 27

Figure 28. Annual movement of five randomly selected individual *C. laevis* in different sex and size categories.

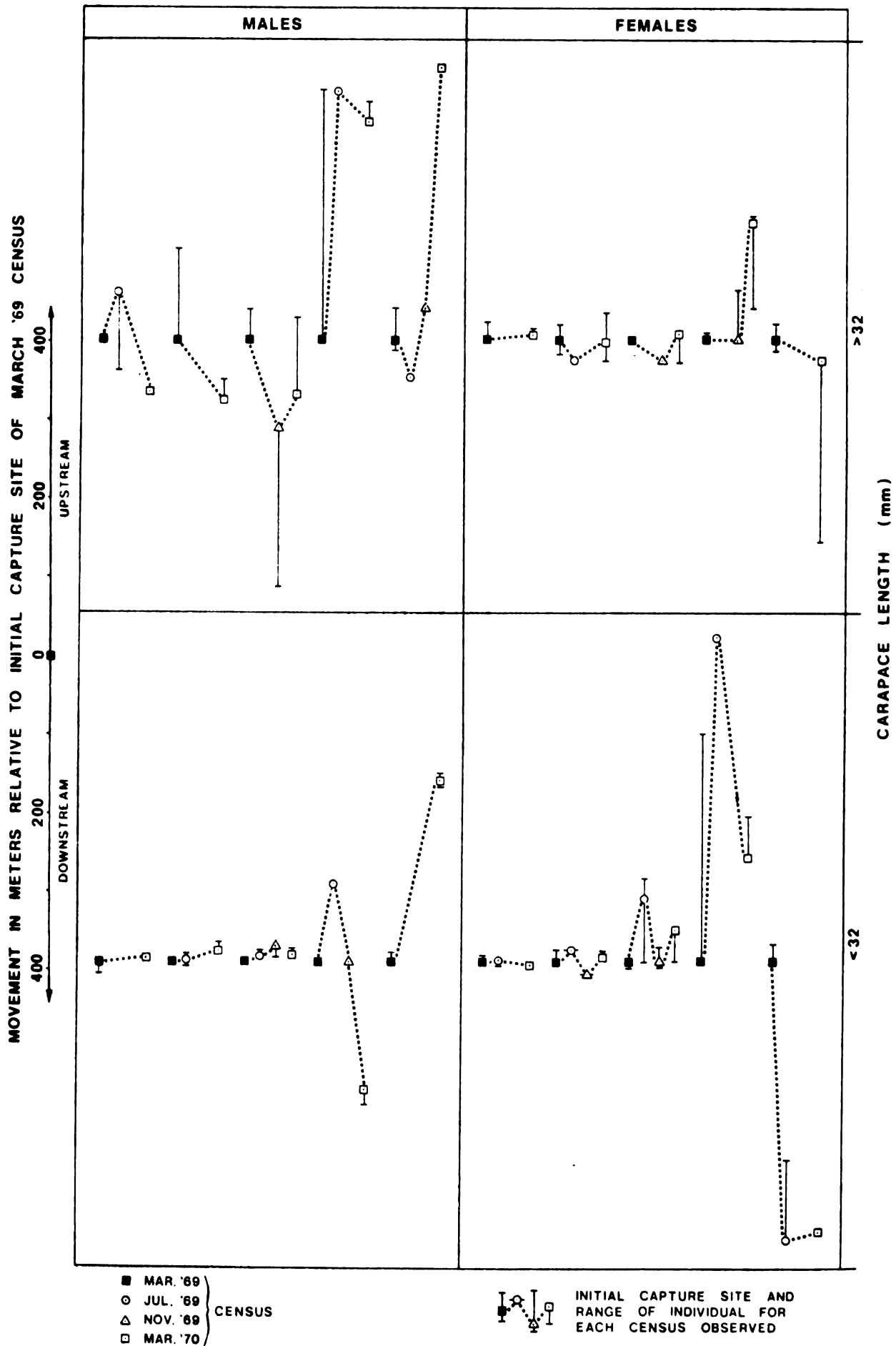


Figure 28

They seemed most extensive in the larger sized males, but the fact that in some cases the range approached the length of the 500 meter census area throws some doubt on their validity as examples of home range behavior. It is possible that in some instances the home range was underestimated, because it was of greater size than the census area. This possibility was supported by the fact that many *C. laevis*, presumably possessing strong locomotive behavior, were encountered in only one census during the year of study. It is also possible that these individuals were undergoing migration or kinesis. Crayfish displaying kinesis behavior in a restricted habitat might have appeared to have a home range, and the extent of available cave stream habitat upstream of the sinkhole entrance is unknown. Home range was certainly operating in the majority of the individuals, but it is uncertain whether it was a universally valid concept for all segments of the population.

Analysis of movement in terms of displacement rate (Table 15) indicated that movement was quite extensive, especially in the middle size ranges from 25.5 to 44.4 mm C L (Figure 30a). Males almost consistently had a greater rate than females (Figure 29a), and there was also seasonal variation with movement depressed during the March censuses (Figure 29b). Movement rates ranged from a low of 0 meters per day for the 5.0 - 12.9 mm C L size category during the March, 1969, census, to a high of 65.1 meters per day for 25.5 - 30.4 mm C L males during July. The mean rate of displacement was 7.6 meters per day.

The percentage of *C. laevis* with a net displacement of more than 10 meters during a census, which in effect measures the magnitude of crayfish in a wandering phase, increased with carapace length, in general, and reached a high of 80.0% in the 38.5 - 41.5 mm C L class (Figure 30b). There was no overall sexual difference, and seasonal variations seemed to be random. The mean wandering frequency was 49.7%.

Figure 29. Daily displacement in *C. laevis*.

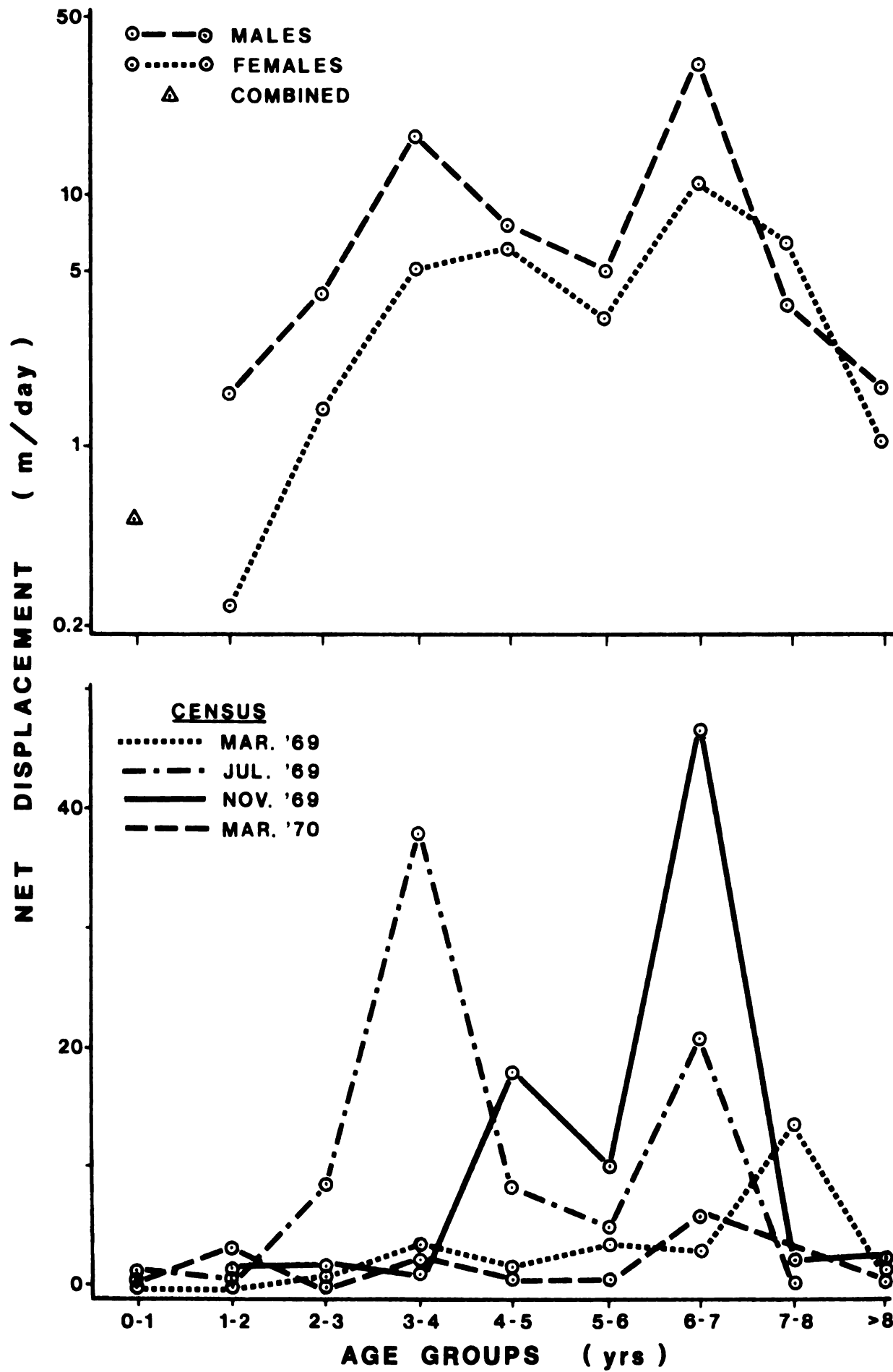


Figure 29

Figure 30. Inter-specific comparison of net displacements occurring during a census.

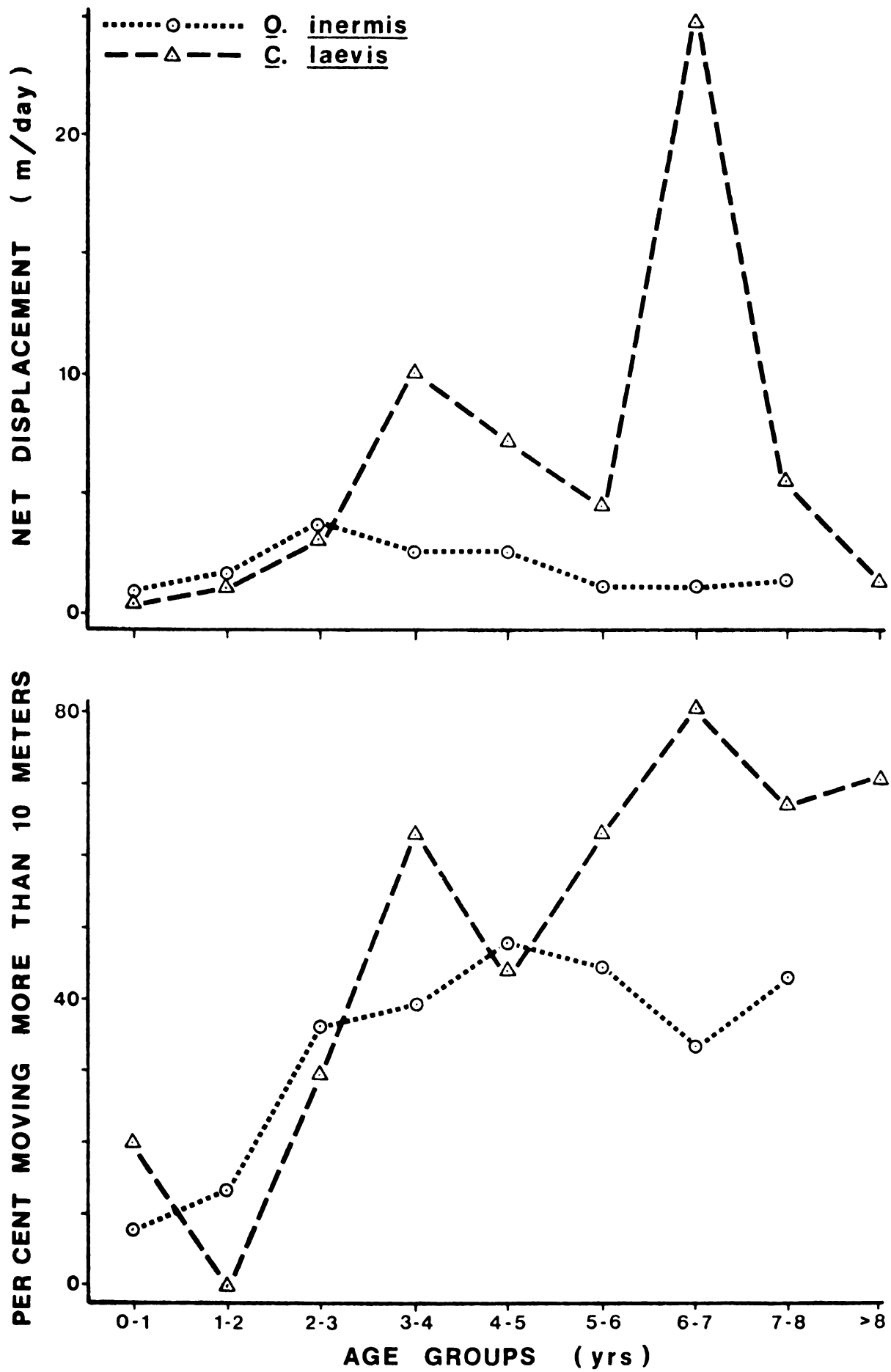


Figure 30

TABLE 15.--LONGITUDINAL MOVEMENTS OF *C. laevis* INDIVIDUALS
Based on net displacement of individual between first and
last sighting during census

CENSUS OR SEX GROUP		STAT OR ANAL *	CARAPACE LENGTH CATEGORIES (mm)									
			5.0- 12.9	13.0- 19.4	19.5 25.4	25.5- 30.4	30.5- 34.5	34.6- 38.4	38.5- 41.5	41.6- 44.4	over 44.4	ALL LENGTHS
CENSUS	MARCH 1969	$\bar{x}$	0	0.1	0.6	3.3	1.2	3.7	3.1	13.7	1.4	2.8
		s	..	0.2	0.5	4.3	2.0	5.2	3.6	18.5	0.3	5.7
		n	1	7	8	12	4	9	4	3	3	51
		% ₁	0	0	37.5	66.7	25.0	77.8	75.0	100.0	100.0	54.9
		% ₂	..	..	33.3	37.5	0	42.9	6.7	0	33.3	35.7
	JULY 1969	$\bar{x}$	0.7	0.5	8.4	37.8	8.2	4.6	20.9	0.4	..	14.2
		s	0.7	0.4	19.7	75.6	16.2	7.0	21.8	0.5	..	39.0
		n	3	3	9	9	6	3	4	3	0	40
		% ₁	33.3	0	44.4	66.7	50.0	33.3	100.0	0	..	47.5
		% ₂	100.0	..	75.0	33.3	0	75.0	..	..	..	52.6
	NOV. 1969	$\bar{x}$	..	1.0	1.2	0.7	18.0	9.8	46.8	2.5	1.7	13.2
		s	..	1.4	1.9	0.6	28.0	4.6	117.5	1.5	1.5	56.2
		n	0	2	6	9	3	2	8	3	3	36
		% ₁	..	0	16.7	44.4	66.7	100.0	75.0	100.0	66.7	55.6
		% ₂	..	..	100.0	75.0	50.0	50.0	50.0	66.7	100.0	65.0
	MARCH 1970	$\bar{x}$	0.3	3.3	0.4	1.2	0.7	0.6	5.8	..	0.6	1.6
		s	..	6.1	0.5	1.3	0.6	0.4	9.6	..	0.7	3.8
		n	1	4	8	10	3	2	4	0	4	36
		% ₁	0	0	12.5	70.0	33.3	0	75.0	..	50.0	38.9
		% ₂	..	..	0	85.7	0	..	66.7	..	100.0	71.4
SEX	MALES	$\bar{x}$	..	1.6	3.9	17.2	7.4	4.9	33.4	3.75	1.7	11.2
		s	..	3.9	13.7	57.7	17.4	5.8	95.9	0.8	..	46.1
		n	..	10	19	16	8	10	12	3	1	79
		% ₁	..	0	31.6	68.8	37.5	60.0	83.3	100.0	100.0	50.6
		% ₂	..	..	50.0	45.5	33.3	33.3	70.0	33.3	0	47.5
	FEMALES	$\bar{x}$	..	0.2	1.5	5.2	6.3	3.2	11.5	6.4	1.1	4.5
		s	..	0.3	2.3	12.6	14.1	4.7	18.0	12.9	1.0	10.9
		n	..	6	12	24	8	6	8	6	9	79
		% ₁	..	0	25.0	58.3	50.0	66.7	75.0	50.0	66.7	50.6
		% ₂	..	..	66.7	64.3	25.0	50.0	50.0	33.3	83.3	57.5
ALL GROUPS		$\bar{x}$	0.5	1.1	3.0	10.0	6.9	4.3	24.7	5.5	1.2	7.6
		s	0.6	3.1	10.8	37.5	15.3	5.3	74.6	11.2	1.0	33.1
		n	5	16	31	40	16	16	20	9	10	163
		% ₁	20.0	0	29.0	62.5	43.8	62.5	80.0	66.7	70.0	49.7
		% ₂	100.0	..	55.6	56.0	28.6	40.0	62.5	33.3	71.4	53.1

*STATISTIC OR ANALYSIS

 $\bar{x}$ = mean (m/day)

s = standard deviation (m/day)

n = number of individuals observed

%₁ = % moving more than 10 meters (% wandering)%₂ = % of wandering individuals that were moving downstream

During the March, 1969, census, 64% of the wandering crayfish were moving upstream. By the July census there was no obvious trend, with 53% moving downstream. Both the November, 1969, and March, 1970, censuses showed downstream displacement, with rates of 65 and 71%, respectively. Males showed a slight net tendency to move upstream (53%), whereas the females generally moved downstream (57%); the mean displacement was 53% downstream. These directional movements were tested by Chi-square, and none were found to be significantly different from random behavior.

There was some evidence for mixing of the epigean and hypogean *C. laevis* populations. A 39.5 mm C L female that had been last observed in the cave at 34 meters was captured in the surface stream 14 months later. However, eighteen epigean crayfish that were tagged never appeared in cave surveys.

Effect at population level

Some additional light was shed on movement by analyzing the gradual decrease of marked crayfish in the census area populations. This was accomplished by monitoring for a year the percentages of crayfish in the various age classes that were first captured and coded during the first census. The initial ratio was determined by applying the Schumacher mark-recapture procedure to the first census data; the resulting estimated population was divided into the number of individuals marked. The final ratio was derived directly from the percentage of first census-marked crayfish in the crayfish captured during the fourth census, which was conducted a year later. These procedures were performed independently for different carapace length cohorts, which were followed throughout the year by application of measured growth rates.

Although crayfish so marked underwent an absolute decrease during the year, this resulted not only from migration, but also from mortality. On the other hand, the annual decrease of marked crayfish relative to the total number comprising a year class cohort resulted only from migration. There was no evidence that differential mortality of marked individuals or loss of marks were factors.

The decrease in the ratio resulted both from emigration of marked individuals and from immigration of unmarked individuals. The percentage decrease in the ratio was a measure of migration across the census area boundaries. It was, in fact, equal to both emigration and immigration, if these two rates were equal. If there was net immigration, then the percentage decrease would be greater than the actual emigration rate of marked crayfish; if, on the other hand, there was net emigration, then the percentage decrease would be less than the percentage of marked crayfish leaving the census area.

In *O. inermis*, crayfish of small (5.0 - 16.9 mm) and medium (17.0 - 24.4 mm) carapace lengths had similar annual migration rates of 50.4% and 51.5%, respectively. Larger crayfish had a lower rate of 37.5%.

Migration rates were much greater in *C. laevis*. Crayfish with a carapace length of 25.5 mm and larger migrated at a rate of 68.4%. Smaller crayfish migrated at the even higher rate of 83.1%.

Regression analysis (Figure 31) indicated that, for both species, the migration rate, as measured by the percentage decrease in the ratio of marked crayfish in the population, was linearly related to the log of the displacement rate. This relationship, however, resulted in a migration rate that was less than doubled when the displacement rate was increased ten-fold. This fact reflected the home range behavior observed in the movement study of individual crayfish.

Figure 31. Relationship between longitudinal movement rates and the annual loss of marked crayfish from the census area population. Loss based on the percentage decrease in cohorts of 1st survey-marked individuals in the population inhabiting the census area a year later.

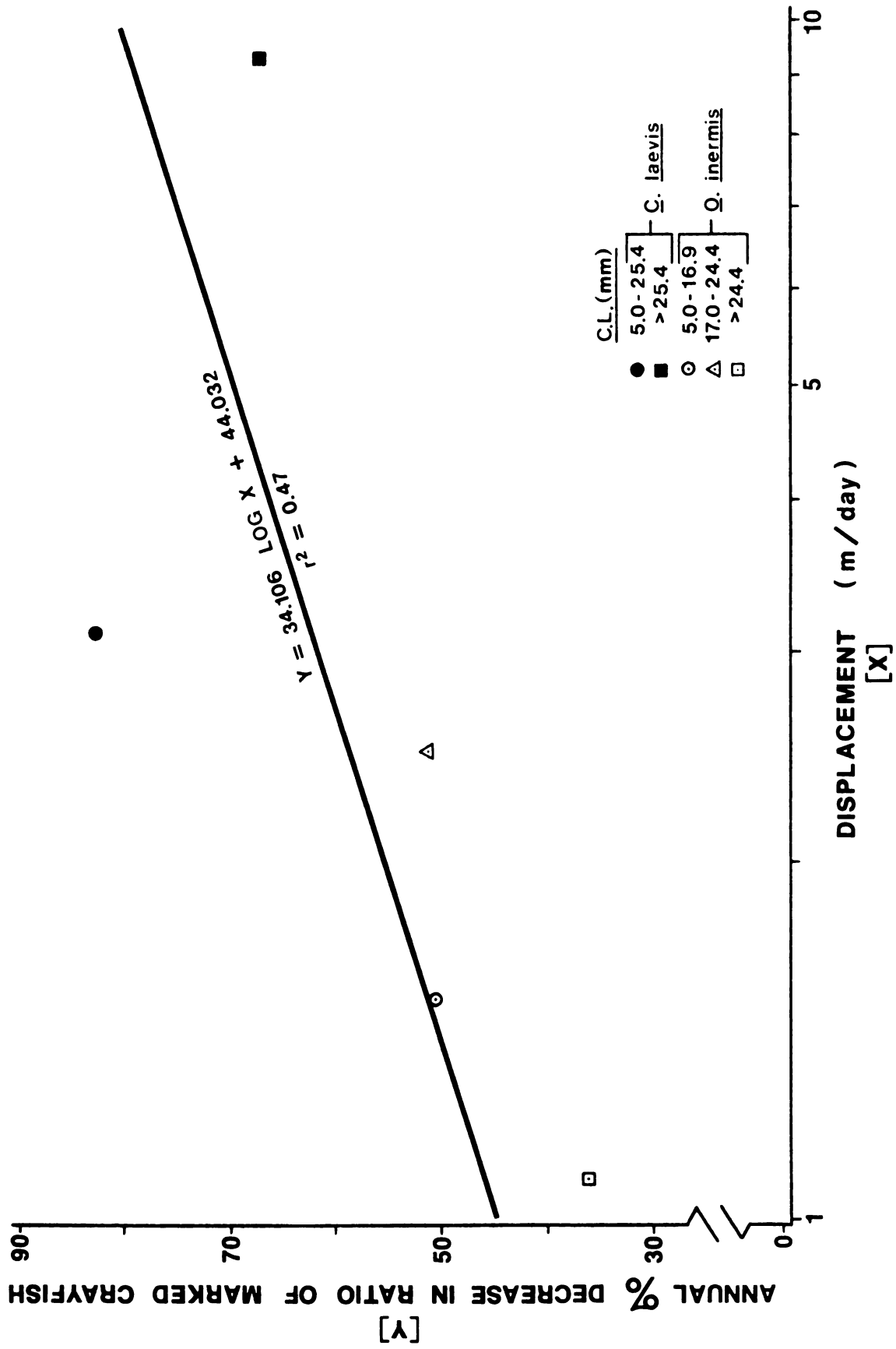


Figure 31

The migration rates represent an approximation of marked crayfish emigration from the census area. The two values would be equivalent only if there was a lack of net population change across the boundaries. Whether or not emigration was balanced by immigration can best be judged from census data on temporal changes in the size of the crayfish populations (see section on population size and structure below). Population sizes and structures were, in general, temporally stable. Consequently, in most cases migration between the census area and other reaches of the stream probably consisted most often of equivalent two-way flow rates between areas of similar population densities.

A notable exception was an apparent net immigration of smaller-sized *C. laevis*. This conclusion, based on population changes that are discussed in more detail below, gained a measure of support from information derived from displacement and migration rates. The population group in question consisted of *C. laevis* with carapace lengths ranging from 5.0 to 25.4 mm. This group had the highest observed migration rate. Figure 31 illustrates that, unlike the other population groups, its apparent migration rate was greater than the regression line. Thus, migration, which was based on loss of marked crayfish, seemed to be higher than would have been expected from the displacement rate. The actual rate of migration for this group may have been less than that indicated by the measuring procedure employed. This possibility was due to the over-estimation of the migration rate that would have resulted if immigration was greater than emigration. Accordingly, the net immigration for this group, that was suggested by the Schumacher-estimated population data, would have been expected to result in the situation illustrated in Figure 31. Likewise, Schumacher-estimated data indicated that emigration of larger *C. laevis* may have occurred,

and this conclusion was consistent with the position of this group below the regression line of Figure 31.

Discussion

Diel capture rates indicated that a circadian rhythm of activity may have existed at the population level, but, if so, only to a slight extent. The peak of activity at 3:00 P.M. may have resulted from an exogenous cue, such as entrainment by light in the twilight zone. On the other hand, differences in the pattern of investigative interference preceding the three time periods may have accounted for the differences in capture rates. Emphasis should rather be focused on the nearly equivalent activity in different segments of the diurnal cycle. Endogenous rhythms of epigeal crayfish are cued by Zeitgebers that generally produce a nocturnal activity pattern; this probably functions to reduce predation. In hypogean crayfish an endogenous rhythm probably lacks entraining Zeitgebers, and this would result in a free-running activity pattern not strictly phased to the diurnal cycle. Although the individual would retain a circadian activity pattern, there would not be a synchronized pattern at the population level. This behavior pattern would have the functional advantage of temporal partitioning of space and food resources. Circadian rhythms at the population level would have no apparent function in the cave stream habitat, unless inter-specific crayfish predation is a significant ecological interaction.

The fundamental pattern of longitudinal movement for both species supported the operation of the home range phenomenon, although it was of a peculiar character. These crayfish resided in a home pool, usually of about 10 meters in extent, in which movement was restricted for extended periods of time; this may be regarded as the primary home range.

The crayfish periodically wandered from the home pool and habitually traveled a secondary home range. The extent of this secondary home range showed both inter-specific, and intra-specific variations. Intra-specifically, the home range was larger in males and young adults in both species, and inter-specifically, *C. laevis* possessed much larger home ranges than *O. inermis*. Both species also exhibited seasonal variations, with a decreased rate of travel during the March censuses.

Although several dams and waterfalls occur in the cave, they apparently are not significant barriers to crayfish movement. *C. laevis* individuals were frequently observed climbing the cave wall to by-pass the weir, and several *O. inermis* were found that had fallen into cavities while traversing the top of the weir. Similar cases of cave crayfish traveling overland were reported by Cooper (1975) and Hobbs (1973). The high humidity of the cave atmosphere is obviously conducive to this type of behavior.

The proportion of time spent wandering the secondary home range varied with size in both species, being much lower in the younger crayfish; no sexual differences were observed. There was a large difference in wandering frequency between the two species, with a mean value of 33% for *O. inermis* and 50% for *C. laevis*. The greater wandering rate of *C. laevis* was observationally obvious. Most *O. inermis* inhabited pools, and only a very few were found moving through riffle areas. *C. laevis* crayfish, on the other hand, were frequently encountered moving through riffles, although they also most often occurred in pools. Trophic studies (see section on diet analysis below) have shown that *C. laevis* had a higher percentage of animals in its diet than did *O. inermis*, and *C. laevis*, therefore, would be expected to be more frequently encountered in the riffles, which supported a richer

benthos than did the pools. In any event, wandering was so prevalent in *C. laevis* that the concept of a home pool or primary home range was not as well established as in *O. inermis*. Nevertheless, the fundamental pattern for both species was a home pool from which the crayfish made periodic forays, the extent of which was partially sex and size dependent.

Other studies on the occurrence of home range in crayfish have produced varying results. The pond-inhabiting *Orconectes virilis* was studied by Camougis and Hichar (1959), who concluded that it did not display a home range. Likewise, Mobberly and Pfrimmer (1967) reported a lack of home range in a population of ditch-dwelling *Orconectes clypeatus*. In both studies, however, the investigators were testing for the presence of a highly confined home range, with 40 and 15 meter limits, respectively. In addition, both of these studied habitats lacked a complex structure, such as the riffle-pool sequence characteristic of the cave stream. In contrast, Black (1963) studied two stream-inhabiting species of *Procambarus* and concluded that they exhibited a home range with limits less than 30 meters. A mean home range of 21.5 meters has been reported by Hobbs (1973) for *O. inermis* of Pless Cave.

Although the home range concept adequately described the behavior of the majority of the crayfish, there was also good reason to believe that other locomotive phenomena may have been operating, especially in *C. laevis*. The apparently huge home ranges of certain individuals may actually have been kinesis behavior confined to the limited cave habitat. Uncertainty exists because of a lack of knowledge of crayfish movement beyond the census area. The gradual loss of marked crayfish from the census area indicated that a degree of migration was operating in both species, especially *C. laevis*. Apparently, home range behavior patterns

were applicable to individuals on a short-term basis, but the home ranges were not spatially immutable throughout their life spans.

Hobbs (1973) has made similar observations of *O. inermis* in nearby Pless Cave.

Some short-term movements of cave-inhabiting *C. laevis*, at least at the level of the individual, appeared to be unidirectional and directed. Several were observed to travel more than 100 meters per day, with the greatest being 337 meters per day. Of those *C. laevis* moving more than 10 meters during a census, the average displacement was 11.9 meters upstream. The majority of these same crayfish, however, were moving in a downstream direction, so it was apparent that no simple migratory mechanism was operating on the population as a unit. Migration at the population level has been noted in other crayfish. Semiannual migration of *Astacus klamathensis* has been observed between a river and a creek (Henry, 1951). An upstream migration of *Orconectes nais* was regarded by Momot (1966) to be a means of stream repopulation.

Migration between epigean and hypogean habitats has also been shown. The only direct evidence was the recovery in the surface stream of a single *C. laevis* individual that had been previously observed in the cave. However, indirect evidence, derived from the peculiar population structure of the cave population (see Figure 47 below), indicated that certain members of the surface population were migrating into the cave. The nocturnal nature of most crayfish preadapts them to the cave habitat. Chidester (1908) observed *Cambarus bartonii* to be negatively phototactic to strong light; this behavior might influence them to enter caves, and negative photokinesis might lead to their incorporation into the cave population. Hobbs and Barr (1960) pointed out that cambarids characteristically explore the sources of springs,

and Weingartner (1962) observed *Cambarus laevis* moving into a spring of a nearby cave. Other factors might also have been operating to initiate movement into the cave. The density of the epigean population was quite high; it was much greater than the cavernicolous population of *C. laevis* (see section on population size and structure below). Bovbjerg (1964) stated that, as a result of intra-specific aggression, members of a population of *Procambarus alleni* disperse at rates directly related to density. It was also reported that the larger and older individuals of this species are more dominant (Bovbjerg, 1956). This behavior, if applicable to *C. laevis*, might explain the peculiar structure of the cave population, which was inflated by those size categories corresponding to young-of-the-year and year-old epigean *C. laevis*.

Flooding has been suggested as an important agent in controlling the movement of crayfish. Wickliff (1940) reported that crayfish rapidly repopulate riffles denuded by floods. In the present study, although floods may have displaced riffle-inhabiting crayfish, they probably had a negligible long-distance displacement effect; no mass downstream displacement associated with flooding was observed. Black (1963) stated that floods did not appear to disperse crayfish.

The functional aspects of crayfish movement within the cave are uncertain. Movement might have been related to sexual activity. Movement was quite extensive in both the July and November censuses, and, based on the fact that form I males of both species were proportionally most abundant during the November census (see section on population size and structure below), mating apparently occurred during the fall. In addition, the greatest movement occurred in young adult crayfish, although there was no abrupt change in locomotive behavior with attainment of sexual activity. It can probably be more logically assumed

that movement was primarily a part of feeding behavior. There was a low rate of movement for both species during the March censuses. At this time of year food was probably quite plentiful, with relatively large quantities of detritus and riffle-inhabiting benthos having been washed into the pools by the frequent winter floods. Evidence for increased drift of benthos and increased allochthonous input during this period of the year was gained from drift net samples (see page 70 above). After flooding subsided in late spring, this reservoir of food in the pools was gradually depleted, and crayfish probably began moving into adjacent riffle areas in search of food. This increased activity was evident in the July and November censuses, which were conducted during, and preceded by, periods of stable, low stream flow. The greater movement and more frequent wandering into riffle areas that characterized *C. laevis* were also probably trophic responses. As discussed in the following section, this crayfish had a higher ingestion rate and a higher proportion of riffle-inhabiting benthos in its diet than did *O. inermis*.

Trophic Ecology

Results

Predator-prey interactions

The various relationships among predator (either *O. inermis* or *C. laevis*), prey (either isopods or amphipods) and habitat (either bare or rocky substrate) were examined. Factorial analysis (Table 16) indicated that only the main effect involving prey was significant ($P < .005$). Isopods were preyed upon at a higher rate than amphipods by both predators and in both types of substrate.

TABLE 16.--RESULTS AND FACTORIAL ANALYSIS OF PREDATOR-PREY INTERACTION STUDY

SUBSTRATE		BARE				ROCKY			
PREDATOR		<i>O. inermis</i>				<i>C. laevis</i>			
PREY		ISOPODS		AMPHIPODS		ISOPODS		AMPHIPODS	
PREY EATEN	$\bar{x}$	2.67	1.75	2.67	2.08	2.83	2.00	3.00	2.25
	s	.65	.87	.49	.90	.39	.95	.0	.75
n		12	12	12	12	12	12	12	12

SOURCE	MAIN EFFECTS			INTERACTIONS			ERROR
	SUBSTRATE (A)	PREY (B)	PREDATOR (C)	AB	AC	BC	
d f	1	1	1	1	1	1	77
M S	1.50	13.50	.67	.04	.04	.37	.49
F	3.06	27.55	1.37	.08	.08	.76	
P	.10 > P > .05	.005 > P	P > .10	P > .10	P > .10	P > .10	
SIGNIFICANCE	ns	HS	ns	ns	ns	ns	
ns=not sign.							
HS=highly sign.							

Prey were consistently captured at a higher rate in the rocky substrate, but the difference was not significant ($.10 > P > .05$). The facts, that the difference was near the level of significance and that all four predator-prey interaction sample means were higher under conditions of rocky substrate, indicated the possibility that a more extensive testing program may have shown a significant difference. If there was a substrate effect, it could have been due either to increased artificiality of the bare substrate, which might have diverted the predators from food-search to escape behavior, or to non-random distribution of prey by aggregation in the rocks, which might have made prey capture more efficient. This latter factor might also explain the greater susceptibility of isopods, which display a greater degree of thigmotactic behavior than amphipods. The *C. laevis* crayfish apparently engaged in a more vigorous search activity, as measured by the movement of rocks; *C. laevis* noticeably disturbed the rocks in 83% of the trials, as opposed to 3% for *O. inermis*.

Before testing, the troglobite, *O. inermis*, was assumed to be more efficient in prey capture than the troglophile, *C. laevis*, under cave conditions of darkness and low prey density. The data, however, did not indicate any significant difference between the two species, and, in fact, the predation rate sample means were slightly higher for *C. laevis*. It is possible that the test may have actually measured satiation, and *O. inermis*, with its lower energy requirements, would have been more susceptible to this confounding factor. This seems unlikely, however, because the number of prey presented to *O. inermis* and *C. laevis* was only 27% and 9% of their respective daily ingestion in the field (see following section on diet analysis).

Diet analysis

Fecal material collected from *C. laevis* in the field was found to be correlated to body weight by the regression,

$$\log \text{ mg dry feces/day} = 0.3041 \log \text{ g dry crayfish} + 1.4677.$$

The slope of this regression was used to adjust the egestion rates of both crayfish species, both in the laboratory and in the field, to the value associated with a crayfish weighing 1 dry gram. It was not possible to compute a similar regression for *O. inermis*, because there was an insufficient range in their carapace lengths. The individuals employed in this study, especially the *O. inermis* crayfish, were selected for body weights of approximately 1 dry gram, and adjustments were, therefore, relatively minor.

Troglobite

Laboratory-maintained crayfish ate all three kinds of homogeneous diets, with the animal diet ingested at the highest rate and mud at the lowest (Table 17). The assimilated fraction of the animal diet was quite high, whereas the fraction of plant detritus assimilated was low. The energy content of the feces was in all cases less than that of the ingested material; consequently, the efficiency of energy assimilation was greater than assimilation based on weight. The energy assimilation efficiency was 97.4% for a diet of animals and 51.4% for a diet of plant detritus.

Crayfish supplied with a complete diet, including mud, plant and animal components, ingested 8.1 mg dry food/day/g dry crayfish, with a ratio of 12.6% mud, 28.6% plant detritus and 58.8% animals. The fecal

TABLE 17.--FEEDING RATES, ASSIMILATION EFFICIENCIES, AND FECAL CONTENT OF HOMOGENEOUS LABORATORY DIETS

DIET	SPECIES	STATISTIC	dry mg/day/g dry crayfish		% ASSIM (weight)	FECAL CONSTITUENTS			
			INGESTION RATE	EGESTION RATE		% ASH	% CELLULOSE	% CRUDE PROTEIN	ENERGY CONTENT (cal/mg)
MUD	<i>O. Inermis</i>	$\bar{x}$	..	0.50	..	86.4	3.2	2.6	0.25
		s	..	0.28	..	3.1	1.2	0.7	0.01
		n	..	4	..	4	4	4	4
	<i>C. Laevis</i>	$\bar{x}$	..	0.42	..	78.2	1.7	2.9	0.12
		s	..	0.21	..	6.6	0.7	0.2	0.04
		n	..	7	..	7	7	7	7
ANIMAL (amphipod)	<i>O. Inermis</i>	$\bar{x}$	14.7	2.3	85.0	79.0	2.4	6.7	0.64
		s	3.8	1.0	3.5	0.6	0.3	0.3	0.03
		n	6	6	8	6	6	6	6
	<i>C. Laevis</i>	$\bar{x}$	34.5	5.4	85.9	72.5	6.3	7.3	1.13
		s	11.7	2.0	1.0	6.6	2.5	0.2	0.13
		n	4	4	4	4	4	4	4
MAPLE	<i>O. Inermis</i>	$\bar{x}$	1.6	1.3	16.4	33.5	8.5	3.8	2.14
		s	0.8	0.7	9.4	0.7	0.1	0.3	0.06
		n	3	3	3	3	3	3	3
	<i>C. Laevis</i>	$\bar{x}$	8.2	6.8	12.8	26.0	10.8	2.9	2.50
		s	7.8	6.1	8.7	1.0	1.6	0.4	0.02
		n	3	3	3	3	3	3	3
OAK	<i>O. Inermis</i>	$\bar{x}$	2.8	2.6	5.7	29.4	8.7	3.4	2.65
		s	1.6	1.4	2.1	7.6	0.3	0.1	0.24
		n	3	3	3	3	3	3	3
	<i>C. Laevis</i>	$\bar{x}$	3.9	3.5	8.2	19.7	10.3	3.4	2.97
		s	1.6	1.3	5.2	1.6	0.6	0	0.06
		n	3	3	3	3	3	3	3
BEECH	<i>O. Inermis</i>	$\bar{x}$	1.5	1.5	3.3	32.0	10.4	2.6	2.33
		s	0.2	0.2	0.6	6.9	0.8	0	0.17
		n	3	3	3	3	3	3	3
	<i>C. Laevis</i>	$\bar{x}$	1.1	1.0	9.0	21.0	10.1	3.0	2.68
		s	0.1	0.2	6.7	0.2	0.2	0.1	0.06
		n	3	3	3	3	3	3	3
MEAN FOR LEAVES	<i>O. Inermis</i>	$\bar{x}$	1.9	1.7	9.6	31.6	9.2	3.3	2.37
		s	1.0	0.9	8.5	4.9	0.9	0.6	0.19
		n	9	9	9	9	9	9	9
	<i>C. Laevis</i>	$\bar{x}$	4.4	3.8	10.0	22.3	10.4	3.1	2.71
		s	4.8	3.8	5.8	3.1	0.8	0.3	0.15
		n	9	9	9	9	9	9	9

matter from these crayfish was chemically analyzed, and this information, in conjunction with results from the homogeneous diets (Table 17), provided estimates of ingestion which could be compared to known values; this procedure is illustrated in Table 18. The estimate of the ingestion rate was slightly lower than the actual value, with an error of 11.0%. The estimate of the component ratio was slightly high for mud and animal fractions (3.4 and 4.0 percentage points, respectively) and low for the plant fraction (7.5 percentage points).

This technique, when applied to fecal matter derived from crayfish in the field, gave an estimated ingestion rate of 41.0 mg dry food/day/g dry crayfish — a value which was 5.8 times greater than the estimated ingestion rate of crayfish maintained in the laboratory. The component ratio of the field diet was estimated to be 7.5% mud, 25.5% plant detritus and 67.0% animals; these values were similar to those of the laboratory diet, although the animal and plant fractions were slightly greater, and the mud fraction was less. Field diet data is shown in Table 19 and Figure 32.

Troglophile

Crayfish fed in the laboratory on homogeneous diets ingested animals at the highest rate and mud at the lowest (Table 17). The energy assimilation efficiency for ingested animals was 95.7%, whereas the efficiency was only 44.7% for plant detritus.

The ingestion rate of *C. laevis* crayfish fed on a complete laboratory diet was 16.4 mg dry food/day/g dry crayfish; this diet consisted of 15.4% mud, 18.7% plant detritus and 65.9% animals. Diet reconstruction from analysis of egested matter gave a 7.3% error in ingestion rate, with a reduced value of 15.2 mg dry food/day/g dry

1

TABLE 18.--INGESTION DATA ON COMPLETE (ANIMAL, PLANT AND MUD) LABORATORY DIETS, WITH ILLUSTRATION OF TECHNIQUE FOR ESTIMATING INGESTION RATES AND COMPONENTS FROM EGESTION RATES AND CONSTITUENTS; $\bar{x}$, s and n are tabulated in order, and rates are expressed as dry mg/day/g dry crayfish

SPECIES	COMPLETE LABORATORY DIET			
	RATE	INGESTED COMPONENTS, ACTUAL AND (ESTIMATED)		
		% MUD	% PLANT	% ANIMAL
<i>O. inermis</i>	8.1(7.2)	12.6(16.1)	28.6(21.1)	58.8(62.8)
	1.2	10.2	6.1	6.1
	4	4	4	4
<i>C. laevis</i>	16.4(15.2)	15.4(16.0)	18.7(12.5)	65.9(71.5)
	4.1	2.7	3.9	3.2
	6	6	6	6

1. Digestive transformation during study.

2. Computation of simultaneous linear equations with 3 unknowns, using data from Table 17.

3 & 4. Computation of ingested components from assimilation data extracted from Table 17.

WEIGHT ASSIMILATION EFFICIENCY OF HOMOGENEOUS DIETS		
MUD	PLANTS	ANIMALS
1.0*	9.6	85.0
	8.5	3.5
	9	8
1.0*	10.0	85.9
	5.8	1.0
	9	4

DIETARY SOURCE OF FECE (%)			
RATE	MUD	PLANT	ANIMAL
3.2	36.0	42.8	21.2
0.4	6.7	2.6	6.3
4	9	9	9
5.7	42.7	30.2	27.1
1.7	18.1	9.7	25.4
6	7	7	7

FECAL ANALYSIS OF COMPLETE LABORATORY DIET						
WEIGHT ASSIM. EFFICIENCY	RATE	EGESTED CONSTITUENTS				ENERGY CONTENT (cal/mg)
		% ASH	% CELLULOSE	% CRUDE PROTEIN		
58.1	3.2	62.4	5.5	3.6	1.27	
2.3	0.4	3.7	0.1	0.2	0.04	
4	4	4	4	4	4	
65.8	5.7	64.3	5.8	3.7	1.31	
2.0	1.7	6.0	0.3	0.5	0.10	
6	6	6	6	6	6	

O. inermis

C. laevis

1. Digestive transformation during study.
 2. Computation of simultaneous linear equations with 3 unknowns, using data from Table 17.
 3 & 4. Computation of ingested components from assimilation data extracted from Table 17.

TABLE 19.--INGESTION RATES AND COMPONENTS COMPUTED FROM FECES COLLECTED IN THE FIELD

CRAYFISH POPULATION	STATISTIC	EGESTION RATES AND CONSTITUENTS						COMPUTED DIETARY SOURCE OF FECES			COMPUTED INGESTION RATES AND COMPONENTS			
		RATE*	% ASH	% CELLULOSE	% CRUDE PROTEIN	ENERGY CONTENT (cal/mg)		% ANIMAL	% PLANT	% MUD	RATE*	% ANIMAL	% PLANT	% MUD
<i>O. inermis</i>	$\bar{x}$	16.88	50.54	7.85	3.88	1.05		24.8	56.9	18.3	41.6	67.0	25.5	7.5
	<i>s</i>	9.35	17.27	2.07	1.02	0.58		12.2	19.7	13.9				
	<i>n</i>	20	5	5	5	5		5	5	5				
CAVE <i>C. laevis</i>	$\bar{x}$	32.03	52.04	8.62	4.94	1.90		39.4	50.7	9.9	110.7	80.8	16.3	2.9
	<i>s</i>	15.81	21.70	2.09	1.64	0.39		5.3	5.2	6.8				
	<i>n</i>	20	5	5	5	5		5	5	5				
SURFACE <i>C. laevis</i>	$\bar{x}$	33.73	64.00	4.91	5.25	1.09		50.5	16.0	33.5	138.2	87.4	4.3	8.3
	<i>s</i>	15.12	9.86	1.35	1.89	0.11		12.6	6.0	8.9				
	<i>n</i>	20	5	5	5	5		7	7	7				

*Dry mg/day/g dry crayfish

Figure 32. Comparison of actual laboratory diet with laboratory and field diets estimated by the fecal analysis method.

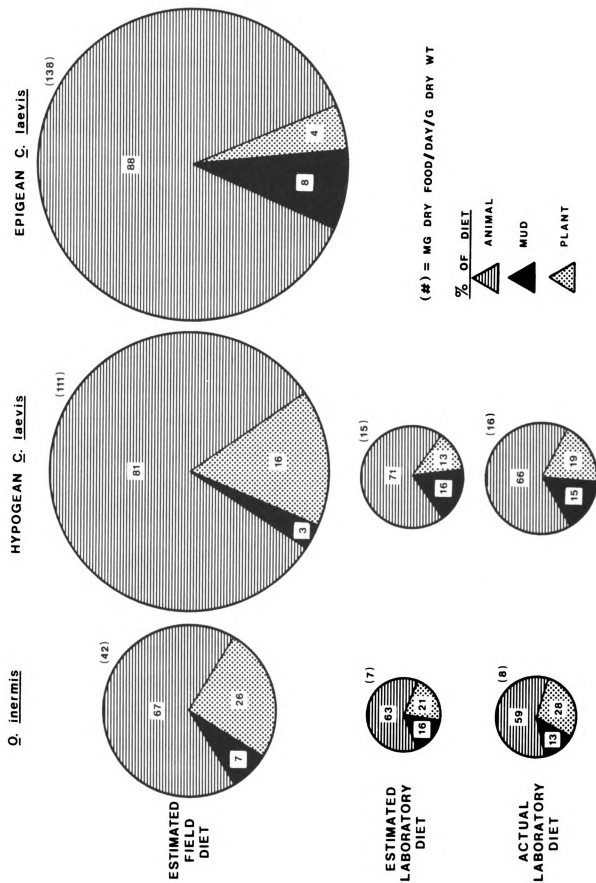


Figure 32

crayfish; the estimate of the proportion of dietary components was 6.2 percentage points low for plant material, and 0.6 and 5.6 percentage points high for the mud and animal fractions, respectively (Table 18).

The field diet of hypogean crayfish, as estimated from fecal analysis, was 110.7 mg dry food/day/g dry crayfish; mud, plant and animal components composed 2.9, 16.3 and 80.8% of the diet, respectively. The ingestion of surface crayfish was greater, with a rate of 138.2 mg dry food/day/g dry crayfish; mud, plant and animal components comprised 8.3, 4.3 and 87.4% of the diet, respectively. The ingestion rates of the field populations were much greater than laboratory maintained individuals — 6.7 times greater for the cave population and 8.4 times greater for the crayfish inhabiting the surface stream. The proportion of animals in the diet was lowest in the laboratory group and highest in the surface population; the consumption of plant material was considerably less in the surface population, and the percentage of mud in the diet varied from a low in the cave population to a high in the laboratory group (Table 19 and Figure 32).

Discussion

Trophic studies based on fecal analysis proved to be a valid method. When compared with known ingestion rates and components, this technique only slightly underestimated the rate and plant component. One of the most interesting findings of the trophic study was the large difference in ingestion rates between field and laboratory-maintained crayfish. The estimated high ingestion rates of the field populations might be suspect, since they are based on assimilation rates determined in the laboratory and extrapolated to the field. In order to check this possibility, the assimilation fractions of both the field and

laboratory crayfish were determined by a method described by Conover (1966), and it was found that the field assimilation efficiency was only 4 percentage points lower than that in the laboratory. It can be concluded that the technique developed in this paper for determining dietary components and ingestion rate was valid in the present case, but care should be exercised in extending its use to other species. Hubbell, Sikora and Paris (1965), for example, found that a terrestrial isopod behaved quite differently; isopods in the laboratory ingested food at a much higher rate and had a much lower assimilation fraction than isopods in the field.

It was determined that the epigeal population of *C. laevis*, although omnivorous, subsisted almost exclusively on an animal diet. This contrasts with the conclusions of other authors that crayfish are primarily herbivores; Tack (1941) found that animals made up only 4.5% of the diet in *Orconectes immunis*, and Momot (1967) observed *Orconectes virilis* to be primarily a herbivore and at times a facultative scavenger. However, both authors used the stomach analysis method, which, because of differential assimilation, underestimates the ingestion rate of animals. In contrast to the epigeal population, those *C. laevis* crayfish inhabiting the cave stream were exposed to a low density of prey, and plant detritus made up a larger portion of the diet. This shift in dependence to a lower trophic level was a more efficient use of available energy. Despite this dietary modification, the ingestion rate was depressed in relation to the value of the surface population. The troglobitic crayfish, *O. inermis*, extended this trend towards reduced consumption and more efficient use of energy, with a low ingestion rate of a diet high in plant material.

Problems of increasing significance with a shift towards a more herbivorous feeding habit are the much lower assimilation efficiency and the decreased proportion of protein in the food. The protein content of leaves at the cave entrance in July was only 6.3%, while leaves in the cave stream had a protein content of 11.4%. This increase in protein content was probably attributable to bacterial and fungal populations that began to grow on the leaves once they entered the water. It was ingestion of bacteria and fungi, along with the plant material, that probably provided the crayfish with significant amounts of assimilable energy and protein.

There was evidence from the homogeneous laboratory diet studies that crayfish might have been able to digest maple leaves more efficiently than either oak or beech, with beech being the least digestible. Analysis of leaves entering the cave indicated that maple had only a 42% content of the highly refractive substances, lignin and cellulose, whereas both oak and beech had a 52% content. The content of cellulose and lignin in leaves may have affected the assimilation efficiency by:

- (1) decreasing the content of assimilable substances;
- (2) physically combining with assimilable substances and preventing their digestion;
- (3) affecting the biomass of bacteria and fungi growing on the leaf.

There was also evidence that crayfish ate leaves selectively, with the preference ranking matching the ranking of assimilation efficiencies. This evidence was based on the fact that uneaten maple, oak and beech leaves, which were left over at the termination of the complete laboratory diets fed to *O. inermis*, occurred in a 1:6:15 ratio; in addition, this ranking was consistent in all four cases. Cummins et al. (1973) cited several papers that indicate that large particle detritivores show preferences for certain leaf types. Kaushik and Hynes (1971)

found that all tested species (2 amphipods and 1 isopod) preferred leaves in the order, maple, oak, beech; this order corresponds with the crayfish results of the present study.

In the cave stream both crayfish species were most commonly found inhabiting the pools, where the substrate was composed of mud mixed with a small quantity of plant-derived detritus. This region of the stream primarily provided the mud and plant components of the diet. Although a portion of the mud in the diet may have been ingested accidentally along with other dietary items, crayfish were observed to ingest mud alone in the homogeneous laboratory diets. Gounot (1960) found that clayey silt was necessary for the growth of young hypogean amphipods of the genus *Niphargus*; it was concluded that the silt, although it has a probable nutritive role, is necessary because of its antibiotic properties. Animal prey species were extremely sparse in the pools and were represented by a few amphipods that, because of a lack of heterogeneity of substrate grain size, were rather homogeneously distributed. From evidence derived from the predator-prey interaction study, it is evident that this combination of a "bare" substrate and lack of isopod prey, together with low prey density, probably resulted in inefficient prey capture in the pools. Prey capture must have been more efficient in the riffle areas, where prey species, especially isopods, were more abundant and had an aggregated distribution based on current and substrate heterogeneity. Accordingly, it is not surprising that crayfish, especially *C. laevis*, which had a higher proportion of animals in its diet, were frequently found foraging in the riffles.

O. inermis had a slightly higher energy assimilation rate of both plant and animal material than did *C. laevis*. This increased

efficiency could have been mediated by a genetic change that occurred during the longer length of time that the troglobite had been exposed to the cave environment with its low level of food resources. On the other hand, it may have simply been related to a reduced ingestion rate; this would have decreased the rate at which food passed through the intestine, and this change, in turn, would have permitted more complete digestive processing. The difference in their assimilation efficiencies was partly responsible for a 2.7-fold difference in ingestion rates, despite only a 2.3-fold difference in metabolism.

It must be pointed out that assimilation, as measured in this study ($\text{ingestion} = \text{egestion} + \text{assimilation}$), was probably not a true measure of energy available to the crayfish. Bacteria inhabiting the gut and feces probably siphoned off a portion of the "assimilated" energy. Zhukova (1963) stated that in the chitin-lined crustacean hindgut bacterial activity is high and no digestion occurs. Conover (1966) believed, however, that bacteria did not contribute more than a few per cent to the total organic content of copepod feces. Nevertheless, Newell (1965) has shown that bacteria can rapidly process fresh feces, causing a rise in the nitrogen content and a decrease in the carbon level. For this reason, in order to accurately reconstruct dietary input, the fecal matter had to be collected at frequent and consistent intervals.

One incongruous result was the fact that laboratory-maintained crayfish, despite being subjected to abundant food resources, ingested food at a much lower rate than crayfish inhabiting the food-poor cave stream. The explanation probably lies in the fact that the energy expenditure for locomotion was minimal in the laboratory, where the crayfish were confined and where they found abundant food without the necessity of movement. In the cave, on the other hand, food was scarce

and much energy must have been expended to search for it. Activity associated with interactions with other crayfish was another factor to which the isolated laboratory crayfish were not subjected. These additional energy expenditures of the field populations required an increased rate of energy procurement and accounted for the higher ingestion rate in the field.

It can be seen that a food-poor ecosystem, such as the cave, is potentially subject to a high degree of resource exploitation. This might arise from a negative feedback mechanism in which the increased energy expenditure of food-search activity requires higher ingestion rates. The result would be increased consumption of already deficient food resources. Although the hypogean *C. laevis* maintained a high ingestion rate, it is apparent that this proposed feedback mechanism was not fully developed. Instead, certain energy-requiring processes, such as growth and reproduction, operated at reduced levels (see section on budget changes below). The troglobite, *O. inermis*, has evolved an ecological role that even further minimized such a drain of food resources and, in addition, probably increased the stability of the ecosystem; this was accomplished by a decreased metabolic rate (see section on respiration below), which resulted in both a decreased ingestion rate and an increased efficiency of energy assimilation.

Population Attributes

Results

Tissue growth

Introduction

The recapture of crayfish on a yearly basis provided data on the annual growth increment. This information, collected from November, 1968,

to July, 1970, was regressed on carapace length. The regression formulae for the two species provided the basis for growth curve relationships between carapace length and age. Based on observed carapace lengths, the growth curves indicated the possibility that the crayfish inhabiting the cave may have possessed extraordinary longevity compared to epigean crayfish (see section on longevity below).

The following factors, however, might invalidate this method of indirect aging: (1) errors in the regression formulae were compounded in constructing the growth curves, resulting in increasing age uncertainty with increasing length; (2) the decreasing growth increment with greater length also increased uncertainty at the upper end of the growth curves; (3) the growth rates may have undergone temporal change; (4) migrant crayfish that had undergone different growth rates may have entered the population; (5) the growth curves represented the means, and did not account for individual variation. In the case of *O. inermis*, the occurrence of a few crayfish that were of greater length (up to 29.5 mm C L) than the maximum length (28.0 mm C L) predicted by the regression formulae showed that at least some of these factors had an effect. The discrepancy between actual and predicted maximum length is very slight, however, and the degree of correspondence basically supports the growth curve.

Although the procedures outlined above were satisfactory for establishing the mean values for the annual growth increments and the growth curves, it was found that the variances were quite high. This resulted in confidence intervals that were impracticably broad, at least for individual data points. The primary reasons for the large variances are probably attributable to the following: growth based on molting has a step-like pattern, at least when growth is measured

by increase in length, and it would be expected to have inherently greater variance than a system with gradual growth; secondly, transformation of the annual growth data into a growth curve is burdened with the assumption that a short-term pattern of extraordinarily high or low growth, which is shown by an individual during a year's observation, is maintained throughout its lifetime.

Growth data collected on individuals for longer intervals of 2.0 - 3.5 years made a significant contribution to the determination of growth rates. This information, which overcame the above-mentioned shortcomings of annual data, was used to check the accuracy of the annual growth rate determinations and the growth curves derived from them. The long-term data were also used to construct confidence intervals for individuals, so that the probable age of a given individual could be determined from its carapace length.

The procedure was to compare the actual length of a recaptured crayfish with the length computed from the growth formula. The deviation, expressed as a fraction of the computed growth increment, was regressed against the initial carapace length. The upper 95% confidence limit for individual data points was determined by a one-tailed t-test; this gives, with 95% confidence, the maximum growth deviation to be expected for an individual of any given carapace length. In addition, for any given carapace length, an annual growth increment can be computed. By multiplying this increment by the applicable confidence limit for growth deviation, a factor is derived, which, when added to the increment gives maximum growth, and, when subtracted from the increment gives minimum growth, with 95% confidence. By applying this procedure to two hypothetical individuals, one assumed to have maximum growth and the other assumed to have minimum growth throughout their lifetimes, it is possible

11

to construct a growth curve with a 95% confidence interval for the prediction of individual observations. The confidence intervals so computed were much narrower than those based on annual data, but they would have been even less with additional data gathered over a more extended observation period.

Troglobite

Annual recapture included 88 crayfish with initial carapace lengths ranging from 9.0 to 28.0 mm. Growth was found to fit the regression, $Y = 8.27 - 0.293 X$, where Y is the annual growth increment in mm and X is the initial carapace length in mm (Figure 33). There was no significant difference in the regression between the two sexes (Table 20).

The regression formula was used to construct a growth curve relationship between carapace length and age (Figure 34); the asymptotic nature of the curve is quite obvious. The predicted mean carapace length and their 95% confidence limits were calculated for each year class (Table 21).

Supplementary growth measurements support the validity of the growth curve for the population (Table 22). Measurements of 34 tagged individuals were made over a time interval of 2.0 to 3.5 years, and the results were superimposed on the growth curve (Figure 34). The extended-interval growth measurements were also converted by the growth curve to an annual basis (Table 20). The resulting regression formula was not significantly different from that computed from annual observations. The growth rate was slightly lower, however, resulting in a decrease of approximately one mm in the theoretical maximum carapace length.

TABLE 20.--CRAYFISH GROWTH IN CARAPACE LENGTH BASED ON ANNUAL AND LONGER INTERVAL OBSERVATIONS OF MARKED INDIVIDUALS

SPECIES	DATA SOURCE	SEX	COEFFICIENT OF DETERMINATION (r^2)	NUMBER OF OBSERVATIONS	THEORETICAL MAXIMUM CARAPACE LENGTH (mm)	REGRESSION FORMULA Y=ANNUAL GROWTH INCREMENT (mm) X=INITIAL CARAPACE LENGTH (mm)	STUDENT'S t-TEST OF NULL HYPOTHESIS	
							SLOPE	Y'S ADJUSTED TO X OF X VARIABLE
<i>C. laevis</i>	Annual Observation Interval	Females	0.448	17	61.2	$Y = 8.84 - 0.144 X$	P>.50	P>.50
		Males	0.386	14	64.6	$Y = 7.87 - 0.122 X$		
		Combined	0.417	31	62.6	$Y = 8.38 - 0.134 X$		
<i>O. inermis</i>	Annual Observation Interval	Females	0.421	41	28.6	$Y = 8.50 - 0.297 X$	P>.50	.40>P>.30
		Males	0.554	47	28.0	$Y = 7.97 - 0.285 X$		
		Combined	0.466	88	28.2	$Y = 8.27 - 0.293 X$		
	2.0-3.5 Year Observation Interval Converted to Annual by Growth Curve	Combined	0.659	34	27.3	$Y = 7.98 - 0.293 X$	P>.50	.20>P>.10
		Females	0.742	14	27.1	$Y = 9.04 - 0.333 X$		
		Males	0.575	20	29.2	$Y = 6.33 - 0.217 X$		

Figure 33. Annual growth in length of *O. inermis* and *C. laevis*

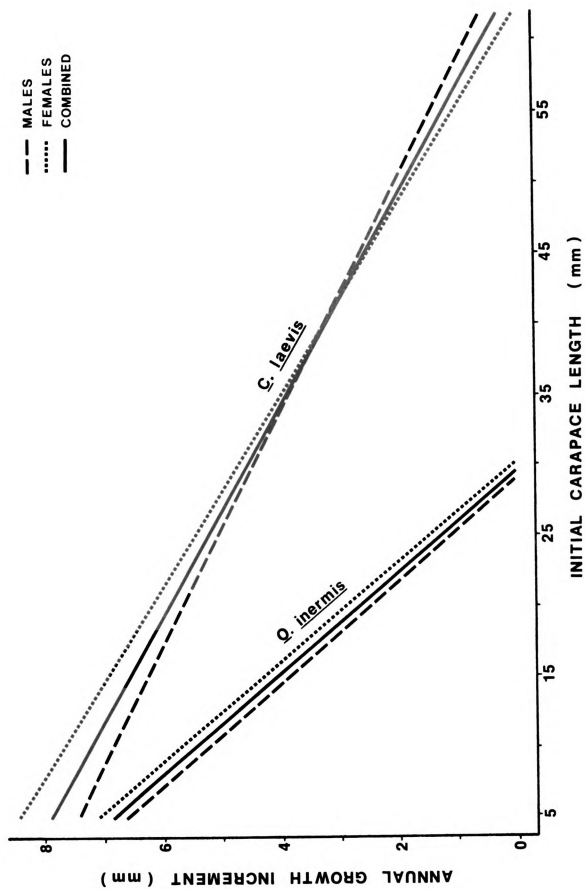


Figure 33

Figure 34. Comparison of annual and longer interval growth data. The growth curves for *O. inermis* and *C. laevis* are based on annual data from Figure 19; longer interval (2.0-3.5 years) data for individual *O. inermis* and *C. laevis* are based on the placement of initial length (represented by $\square$ in the procedural example) on the applicable growth curve.

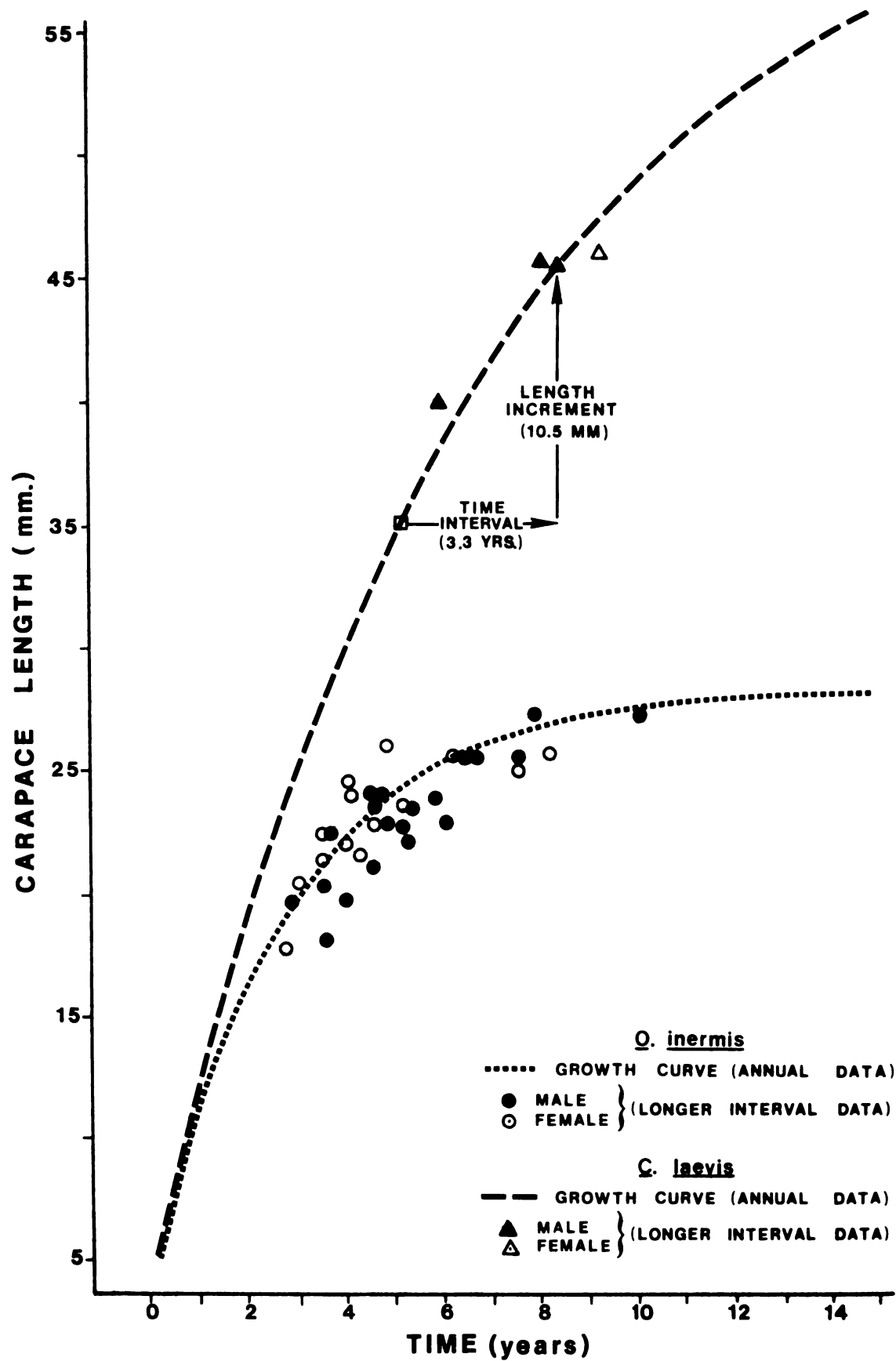


Figure 34

TABLE 21.--AGE-SPECIFIC CARAPACE LENGTHS (mm), WITH MEANS AND 95% CONFIDENCE LIMITS
The means and their confidence limits are based on the annual growth regression
formulas of Table 20; the confidence limits for individuals are based on the devia-
tions of long-term (2.0-3.5 years) growth from the curve of Figure 34 (Table 22)

AGE IN YEARS	O. inermis						C. laevis					
	MEAN	95% CONFIDENCE LIMITS						MEAN	95% CONFIDENCE LIMITS			
		MEAN		INDIVIDUAL					MEAN		INDIVIDUAL	
		LOWER	UPPER	LOWER	UPPER	LOWER	UPPER		LOWER	UPPER	LOWER	UPPER
RELEASE	5.0	..	..	..	..	5.0	..	..	..	..	..	
1	11.8	11.2	12.4	10.0	13.6	12.7	11.1	14.3	10.6	14.9		
2	16.6	15.6	19.1	13.5	19.6	19.4	16.6	22.1	15.1	23.9		
3	20.0	18.8	22.3	16.1	23.4	25.2	21.4	28.8	18.8	31.9		
4	22.4	21.0	24.6	18.1	25.6	30.2	25.6	34.6	21.9	38.7		
5	24.1	22.6	26.2	19.6	26.9	34.5	29.3	39.6	24.6	44.3		
6	25.3	23.7	27.3	20.9	27.5	38.3	32.5	44.1	26.8	48.9		
7	26.2	24.5	28.2	21.9	27.9	41.5	35.3	48.0	28.8	52.4		
8	26.8	25.1	28.8	22.7	28.0	44.4	37.6	51.5	30.6	55.1		
9	27.2	25.4	29.2	23.5	28.1	46.8	39.6	54.6	32.1	57.2		
10	27.5	25.7	29.5	24.1	28.2	48.9	41.3	57.4	33.5	58.7		
11	27.7	25.9	29.8	24.6	28.2	50.7	42.8	59.9	34.7	59.8		
12	27.9	26.0	29.9	25.0	28.2	52.3	44.0	62.1	35.8	60.6		

TABLE 22.--COMPARISON OF MEASURED LONG-TERM GROWTH OF CRAYFISH CARAPACE LENGTHS WITH COMPUTATIONS FROM ANNUAL GROWTH DATA

SPECIES	SEX	TIME INTERVAL (years)	CARAPACE LENGTH (mm)			GROWTH DEVIATION (mm)
			INITIAL	FINAL		
				ACTUAL	COMPUTED	
<i>O. inermis</i>	Female	2.0	10.8	17.5	18.9	1.4
<i>O. inermis</i>	Female	2.0	19.0	22.8	23.3	0.5
<i>O. inermis</i>	Female	2.0	21.5	23.5	24.3	0.8
<i>O. inermis</i>	Female	2.0	12.5	20.2	19.8	0.4
<i>O. inermis</i>	Male	2.0	19.0	24.0	23.3	0.7
<i>O. inermis</i>	Male	2.0	14.8	20.0	21.3	1.3
<i>O. inermis</i>	Male	2.0	13.0	19.7	19.6	0.1
<i>O. inermis</i>	Male	2.0	15.5	22.5	21.7	0.8
<i>O. inermis</i>	Female	2.2	13.7	21.5	21.3	0.2
<i>O. inermis</i>	Female	2.3	24.5	25.0	26.5	1.5
<i>O. inermis</i>	Female	2.3	13.3	22.5	21.3	1.2
<i>O. inermis</i>	Female	2.3	16.0	24.5	22.6	1.9
<i>O. inermis</i>	Male	2.3	25.0	27.2	26.7	0.5
<i>O. inermis</i>	Male	2.3	15.5	19.7	22.8	3.1
<i>O. inermis</i>	Male	2.5	17.0	24.0	23.4	0.6
<i>O. inermis</i>	Male	2.5	22.0	25.5	25.6	0.1
<i>O. inermis</i>	Female	2.6	17.5	26.0	23.8	2.2
<i>O. inermis</i>	Female	2.6	21.5	25.5	25.4	0.1
<i>O. inermis</i>	Male	2.6	24.0	25.5	26.5	1.0
<i>O. inermis</i>	Male	2.6	16.5	23.7	23.4	0.3
<i>O. inermis</i>	Male	2.6	11.5	18.0	21.3	3.3
<i>O. inermis</i>	Female	2.7	15.0	21.5	22.9	1.4
<i>O. inermis</i>	Male	2.7	17.5	22.8	23.9	1.1
<i>O. inermis</i>	Male	2.7	20.5	23.8	25.1	1.3
<i>O. inermis</i>	Female	2.9	24.5	25.7	26.9	1.2
<i>O. inermis</i>	Male	2.9	17.7	22.7	24.4	1.7
<i>O. inermis</i>	Male	3.0	20.2	23.0	25.4	2.4
<i>O. inermis</i>	Male	3.0	14.7	20.8	23.4	2.6
<i>O. inermis</i>	Male	3.0	22.0	25.5	26.0	0.5
<i>O. inermis</i>	Female	3.3	10.0	22.0	22.3	0.3
<i>O. inermis</i>	Female	3.3	11.0	24.0	22.6	1.4
<i>O. inermis</i>	Male	3.3	26.0	27.2	27.5	0.3
<i>O. inermis</i>	Male	3.3	16.7	22.3	24.5	2.2
<i>O. inermis</i>	Male	3.4	16.0	23.5	24.5	1.0
<i>C. laevis</i>	Female	2.0	43.0	46.0	47.9	1.9
<i>C. laevis</i>	Male	2.3	37.5	45.5	44.5	1.0
<i>C. laevis</i>	Male	2.4	28.5	40.0	38.4	1.6
<i>C. laevis</i>	Male	3.3	35.0	45.5	45.3	0.2

*based on regression formulae of Table 20

1

Figure 34 illustrates the extent of deviation between actual and predicted growth over a period of several years. Deviation, expressed in relative terms as a fraction of the predicted growth increment, was correlated ($r = 0.46$) with carapace length; the relationship is represented by the regression formula, $Y = 0.017 X - 0.084$, where Y is the ratio between deviation and the predicted increment, and X is the initial carapace length. For any given carapace length, the upper individual 95% confidence limit for this relationship was applied as a factor to adjust the mean growth increment to maximal and minimal levels.

Beginning with the 5.0 mm carapace length of newly-hatched *O. inermis*, this procedure generated the 95% confidence limits for the prediction of the growth of individuals over their lifetimes (Table 21). For any given individual crayfish, based on its carapace length, age could be assessed with 95% confidence. The ratio of the confidence interval to the mean carapace length ranged from a high of 37% for 2 year-olds to a low of 11% for 12 year-olds (if they occurred in the population); the mean value was 24%. In absolute terms, the confidence interval was broadest at age four. The confidence interval gradually narrowed in older crayfish, as faster growing individuals experienced the highly reduced growth rates characteristic of the longer carapace lengths, while individuals with lower growth rates were undergoing the more rapid growth associated with a lesser carapace length.

In terms of weight, the growth rate, which was based on annual data, was quite low for *O. inermis* (Table 23). The relative annual growth rate varied from a high of 19.04 during the first year of growth to a low of 0.12 during the seventh; the mean was 3.33. The annual absolute growth increment peaked during the fourth year and gradually declined in older crayfish.

TABLE 23.--CRAYFISH GROWTH IN DRY WEIGHT (g)

YEAR OF GROWTH	<i>O. inermis</i>			HYPOGEAN <i>C. laevis</i>			EPIGEAN <i>C. laevis</i> *		
	k	h	g	k	h	g	k	h	g
1st	0.043	19.044	2.998	0.086	29.203	3.408	0.84	284.46	5.65
2nd	0.104	2.295	1.192	0.330	3.687	1.545	2.24	2.65	1.30
3rd	0.136	0.915	0.649	0.667	1.592	0.952	3.97	1.28	0.83
4th	0.139	0.487	0.397	1.022	0.941	0.663	4.43	0.63	0.49
5th	0.123	0.290	0.255	1.336	0.634	0.491	..	..	..
6th	0.101	0.185	0.169	1.582	0.459	0.378	..	..	..
7th	0.079	0.122	0.115	1.748	0.348	0.298	..	..	..
8th	..	..	..	1.838	0.271	0.240	..	..	..
MEAN	0.103	3.334	0.825	1.076	4.642	0.997	2.87	72.26	2.07

k = Annual absolute growth rate
h = Annual relative growth rate
g = Instantaneous growth rate

*Growth is only approximated

It was also found that the growth rate varied in different sections of the stream (Table 24). The census area of the cave stream was longitudinally divided into five 100-meter sections, and the growth rates of crayfish living in each section were compared. Analysis by Tukey's honestly significant difference procedure (Steel and Torrie, 1960) provided evidence that growth was not significantly different for four of the sections, but that crayfish inhabiting the upstream section (0-99 meters) grew at a significantly higher rate.

Troglophile

Annual growth was monitored in 31 individuals of the cave population, with carapace lengths ranging from 11.0 to 45.5 mm. Growth best fit the regression formula, $Y = 8.38 - 0.134 X$, where Y is the annual growth increment in mm and X is the initial carapace length in mm (Figure 33). There was no significant difference in sex-specific regressions (Table 20).

The regression formula was used to construct a growth curve relationship between carapace length and age (Figure 34); the growth rate gradually decreased with age, but was not asymptotic. The predicted mean carapace length and their 95% confidence limits were calculated for each age group (Table 21).

A largely unsuccessful attempt was made to collect long-term growth data. The extreme movement characteristic of *C. laevis* is probably the reason only four long-term (2.0 - 3.5 years) recaptures were achieved. Premature death, due to insufficient adaptation to the cave environment, is possibly another factor, but trophic studies (see page 164 above) have shown that members of the cave population ingested food at almost the same rate as the surface population, and

TABLE 24.--SPATIAL VARIATIONS IN CRAYFISH GROWTH

STREAM SECTION (Meter location)	% OPTIMUM (slow flow) HABITAT	O. inermis				C. laevis			
		% OF STUDY AREA POPULATION	ANNUAL GROWTH INCREMENT IN MM*			% OF STUDY AREA POPULATION	ANNUAL GROWTH INCREMENT IN MM*		
			$\bar{x}$	s	n		$\bar{x}$	s	n
0-99	39	32.2	4.16	2.03	21	57.4	5.04	1.83	13
100-199	15	15.5	2.28	0.95	16	13.0	} 4.58	1.63	12
200-299	28	21.7	2.51	0.80	18	7.4			
300-399	27	15.3	2.89	0.79	16	9.5			
400-499	13	15.3	2.79	0.84	13	12.7			

*adjusted by increment-length regression to growth for mean carapace length

ANALYSIS OF VARIANCE TABLE

SPECIES	SOURCE OF VARIATION	df	SS	MS	F	P	SIGNIFICANCE HS = highly sign. ns = not sign.
<i>O. inermis</i>	Among sections	4	41.72	10.43	6.63	P < .005	HS
	Within sections	79	124.38	1.57			
<i>C. laevis</i>	Among sections	1	1.28	1.28	0.43	P > .10	ns
	Within sections	23	69.29	3.01			

microsporidian infestations (see section on disease below) were more prevalent in the surface population. Nevertheless, the few long-term observations that were collected, when displayed as data points superimposed on the growth curve (Figure 34), substantiate its accuracy.

The long-term growth data were converted to deviations from the predicted increment and expressed as fractions of the predicted growth increment. These were found to be correlated ($r = 0.61$) with carapace length; the relationship is represented by the regression formula, $Y = 0.015 X - 0.378$, where Y is the ratio between deviation and the predicted increment, and X is the initial carapace length. The upper individual 95% confidence limit for this regression was computed and applied to the calculation of the individual 95% confidence interval for the growth curve (Table 21). Although these confidence intervals for the aging of individual *C. laevis* are greater than similarly derived values for *O. inermis*, they are amazingly narrow considering the small amount of data on which they are based; this is due to the high degree of agreement between actual and predicted long-term growth.

In terms of weight, the relative annual growth rate varied from a high of 29.20 during the first year of growth to a low of 0.27 during the eighth year; the mean value was 4.64. The annual absolute growth increment increased with age.

Differential growth rates for crayfish occupying different longitudinal sections of the cave-stream were examined. Because of the mobility of *C. laevis*, only a few individuals were found to have occupied a restricted area for a year's time. It was found that crayfish inhabiting the upstream section, 0-99 meters, grew at a faster, but not significantly different, rate than crayfish in the section of stream from 100 to 499 meters (Table 24).

Enclosure growth study - The comparative growth study of confined young-of-the-year *C. laevis* in cave and surface habitats resulted in differential growth favoring the latter (Figure 35). Crayfish maintained for a year in the surface stream grew from a mean dry weight of 4.5 mg to 77.8 mg; carapace length increased from a mean of 5.0 mm to 11.8 mm. Visual inspection of the gut contents at the time of sampling demonstrated that the guts of these crayfish were nearly always full. A 13% mortality occurred during the course of the experiment. Quite different results were obtained from the cave-maintained crayfish. No increase in weight was observed, although carapace length increased slightly to 6.0 mm. The guts of these crayfish were often empty or only partially full. The mortality was 47%.

Epigeal population - The rate of recapture of code-injected epigeal *C. laevis* was extremely low. Of the 94 marked crayfish only two were recaptured a year later. One recapture was a male which had undergone an upstream movement of 100 meters; it had increased in size from a carapace length of 44.0 mm to 50.0 mm. This growth, although much greater than that predicted from the regression formula derived from hypogean *C. laevis*, was within the 95% confidence interval for an individual datum point. Another recaptured male increased from 27.5 mm to 37.5 mm. Compared to the increment-length regression of hypogean *C. laevis*, this growth increment was much greater, lying outside the 95% confidence interval for a predicted datum point. Information gained from recaptures and size-frequency analysis (see Figure 45 below) has permitted a tentative estimation of the epigeal growth rate (Table 23). Over a 4-year period the absolute growth rate was approximately 5.5 times that of the cave-inhabiting population (Figure 36).

Figure 35. Comparative growth of confined young-of-the-year *C. laevis* maintained either in the cave stream or in the surface stream.

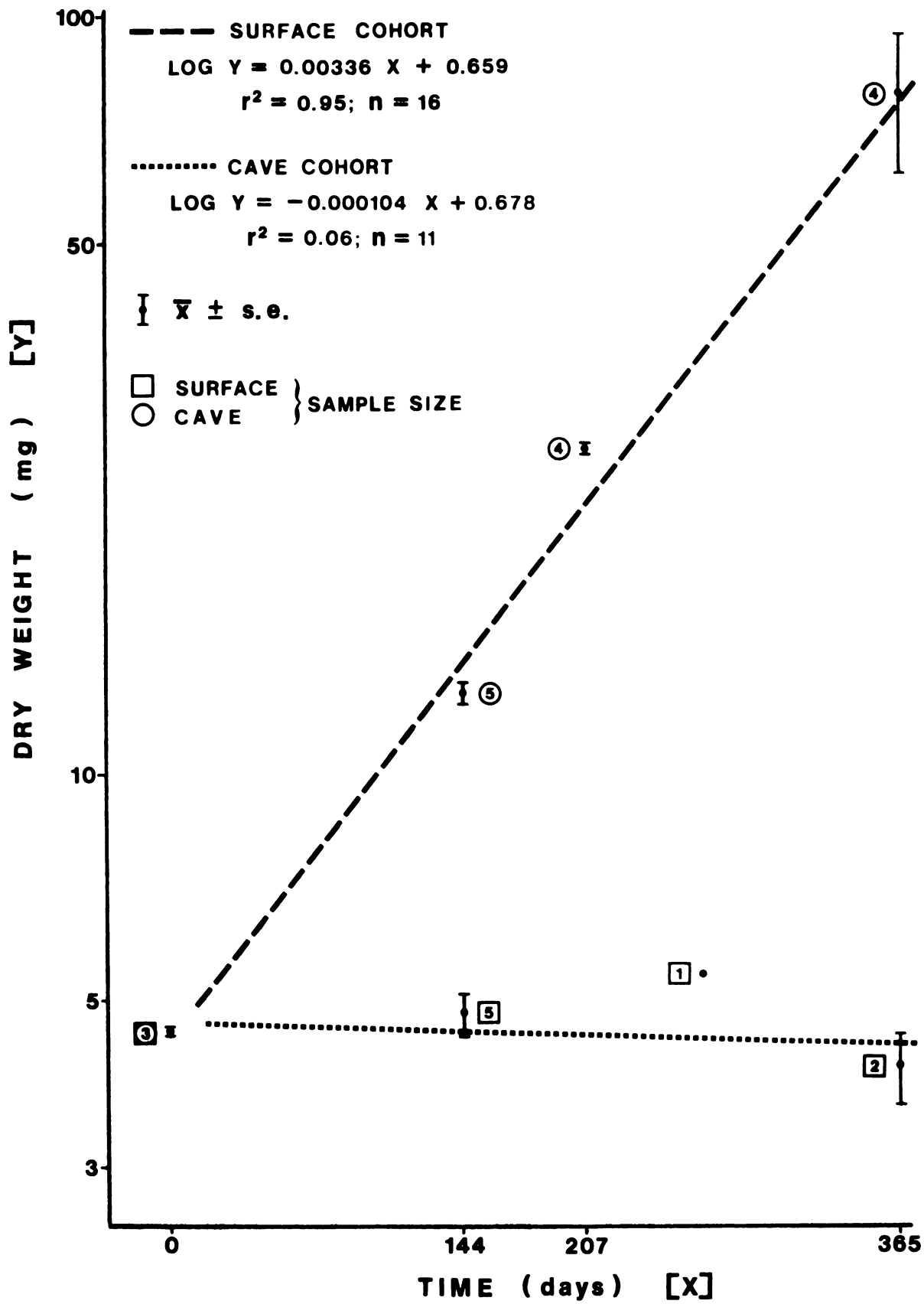


Figure 35

Figure 36. Comparative annual growth increments. The values for epigeal *C. laevis* are only approximated.

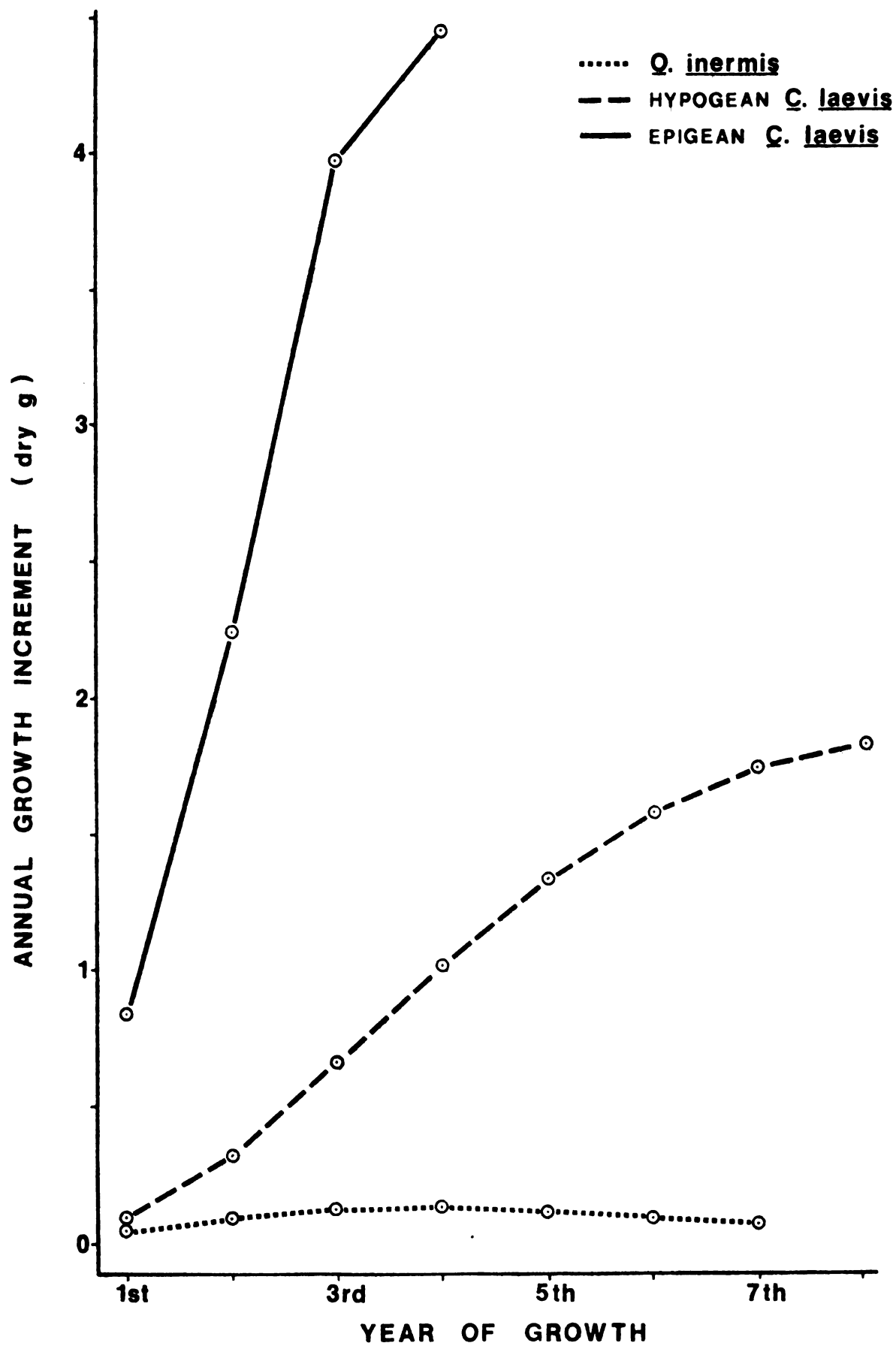


Figure 36

Molting

When a crayfish was first captured during a particular census, it was noted whether this individual had been previously coded. If the same crayfish had been observed during the preceding census, it was determined whether the crayfish had molted during the intervening period and, if so, how much it had increased in carapace length. The data on percentage molting during a 4-month intercensus period were blocked by carapace length, and the factors, sex and season, were tested by analysis of variance (Table 25). The growth increment data were also tested by analysis of variance for the factors, sex and carapace length (Table 26). Both crayfish species were tested, and, in addition, summarizing statistics are displayed for *O. inermis* (Figure 37) and *C. laevis* (Figure 38).

Troglobite

The percentage of *O. inermis* molting during the various seasons was significantly different. The highest molting rate occurred in the 4-month intercensus interval extending from July to November, although no period lacked molting activity (Figure 37a and Table 25). The molting rates for all carapace length categories were consistently highest during the period from July to November, but the other two intercensus periods showed no clear relationship between themselves.

Males showed a slightly higher frequency of molting for most carapace length categories (Figure 37b), but sexual differences were not significant (Table 25). Thus, there did not appear to be a major sexual divergence in molt frequency on an annual basis.

Annual molting frequency decreased with increasing carapace length (Figure 37b and Table 25); differences were highly significant.

TABLE 25. --PERCENTAGE OF CRAWFISH MOLTING DURING A 4-MONTH INTERCENSUS PERIOD

SPECIES STAT	PARTITIONING BY LENGTH, SEX AND TIME									
	CARAPACE LENGTH (mm) CATEGORIES:					SEX		INTERCENSUS TIME PERIOD		
	<i>O. inermis</i> (upper row) <i>C. laevis</i> (lower row)					MALE	FEMALE	MAR- JUL	NOV- NOV	MAR- MAR
<i>O. inermis</i> s n	5.0-8.9	9.0-12.9	13.0-16.9	17.0-20.9	21.0-24.9	25.0-28.9				
	10.0-16.9	17.0-23.9	24.0-30.9	31.0-37.9	38.0-44.9	45.0-47.9				
	100	92	84	85	64	36	69	65	83	69
<i>O. inermis</i> s n	0	20	11	12	17	20	26	23	23	30
	3*	6	6	6	6	6	15	10	10	10
<i>C. laevis</i> s n	100	95	68	63	60	0	73	70	72	67
	0	13	37	42	37	0	37	39	39	44
	4	6	6	5	5	2	13	15	11	8

*Not included in sex and time partitions or A O V

ANALYSIS OF VARIANCE TABLE

SPECIES (REPLICATION)	SOURCE	df	SS	MS	F	P	SIGNIFICANCE HS = highly significant S = significant ns = not significant
<i>O. inermis</i> (3)	BLOCKS (LENGTH)**	4	1.234	0.309	18.74	P < .005	HS
	TIME	2	0.181	0.091	5.53	P < .025	S
	SEX	1	0.037	0.037	2.25	P > .10	ns
	INTERACTION	2	0.076	0.038	2.37	P > .10	ns
	ERROR	24	0.395	0.016			
<i>C. laevis</i> (3)	BLOCKS (LENGTH)**	2	0.818	0.409	10.76	P < .005	HS
	TIME	2	0.128	0.064	1.68	P > .10	ns
	SEX	1	0.106	0.106	2.79	P > .10	ns
	INTERACTION	2	0.077	0.039	1.03	P > .10	ns
	ERROR	12	0.456	0.038			

** 5 length categories (5.0-8.9 category deleted)

*** 3 length categories: 10.0-23.9, 24.0-37.9, 38.0-47.9

TABLE 26. --CRAYFISH MOLT INCREMENT (mm) WITH DATA PARTITIONED AND ANALYZED BY SPECIES, LENGTH AND SEX

SPECIES	STAT	PARTITIONING BY LENGTH AND SEX									
		CARAPACE LENGTH (mm) CATEGORIES: <i>O. inermis</i> (upper row) <i>C. laevis</i> (lower row)									
		5.0-8.9	9.0-12.9	13.0-16.9	17.0-20.9	21.0-24.9	25.0-28.9				
		10.0-16.9	17.0-23.9	24.0-30.9	31.0-37.9	38.0-44.9	45.0-47.9				
<i>O. inermis</i>	$\bar{x}$	1.50	1.61	1.56	1.31	1.03	0.87				
	<i>s</i>	0	0.47	0.64	0.60	0.54	0.43				
	<i>n</i>	3	25	53	88	66	11				
<i>C. laevis</i>	$\bar{x}$	1.66	2.31	3.34	3.71	3.03	1.75				
	<i>s</i>	0.45	0.82	1.42	1.22	1.24	0.35				
	<i>n</i>	11	14	14	7	7	2				

ANALYSIS OF VARIANCE TABLE

SPECIES (REPLICATION)	SOURCE	df	SS	MS	F	P	SIGNIFICANCE ns = not sign. HS = highly sign.
<i>O. inermis</i> (37)	LENGTH*	2	11.94	5.97	18.04	P < .005	HS
	SEX	1	1.07	1.07	3.23	.1 > P > .05	ns
	INTERACTION	2	0.20	0.10	0.30	P > .10	ns
	ERROR	216	71.50	0.33	..		
<i>C. laevis</i> (10)	LENGTH**	1	19.88	19.88	16.21	P < .005	HS
	SEX	1	0.36	0.36	0.29	P > .10	ns
	INTERACTION	1	1.68	1.68	1.37	P > .10	ns
	ERROR	36	44.16	1.23	..		

* condensed to 3 categories: 5.0-16.9; 17.0-20.9; 21.0-28.9

** condensed to 2 categories: 10.0-23.9; 24.0-37.9

Figure 37. Molting in *O. inermis*

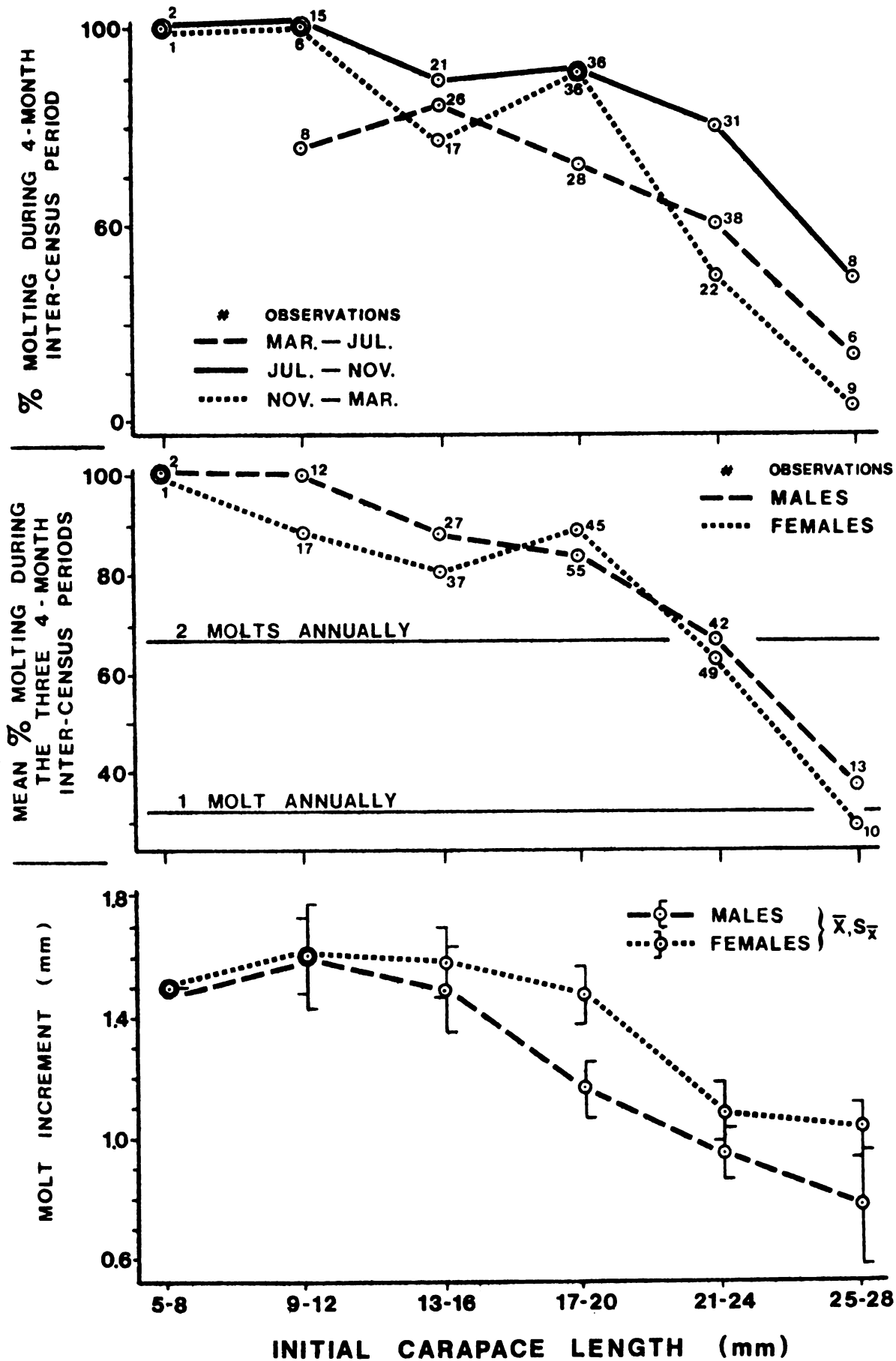


Figure 37



Figure 38. Molting in *C. laevis*.

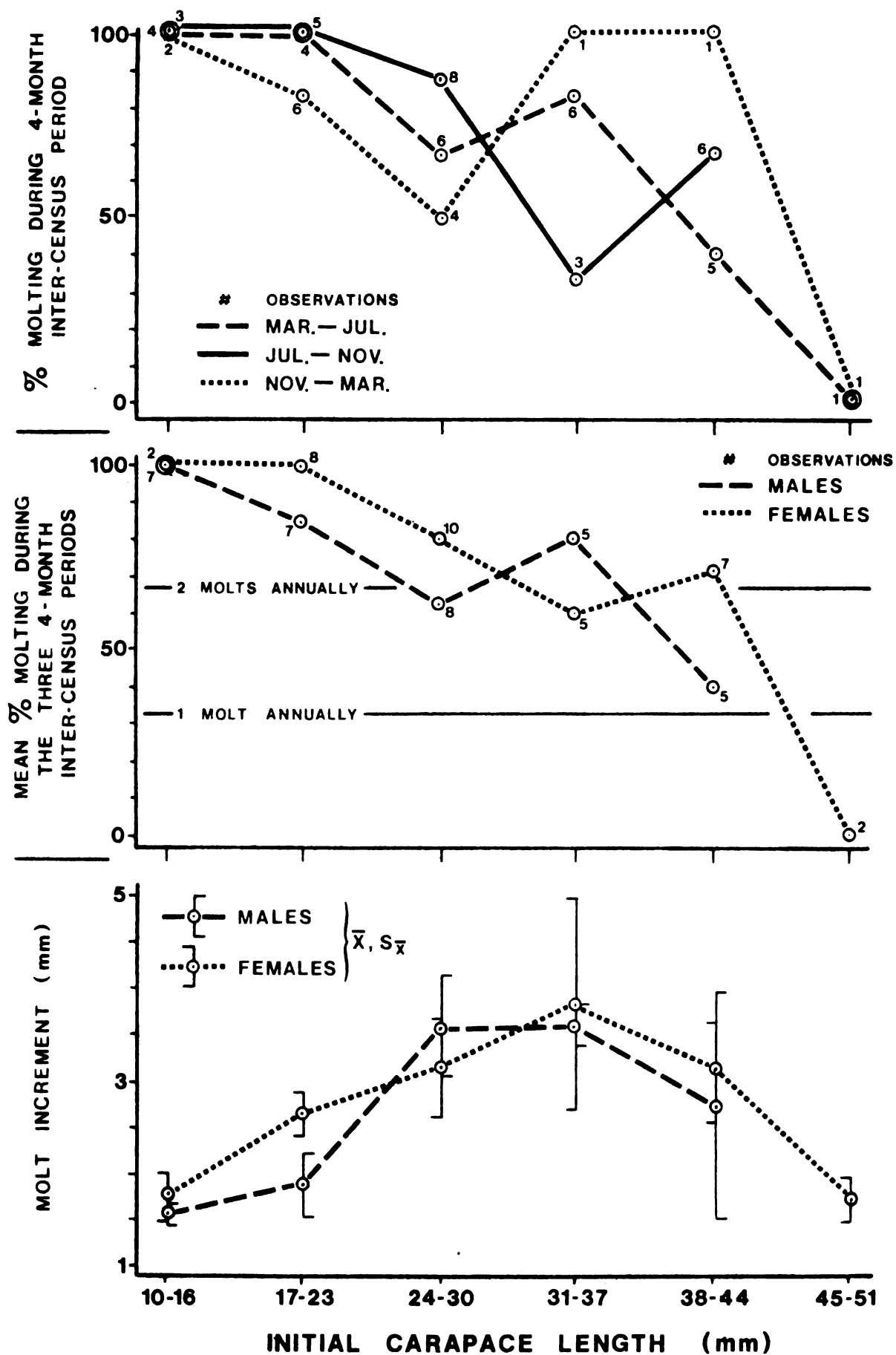


Figure 38

Juveniles molted in excess of twice annually, with those in the 13.0 - 16.9 mm C L and 17.0 - 20.9 mm C L size groups molting about every 4.7 months. Crayfish of the 21.0 - 24.9 mm C L size category molted twice annually, while those in the largest size group molted only once.

Molt increments were determined from 246 observations (Figure 37c and Table 26). The increment reached a maximum of 1.6 mm in the young juveniles, then decreased to 0.9 mm in the largest size category; these differences were highly significant. Females had a slightly greater molt increment than males, but the difference was not significant.

Troglophile

No significant differences were observed in either the seasonal or sexual molt frequency in *C. laevis* (Figures 38a and 38b, and Table 25). Molting frequency generally decreased with increasing size, although the older juveniles and young adults (24.0 - 44.9 mm C L) were stabilized at two molts annually (Figure 38b). These differences in molting frequency associated with increasing size were highly significant (Table 25).

From an initial molt of 1.1 mm, the molt increment increased with increasing length, peaking at a 3.8 mm increment in the largest juvenile stage; the growth increment in larger crayfish decreased to a level equivalent to that in the smallest size categories (Figure 38c). Although differences in length-specific growth increments were highly significant, the greater increments observed in females were not significantly different from those of males (Table 26).

Reproduction

Troglobite

Only a few ovigerous *O. inermis* were encountered during the course of the study. During the egg laying season only 19.4% of the mature females (22.6 mm C L or larger) were in berry. There was evidence that only about two-thirds of the females with developing ovarian eggs and cement glands eventually laid eggs, with the others apparently undergoing resorption (see section on population size and structure below). Eight pleopod egg counts were made; the range was 23 to 61, with a mean of 37. The number of eggs did not appear to increase linearly with parent size. Egg-carrying females ranged in size from 23.0 to 26.0 mm C L, and the greatest number of eggs were carried by a crayfish 24.0 mm in carapace length. *O. inermis* eggs were quite small, having a mean dry weight of 2.56 mg.

Of the total of 11 egg-bearing females encountered, all but one occurred in a 4.5-month time span, from June 27 to November 11. The one exception was a female with well developed ovarian eggs (the eggs can be seen through the translucent carapace) and cement glands that had been removed from the stream. This crayfish was observed to carry eggs from October 25, 1969, to April 7, 1970. The unnatural experimental conditions may have caused the failure of these eggs to hatch. A total of four females carrying young attached to their pleopods were observed. All were encountered in a 2.5-month interval, from September 1 to November 18. The time required for the eggs to hatch was observed by placing a female with well-developed ovarian eggs inside a sealed minnow trap. The trap was positioned in the cave stream so that the flow of

water and food through the 6 mm-mesh openings simulated natural conditions. This 23.5 mm C L female was placed in the trap June 29, 1969. Eggs were attached to the pleopods August 1, and they began hatching 102 days later, on November 11. By November 18 all young had left the pleopods of the mother.

Two of the *O. inermis* that had produced young were found up to 6 months later; consequently, it is assumed that females were capable of reproducing more than once in their lifetime. The size range of ovigerous females indicated that egg production probably occurred over at least a 3-year period, beginning during their fifth year. The largest (28.0 mm C L) female captured, of advanced but highly uncertain age, had developing ovarian eggs in the spring of 1970 and possibly laid eggs later that year.

During the year of study a total of 7 females carrying either eggs or young on their pleopods were captured in the census area; four of these females were found in the quantitative study area. Pleopod egg production was determined from the egg count tally; in the cases of females bearing young, the mean number of pleopod eggs was substituted. It was calculated that the annual pleopod egg production for the census area was 248, and for the quantitative study area 129.

Troglophile

The capture of ovigerous *C. laevis* was quite meager. Only three females ranging in size from 43.0 to 45.5 mm C L were found in berry during the year of study. This represented 25.0% of all females 42.0 mm C L and larger encountered during the egg laying season. There was no evidence of egg resorption. The number of pleopod eggs had a wide

range, from 7 to 183, and had a mean of 98. The eggs were large with a mean weight of 5.44 mg.

Of the total of five egg-bearing females encountered in the cave, all occurred in a 3-month period from July 20 to October 22. A crayfish, observed to be carrying eggs October 22, was found to be carrying newly-hatched young when recaptured four days later. A 43.0 mm C L female with developed cement glands was placed in a sealed minnow trap July 6. This crayfish was carrying eggs 14 days later, and by October 25 the young crayfish were beginning to temporarily detach from the pleopods. Young remained associated with the parent for another 24 days. The total developmental time was 121 days.

C. laevis females did not die after reproduction, as do the females of many crayfish species. Of the three females encountered carrying eggs, two were recaptured 6 to 19 months later.

The annual pleopod egg production for the census area was observed to be 260, and for the quantitative study area 70.

Mortality

Troglobite

Mortality rates in *O. inermis* were very consistent, with no excessive mortality associated with any particular age group (Table 27). Mortality was generally greater at the age extremes and lowest for the 2-3 age class, which was transitional between juvenile and adult. There was, however, a definite pattern of differential mortality between the sexes (Figure 39). Mortality, based on horizontal sampling, was higher for males in the 2-3 and 3-4 age groups; female mortality was greater in both younger and older age groups (Table 27). The general pattern

TABLE 27.--MORTALITY RATE AND SURVIVORSHIP IN *O. inermis*

AGE GROUP (yrs)	ANNUAL MORTALITY RATE: ACTUAL, (INSTANTANEOUS)				SURVIVORSHIP					
	HORIZONTAL SAMPLING*		VERTICAL SAMPLING**		HORIZONTAL SAMPLING*			VERTICAL SAMPLING**		
	FEMALES	MALES	BOTH	FEMALES	MALES	BOTH	FEMALES	MALES	BOTH	FEMALES
PLEOPOD EGGS	..	..	0.541 (0.779)	..	..	0.444 (0.587)	100	100	100	100
0-1	0.267 (0.311)	0.262 (0.304)	0.266 (0.309)	0.400 (0.511)	0.372 (0.465)	0.388 (0.491)	[45.9]	[45.9]	45.9	[55.6]
1-2	0.498 (0.689)	0.164 (0.179)	0.360 (0.446)	0.473 (0.641)	0.234 (0.267)	0.365 (0.454)	33.6	33.9	33.7	34.9
2-3	0.091 (0.095)	0.304 (0.362)	0.224 (0.254)	0.346 (0.425)	0.281 (0.330)	0.310 (0.371)	16.9	28.3	21.6	17.6
3-4	0.542 (0.781)	0.662 (1.085)	0.604 (0.926)	0.068 (0.070)	0.617 (0.960)	0.381 (0.480)	15.4	19.7	16.7	11.5
4-5	0.568 (0.839)	0.262 (0.304)	0.466 (0.627)	0.464 (0.624)	0.398 (0.507)	0.441 (0.582)	7.0	6.7	6.6	10.7
5-6	0.719 (1.269)	0.507 (0.707)	0.642 (1.027)	0.682 (1.146)	0.636 (1.011)	0.664 (1.091)	3.0	4.9	3.5	5.7
6-7	..	..	..	..	..	..	0.9	2.4	1.3	1.8
MEAN	0.448 (0.664)	0.360 (0.490)	0.443 (0.624)	0.406 (0.570)	0.423 (0.590)	0.428 (0.579)				

*a modified form, based on annual observations of each year cohort

**based on mean of 4 censuses

[] based on the assumption that pleopod egg mortality is equivalent for both sexes

Figure 39. Comparison of sex-specific mortality rates in *O. inermis*, based on both horizontal and vertical sampling.

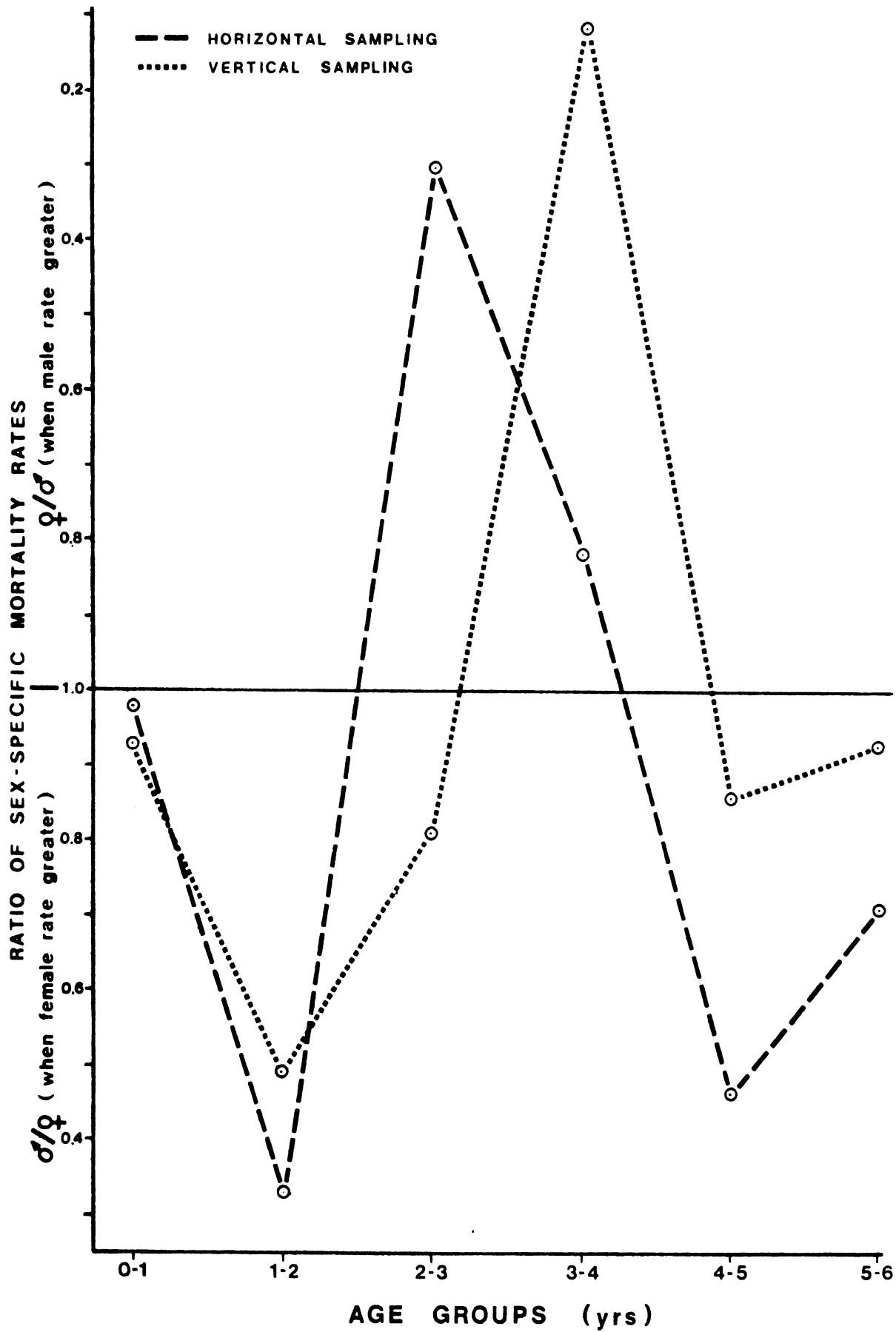


Figure 39

seemed to be as follows: female mortality was greater than male mortality in the juvenile stages; male mortality became greater with the onset of sexual maturity, which occurred a year earlier than in females; this shift resulted not only from increased mortality in males, but also from a dramatic decrease in female mortality; female mortality once again became dominant when females matured, and remained greater than male mortality throughout adult life. Figure 39 illustrates that the sex-specific mortality pattern, as deduced from horizontal sampling, was quite similar to sex-specific changes in successive year groups (vertical sampling).

Survivorship curves based on horizontal and vertical sampling were very similar (Figure 40). The slightly decreased survivorship, based on horizontal data, might have resulted from accidental study-associated deaths.

Troglophile

Mortality determinations in *C. laevis* were complicated by the confounding effects of communication between the epigean and hypogean populations; such determinations actually measured the summation of mortality and migration. Mortality in the 0-1, 1-2, and 2-3 age groups was masked by an apparent immigration of a portion of the 0-1 age group from the surface stream (Table 28). The survivorship curve based on vertical sampling data indicated an increase in the size of the 6-7 age group; this was possibly the result of instability in annual recruitment, since, in the 3-4, 4-5 and 5-6 age groups, mortality rates based on horizontal sampling appeared to be relatively free of immigration.

TABLE 26.--MORTALITY RATE AND SURVIVORSHIP IN *C. laevis*

AGE GROUP (Yrs)	ANNUAL MORTALITY RATE: ACTUAL, (INSTANTANEOUS)						SURVIVORSHIP					
	HORIZONTAL SAMPLING*			VERTICAL SAMPLING**			HORIZONTAL SAMPLING*			VERTICAL SAMPLING**		
	FEMALES	MALES	BOTH	FEMALES	MALES	BOTH	FEMALES	MALES	BOTH	FEMALES	MALES	BOTH
PLEOPOD EGGS	..	..	0.929 (2.645)	..	..	0.951 (3.016)	100	100	100	100	100	100
0-1	0.331 (0.402)	I	I	0.352 (0.434)	I	0.141 (0.152)	[7.1]	[7.1]	7.1	[4.9]	[4.9]	4.9
1-2	0.498 (0.609)	I	0.181 (0.200)	I	I	I	4.8	12.8	8.8	3.2	5.2	4.2
2-3	I	0.254 (0.293)	I	I	0.190 (0.211)	I	2.4	19.7	7.2	3.5	5.6	4.5
3-4	0.521 (0.736)	0.753 (1.398)	0.683 (1.149)	0.470 (0.635)	0.282 (0.331)	0.388 (0.491)	4.2	14.7	8.8	5.9	4.6	5.2
4-5	0.861 (1.973)	0.550 (0.799)	0.711 (1.241)	0.638 (1.016)	0.254 (0.293)	0.441 (0.582)	2.0	3.6	2.8	3.1	3.3	3.2
5-6	0.568 (0.839)	0.551 (0.801)	0.559 (0.819)	I	0.060 (0.062)	I	0.3	1.6	0.8	1.1	2.4	1.8
6-7	1.00	1.00	1.00	I	0.676 (1.127)	0.393 (0.499)	0.1	0.7	0.4	1.5	2.3	1.9
7-8	..	..	..	..	..	..	0	0	0	1.6	0.7	1.2
MEAN	0.630 (0.928)	0.622 (0.823)	0.677 (1.211)	0.487 (0.695)	0.292 (0.405)	0.463 (0.948)						

*a modified form, based on annual observations of each year cohort

**based on mean of 4 censuses

[] based on assumption that plicopod egg mortality is equivalent for both sexes

I immigration > mortality + emigration

Figure 40. Inter-specific comparison of survivorship curves,
based on both horizontal and vertical sampling.

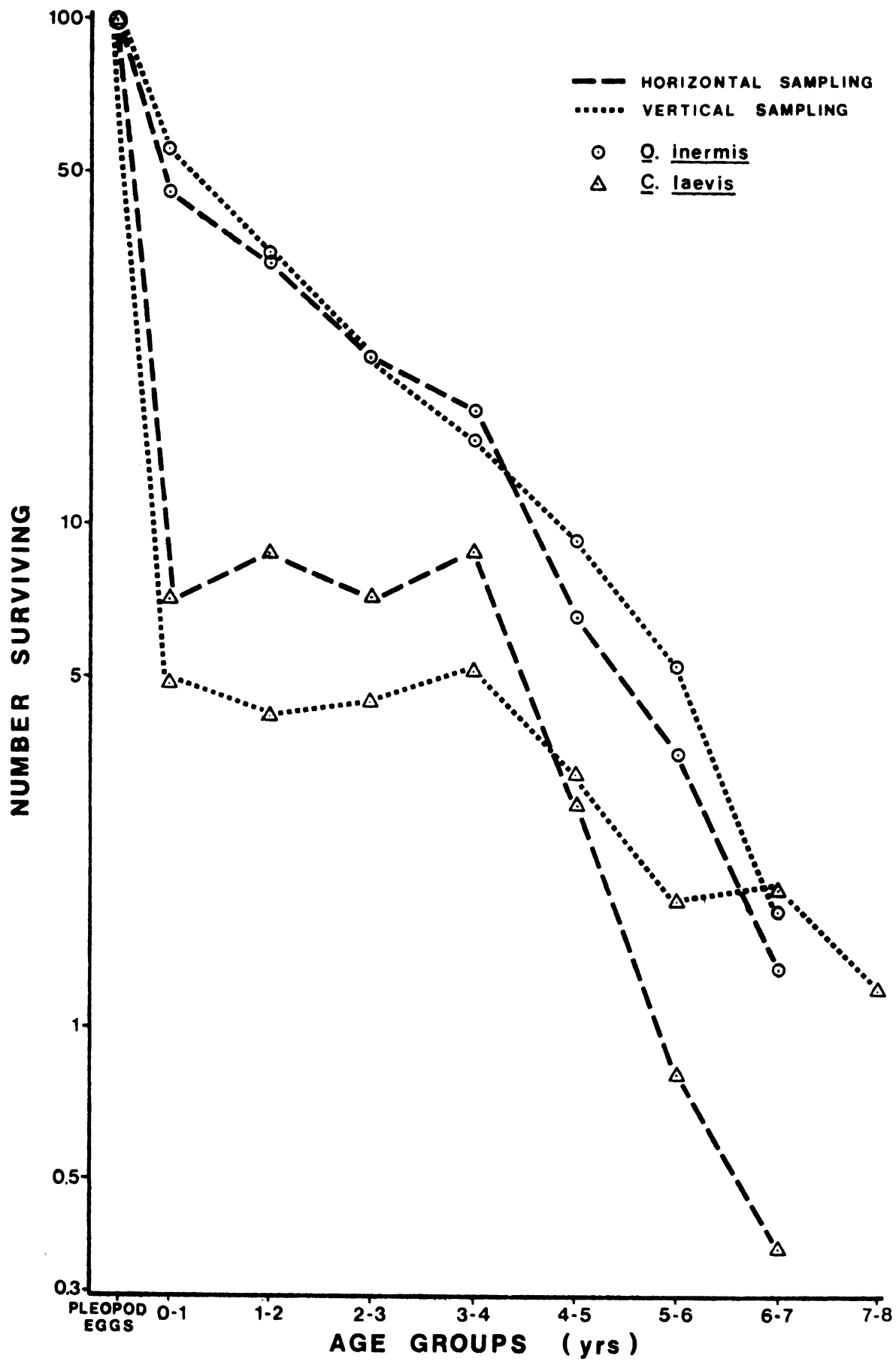


Figure 40

Survivorship curves based on horizontal and vertical sampling were slightly dissimilar, especially in the older age groups where the horizontal-based survivorship was less. It was judged that study-related mortalities, which occurred primarily in the younger age groups, did not contribute to this difference in survival.

Longevity

The longevity of the crayfish living in Shiloh Cave could only be approximated. Based on growth rates measured during the year of study, age was equated to length. Because of variation in the growth rate of individuals, this aging method was accurate only at the population level, as a mean value, and then only under certain assumed conditions. It was especially fallible for accurately aging very large individuals. The maximum carapace lengths attained by *O. inermis* were 29.5 mm for a male and 28.0 mm for a female. Because these individuals probably had higher than average growth rates, they could not be used in estimating the maximum age. A more reliable estimation was reached by taking the mean length of the 12 largest crayfish of both sexes; this number represented 5% of the sampled population. The resulting carapace lengths of 27.2 mm for males and 27.0 mm for females, indicated a probable age of 9 years. In the case of *C. laevis*, carapace lengths of 47.8 mm for the males and 46.7 mm for the females were the means of the largest 5% of the sampled population. According to the growth curve, this indicated an age of approximately 9 years. The asymptotic nature of the growth curve, which was especially well-developed in *O. inermis*, caused severe problems in accurately determining longevity from maximum carapace length data, since crayfish could have lived for several years with no measurable increase in length.

The life span was best judged from long-term recaptures of tagged individuals, although this also relied in part on the age-length relationship. Using this method, the oldest *O. inermis* was a male that was originally tagged when its carapace length was 26.0 mm, and its age, by length-age conversion, was assumed to be 6.8 years. This individual was recaptured 3.3 years later, and was assumed to be 10 years old (with 95% confidence that the crayfish was at least 7.6 years). The oldest female was monitored for 2.9 years, and it was judged to be over 8 years of age (with 95% confidence of at least 6.4).

There were very few long-term recapture of *C. laevis*; this probably resulted from the higher mortality rates and greater movement that were characteristic of this species. The oldest, followed for only two years, was a female judged to be over 9 years of age at the end of the period (95% confidence of at least 6.8). The oldest male, which was monitored for 3.3 years, attained an estimated age of almost 8.5 years (95% confidence of at least 6.8). These age estimates for *C. laevis* are especially questionable, on account of the possible movement between the epigean and hypogean streams. Such movement would invalidate initial aging by the length conversion method, because the length-age relationship differed greatly for crayfish inhabiting these two habitats.

Population size and structure

Troglobite

According to growth data gathered during the year of study, the population, based on carapace length, was partitioned into seven year groups (Table 29). Another group, represented by crayfish greater than 7 years of age, was not an annual age grouping. Information from

1

TABLE 29.--ESTIMATED POPULATION SIZE AND STRUCTURE OF *O. inermis* INHABITING THE 750 m² STUDY AREA DURING THE YEAR OF STUDY

Age (yrs)	C L (mm)	CENSUS												MEAN $\pm$ S D			
		MARCH '69			JULY '69			NOVEMBER '69			MARCH '70						
		A (A)	B (B)	C (B)	A (A)	B (B)	C (B)	A (A)	B (B)	C (B)	A (A)	B (B)	C (B)	A (A)	B (B)	C (B)	D (B)
0-1	5.0- 11.9	57	72	38.8	63	80	37.9	73	92	41.2	50	64	34.6	61	77	38.6	44.1 ± 3.5 ± 0.2
1-2	12.0- 16.9	30	42	21.6	53	48	26.7	28	42	18.3	42	52	28.6	38	46	23.8	44.7 ± 1.1 ± 5.5
2-3	17.0- 20.4	29	18	14.1	27	29	14.8	36	29	17.0	25	21	14.0	29	24	15.0	54.9 ± 15.2 ± 1.4 ± 5.5
3-4	20.5- 22.5	22	20	12.6	24	12	9.5	19	15	8.9	21	16	11.2	21	16	10.5	57.9 ± 21.1 ± 6.7 ± 1.7 ± 6.1
4-5	22.6- 24.4	8	17	7.5	3	17	5.3	13	17	7.9	7	10	5.2	8	15	6.5	32.9 ± 37.5 ± 18.8 ± 11.8 ± 14.1 ± 12.9
5-6	24.5- 25.5	4	7	3.3	6	8	3.7	3	9	3.1	6	7	4.0	5	8	3.5	37.6 ± 75.0 ± 28.6 ± 100.0 ± 12.5 ± 25.0 ± 66.7 ± 28.6 ± 7.3
6-7	25.6- 26.5	2	4	1.8	2	3	1.3	1	1	0.5	2	2	1.2	2	3	1.2	43.3 ± 100.0 ± 25.0 ± 50.0 ± 50.0 ± 50.0 ± 57.1 ± 20.0 ± 0.5 ± 8.2
>7	>26.5	1	0	0.3	1	2	0.8	3	1	1.1	2	2	1.2	2	1	0.9	64.6 ± 100.0 ± 50.0 ± 71.4 ± 20.0 ± 0.4 ± 29.2
TOTAL POPULATION (SEXES COMBINED)		333			378			382			329			356 $\pm$ 28			

A = number of males
(A) = % first form

B = number of females
(B) = % with developed cement glands
[B] = % in berry

C = % of population
D = % male

long-term growth studies (see page 208 above) indicated that longevity may have been greater (longevity of approximately 10 years) than that indicated by the above method, that is, age categorization of the population based on the relationship between carapace length and growth rate.

The proportions of the various age classes in the *O. inermis* population showed a consistent decrease with age (Figures 41 and 47). The decrease was very gradual, and the young-of-the-year crayfish made up only 38.6% of the population.

Sexual maturity in males, indicated by presence of the form I gonopod, was generally reached during the third year, when crayfish increased to carapace lengths greater than 18.0 mm (Figure 41). One exception was a form I male with a carapace length of 16.0 mm, which indicated an age of less than two years. Upon reaching maturity during the third year, form I males commonly occurred in all succeeding age groups. The percentage of mature males in the form I condition generally increased from the third (15.2%) to the sixth year (63.2%). Based on the capture of only a few individuals, the two oldest age categories seemed to show a leveling off of this trend.

Mature females often possessed developed cement glands and maturing ovarian eggs visible through the translucent carapace. They first appeared during the fourth year, with the smallest mature females having a carapace length of 21.0 mm. This indicated a pattern in which female maturity lagged a year behind the attainment of maturity in males. Cooper (1975) found a similar, but even more pronounced, lag in the cave crayfish, *O. australis*, in which male maturity was reached at a carapace length only 74% of that of females reaching maturity. The percentage of females with developed cement glands increased from a

Figure 41. Mark-recapture estimates of *O. inermis* population in the census area (750 m^2) during the year of study; age 0-1 not sexed and arrows indicate growth of a cohort.

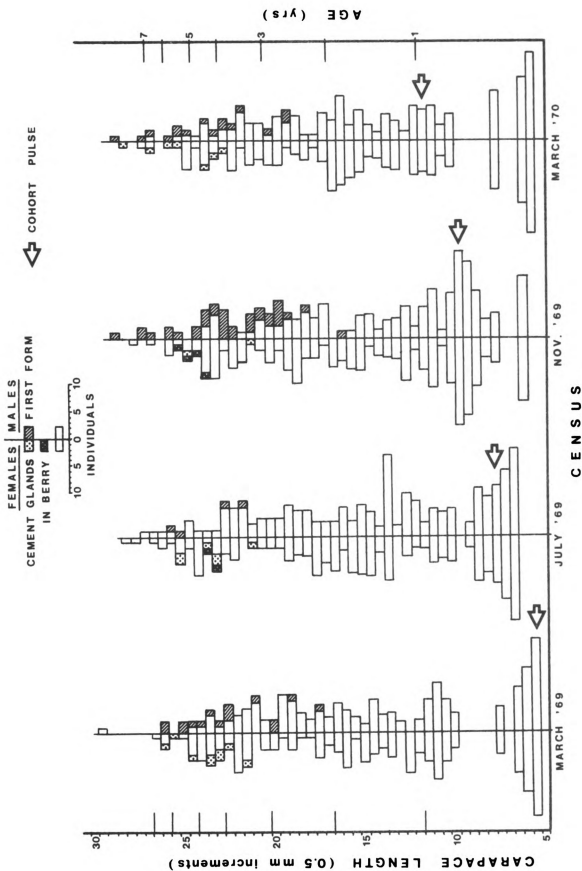


Figure 41

mean of 8.5% of fourth year females to 23.0% for those in their sixth year, while the two older age categories were stabilized at the 20.0% level. The pattern of change of crayfish in the active sexual state was similar in both sexes, but the level was always lower in the females. The production of pleopod eggs was observed in only the fifth and sixth age groups, but it is assumed that the seventh and over-seven age groups would have shown reproductive capacity if a larger population had been observed.

The sex ratio varied greatly among the age classes (Table 29). The proportion of males in young-of-the-year crayfish was $44.1 \pm 0.2\%$. This value only represented the ratio of crayfish 10.0 mm C L or greater, since smaller individuals could not be reliably sexed. It is uncertain whether this value represented the ratio at hatching or resulted from a differential rate of mortality in the unsexed sizes. The proportion of males steadily increased with age, reaching a high of $57.9 \pm 6.1\%$ during the fourth year. The ratio underwent an abrupt change during the next year, and the proportion of males declined to its lowest level of $32.9 \pm 12.9\%$. The ratio of males again increased during the remaining age groups and reached a high of $64.6 \pm 29.2\%$. This complex pattern was the result of sex-specific mortality rates that, except for young adults, favored males.

There were seasonal changes in the sexual condition of both sexes. Form II males predominated in July, with only 6.6% of males older than two years with form I gonopods. By November the percentage of form I males increased to 49.4%. Intermediate levels occurred in March, with 25.8% in 1969 and 27.0% in 1970. March appeared to be a molting period with males changing from first to second form. The reverse molt occurred between July and November. It should be emphasized that the annual

alternation of form did not involve the entire population of mature males (Figure 42).

In females the cement glands and oocytes underwent seasonal changes in development. During March, 1969, cement glands were developed in 19.5% of females older than three years. By July cement glands were developed in only 15.0% of the mature females, but the change was partially accounted for by the presence of females in berry (5.1%). By November developed cement glands had decreased to only 5.1%, and 9.9% of mature females were in berry. Of those individuals with developed glands in July, by November approximately a third were carrying eggs, another third were unchanged, and the final third had apparently undergone resorption. In March of 1970 the proportion of females with developed glands had increased to 19.7%, which was comparable to the level of a year previously.

These changes in the sexual conditions of both sexes were phased to the reproductive pattern of this species. Mating apparently occurred in late fall when the breeding form male (form I) was most abundant. Ovarian eggs and cement glands developed during the spring, and pleopod eggs were carried in summer and early fall.

Seasonal fluctuations in the carapace length structure of the *O. inermis* population were minimal. Recruitment was spread over a period of several months, and this, combined with differential growth, resulted in a near constant flow through the age groups. Only one definite cohort pulse was observed passing through the population; this consisted of an increase in the mean carapace length of the young-of-the-year crayfish (Figure 41).

The population size for the census area was 356 ± 28 ; this was equivalent to a density of 4747 crayfish per hectare. The estimated

Figure 42. Seasonal percentages of form I male *O. inermis*.

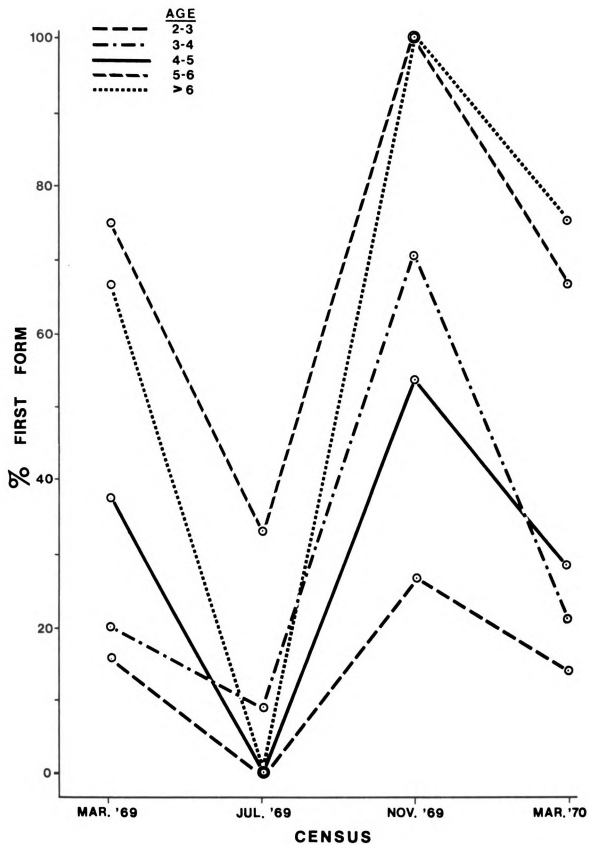


Figure 42

population showed only a slight numerical variation seasonally. The lowest population levels occurred in the March censuses — 333 in 1969 and 329 in 1970. The largest population, 382, occurred during the November census. These changes in population level paralleled similar slight changes in young-of-the-year crayfish, which probably generated the fluctuations. From knowledge of the time of egg-laying, it would be expected that the young-of-the-year would be most numerous during the March census, instead of least numerous. However, at this time they were mostly quite small, and this probably permitted them to escape representative detection.

The population size also varied in different longitudinal sections of the stream (Figure 46b). The highest density was found in the upstream section, where 32% of the census area population resided. The middle section, 200-299 meters, was inhabited by 22% of the population, and the other three sections contained equivalent levels of 15%. The population levels of the various sections were compared to the following variables: (1) the annual growth rate; (2) the number of *C. laevis*; (3) the extent of optimal habitat (subjectively judged to be the non-riffle areas); and (4) the food level, as determined by measurement of the benthos, microseston and mud energy content (Table 31). Positive simple correlations, with correlation coefficients above 0.83, existed between the number of *O. inermis* and three of the variables. Only the food level, with a value of -0.43, did not fit the general pattern. Partial correlations were computed for the three variables showing positive correlation. Both optimal habitat and the number of *C. laevis* still showed high positive correlations ($r = 0.80$) with the number of *O. inermis*; the growth rate, however, had a coefficient of -0.54.

Because of the small number of stream sections observed, none of these partial correlations, although some were high, were found to be significant by the *t*-test.

Troglophile

All sizes, from 7.0 to 47.0 mm C L, were well represented in the *C. laevis* population. There was an isolated occurrence of one exceptionally large individual of 58.5 mm C L. With the aid of growth data this population was categorized into 9 age groups, with the last group including all crayfish over 8 years of age (Table 30). Long-term growth studies (see page 208 above) reinforced longevity estimates of approximately 9 years. Aging was based on the relationship between the mean annual growth increment and the initial carapace length of members of the cave population; this relationship provided an estimation of the age of individuals. In contrast, based on population structure and limited growth data, the epigean population was roughly estimated to be composed of four year groups (Figure 45).

The population structure of the cave-inhabiting *C. laevis* was quite complex (Figures 43 and 47). Several age groups, such as the third, fourth and seventh, were numerically inflated above the levels of the preceding age groups. This would superficially appear to result from variations in annual recruitment. The survivorship curve based on horizontal sampling showed a similar pattern, however, with several cohorts increasing numerically with time (see Figure 40 above). In all such cases the data had overlapping 95% confidence limits, and the increases in numbers could simply be ascribed to sampling error. The following observations, however, provide arguments against drawing this conclusion: (1) the patterns of the horizontal and vertical

TABLE 30.--ESTIMATED POPULATION SIZE AND STRUCTURE OF HYPOGEAN *C. Jaevii* INHABITING THE 750 m² STUDY AREA DURING THE YEAR OF STUDY AND A ROUGH ESTIMATION OF THE EPIGEAN POPULATION INHABITING AN EQUIVALENT AREA

AGE (yrs)	C L (mm)	CENSUS										MEAN $\pm$ S D			
		MARCH '69			JULY '69			NOVEMBER '69			MARCH '70			A (A)	B (B)
		A (A)	B (B)	C (C)	A (A)	B (B)	C (C)	A (A)	B (B)	C (C)	A (A)	B (B)	C (C)		
0-1**	5.0-12.9 5.0-27.4*	10	13.2		4680*	26 5972*	27.1 81.6*	4	6.1	12	19.7	13	16.5 49.0		
1-2	13.0-19.4 27.5-37.4*	5	7	15.8	786*	461*	16.7 9.6*	4	0	6.1	18.0	7.5	3.3 14.2 74.6		
2-3	19.5-25.4 37.5-44.4*	5	5	13.2	11 277*	5 379*	16.7 5.0*	5	6	16.6	16.4	6.8	5.0 15.7 58.1		
3-4	25.5-30.4 >44.4*	9	4	17.1	186*	316*	16.7 3.8*	6	8	21.2	19.7	6.8	7.0 18.7 48.9		
4-5	30.5-34.9	6	4	13.2	3	3	6.3	6	7	19.7	2	4.3	4.0 11.4 51.5		
5-6	35.0-38.4	5	4	11.8	3	2	5.2	3	0	4.5	2	3.3	1.8 56.4 45.9		
6-7	38.5-41.9	3	3	7.9	2	2	4.1	4	2	9.1	2	2.8	2.3 6.9 54.2		
7-8	42.0-44.4	1	2	3.9	0	4	4.1	3	2	9.1	0	1.0	2.0 4.3 31.1		
>8	>44.4	1	2	3.9	1	2	3.1	0	4	7.6	2	1.0	2.8 5.7 26.7		
TOTAL POPULATION (sexes combined)		76			96			64			61	74.2 $\pm$ 15.9			

*Epigeal population

**0-1 age group of hypogean population

not sexed

A = number of males

(B) = % with developed cement glands

(C) = % in berry

B = number of females

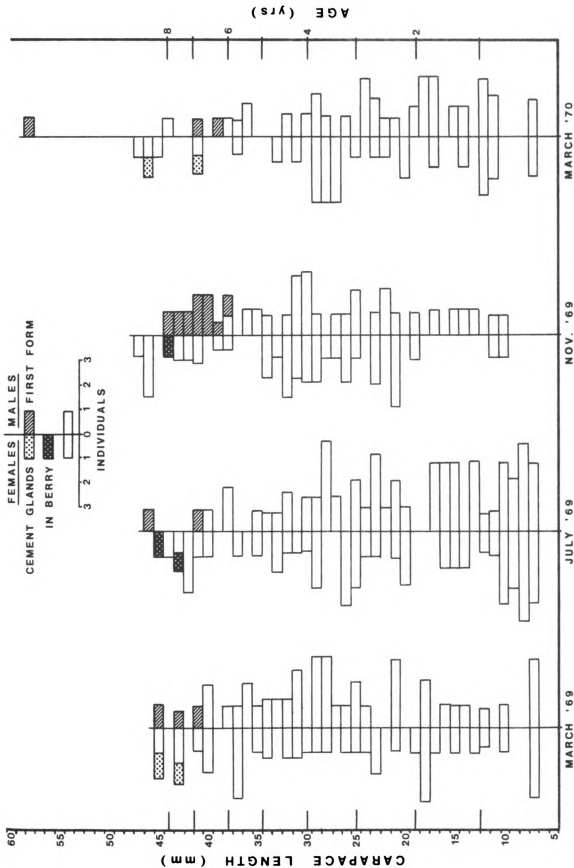
(B) = % with developed cement glands

(B) = % in berry

C = % of population

D = % male

Figure 43. Mark-recapture estimates of *C. laevis* population in the census area (750 m²) during the year of study; age 0-1 not sexed.



CENSUS
Figure 43

survivorship curves were quite similar; (2) the standard deviations in age class percentages among the four censuses were small, especially when it is considered that a portion of this variation was due to real seasonal changes in the population structure (Figure 47); and (3) even if the increases were spurious, the numerical stability of an age class during a year's time seems contrary to an expected mortality-based decrease in numbers. Accepting these arguments, and with fluctuations in annual recruitment being ruled out as the prime causal agent, the increases in certain age classes can only be explained by immigration. The survivorship curve based on horizontal sampling indicated that the cave *C. laevis* population was probably supplemented by immigration in the second, third and fourth age groups. The significance of immigration, either from other reaches of the cave stream or, more probably, from the surface stream, was demonstrated by the fact that young-of-the-year crayfish made up only 17.3% of the cave population.

The influence of immigration was also observed in the sex ratio. The sex ratio of the cave population varied in a very complex manner (Figure 44) that seemed to be primarily influenced by immigration in the younger age classes and other factors in the older groups. Immigration was assumed to be operating when the age class in question was both numerically inflated and locomotively active. Although the measurement of age-specific movement rates of the cave population did not constitute a direct measure of immigration, extensive movement, expressed as a displacement rate, appeared to be associated with an influx of crayfish into the census area. There is a logical connection. Assuming movement patterns to be specific for various carapace length categories whether the crayfish were residing in the epigean or hypogean stream,

Figure 44. Relationship between movement, sex ratio, and relative change in the size of succeeding age groups in hypogean *C. laevis*.

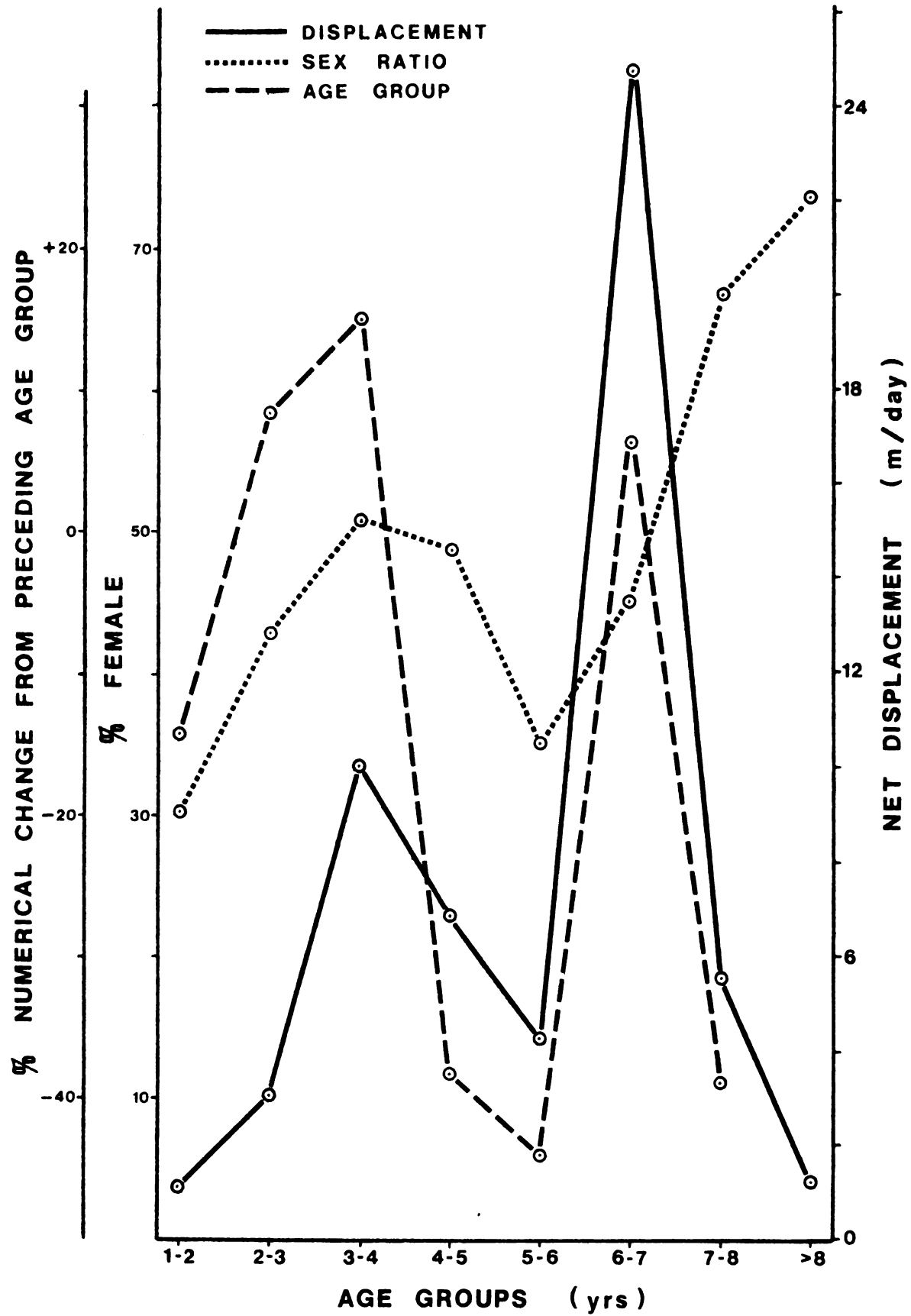


Figure 44

extensive non-directed displacement would result in a net movement from the high density surface population to the low density cave population.

The young-of-the-year *C. laevis* were not sexed because of the uncertainty of proper sex identification. Crayfish of the second year class were 69.8% male, and this ratio gradually decreased to a value of 49.1% in the fourth year class; moreover, the sizes of the age classes and the length-specific movement rates were concurrently increasing. All of these changes were probably associated with immigration of females into the population. After the fourth age class the proportion of males increased until it reached 65.0% in the sixth. This was associated with both decreasing movement rates and decreasing age class sizes. For these age classes immigration seemed to be curtailed, and differential mortality favoring males apparently accounted for the shift in the sex ratio. A decline in the proportion of males to 55.0% of the seventh age class was correlated with increases in both the size of the age class and movement. Once again immigration may have been involved, but it is uncertain whether the increased proportion of females was due to immigration of females or to other factors that were apparently operating in older age groups. The eighth and over eight age groups had sex ratios of 33.3 and 26.7% males, respectively. This decreasing proportion of males was associated with both decrease in movement and decrease in size of the eighth age class; this relationship runs counter to trends in younger classes. Immigration seemed to be insignificant in these groups, and the changes in the sex ratio may instead have been caused by higher mortality rates in males. Another possibility is that slight differences in the growth rate favoring females may have had a cumulative effect, and this may have been responsible for the lower proportion of males reaching the two largest size groups.

The length-specific sex ratios of trap-captured epigean *C. laevis* were similar to those of the cave population. Females were in the majority, except for the second year class. The ratio of the first year class, which corresponded in its length range to the age classes of the cave population experiencing immigration, was only 43.9% male. The preponderance of first year class females in the surface population probably resulted in their entering the cave to the greatest extent; this migration would explain the inflated numbers and shifted sex ratios of corresponding size groups in the cave population.

Both males and females in the cave population reached maturity during their seventh year. The percentage of males with form I gonopods varied in the mature age classes from 66.6 to 100.0%, but no trend with length was obvious. The proportion of females with developed cement glands increased with increasing length, from 11.1% of seventh year females to 22.9% of those over eight years old. Pleopod eggs were observed being carried by females in the eighth and over eight age groups.

In epigean populations maturity was probably attained when the crayfish were two years of age (Table 30). Not only did epigean populations reach maturity at a much younger age, but also at a smaller size. In the cave the smallest form I male was 38.5 mm C L and the smallest female with developed cement glands was 41.5 mm C L. In the surface stream flowing from Shiloh Cave the lengths were 34.0 and 34.5 mm C L respectively, and in the stream that issues from Sullivan's Cave the lengths were 32.0 and 38.5 mm C L.

The sexual condition of hypogean males varied to some extent with the seasons. During November form I gonopods were present in 100% of males older than six years. Form I males were present throughout the year, however, and never were less than 58.4%. The 66.7% presence

of first form males in July was comparable to the level of first form males in the surface population.

In hypogean females the presence of either cement glands or pleopod eggs was found throughout the year. Cement glands occurred only during the March censuses, varying from 23.8 to 35.0% of the females among the age groups older than six years. Pleopod eggs were present in both July and November, varying from 22.5 to 13.3%, respectively. In July cement glands and pleopod eggs combined were present in a larger proportion of mature epigean females than in their hypogean counterparts (48.5 versus 22.5%).

This pattern of change in the sexual state of the cave population indicated that mating probably occurred in late fall, when the first form males were most abundant. In females, mating was followed by the developing of cement glands in the spring and the carrying of pleopod eggs during late summer and early fall.

There were only small seasonal fluctuations in population structure. The low growth rate and extreme longevity characteristic of the cave population tended to minimize seasonal changes in the structure of the population. Although young-of-the-year crayfish were present throughout the year, the largest pulse occurred in July. These crayfish were approximately eight months old, and had entered a size range that was more representatively sampled than the smaller sizes.

The mean size of the estimated population in the census area was 74 ± 16 , which was equivalent to a density of 987 crayfish per hectare. The density of the epigean population was roughly estimated to be 174,000 crayfish per hectare, or 176 times the density of the cave population (Figure 45). The size of the cave population underwent substantial seasonal changes that probably resulted from fluctuation

Figure 45. Size and structure of epigean (approximated) and hypogean populations of *C. laevis*.

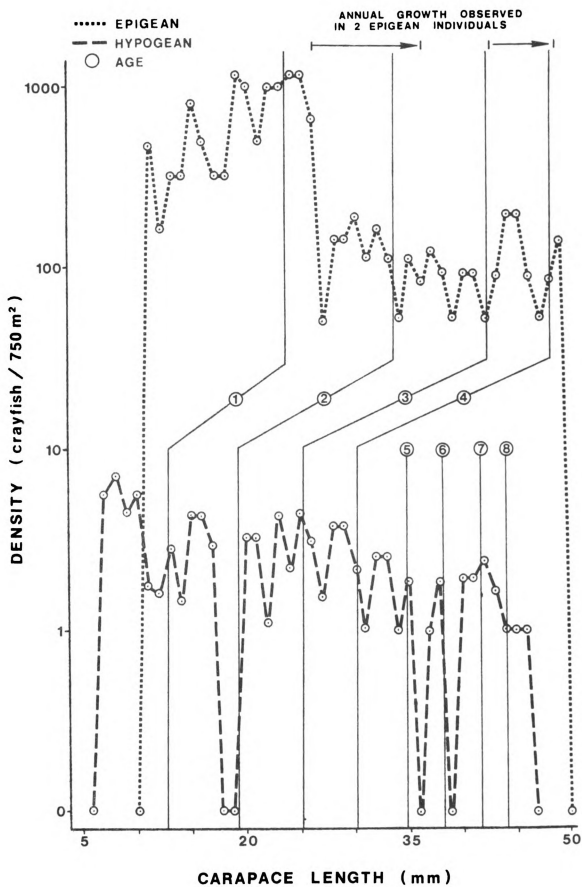


Figure 45

of the first year class and migration. In March of 1969 the population size was 76. Measurements of movement showed that 64% of wandering *C. laevis* were moving upstream at this time. It is reasonable that, if the denser epigeal population was undergoing similar movements, this would result in increasing the size of the upstream cave population; the July census did show an increased population of 96. By July, however, 53% of wandering crayfish were moving downstream, and, apparently as a consequence, the population had decreased to 64 in the November census. Movement of the majority of the crayfish at this time was still downstream (at a rate of 65%), and by the following March the population had decreased to 61.

Population densities varied in different longitudinal zones of the cave stream. Density was highest in the upstream section, from 0 to 99 meters. This section, which was a fifth of the census area, contained an annual mean of 57.4% of the population. Density was lowest in the middle section, with densities progressively increasing in both upstream and downstream sections (Figure 46b). The population levels of the various sections were compared to associated variables (Table 31). The number of *C. laevis* showed positive simple correlations with the number of *O. inermis* ($r = 0.90$), the growth rate of *O. inermis* ($r = 0.94$), and the extent of non-riffle habitat ($r = 0.71$). Only the food level, with a value of -0.30 , did not fit the pattern. The growth rate of *C. laevis* was not tested, because insufficient numbers remained within one stream section throughout the year. Partial correlations, which were calculated for the three variables showing positive correlation, were positive for a number of *O. inermis* ($r = 0.80$) and the growth rate of *O. inermis* ($r = 0.87$); the extent of non-riffle habitat, however, was transformed to a negative correlation ($r = -0.66$).

Figure 46. Inter-specific comparison of longitudinal population levels.

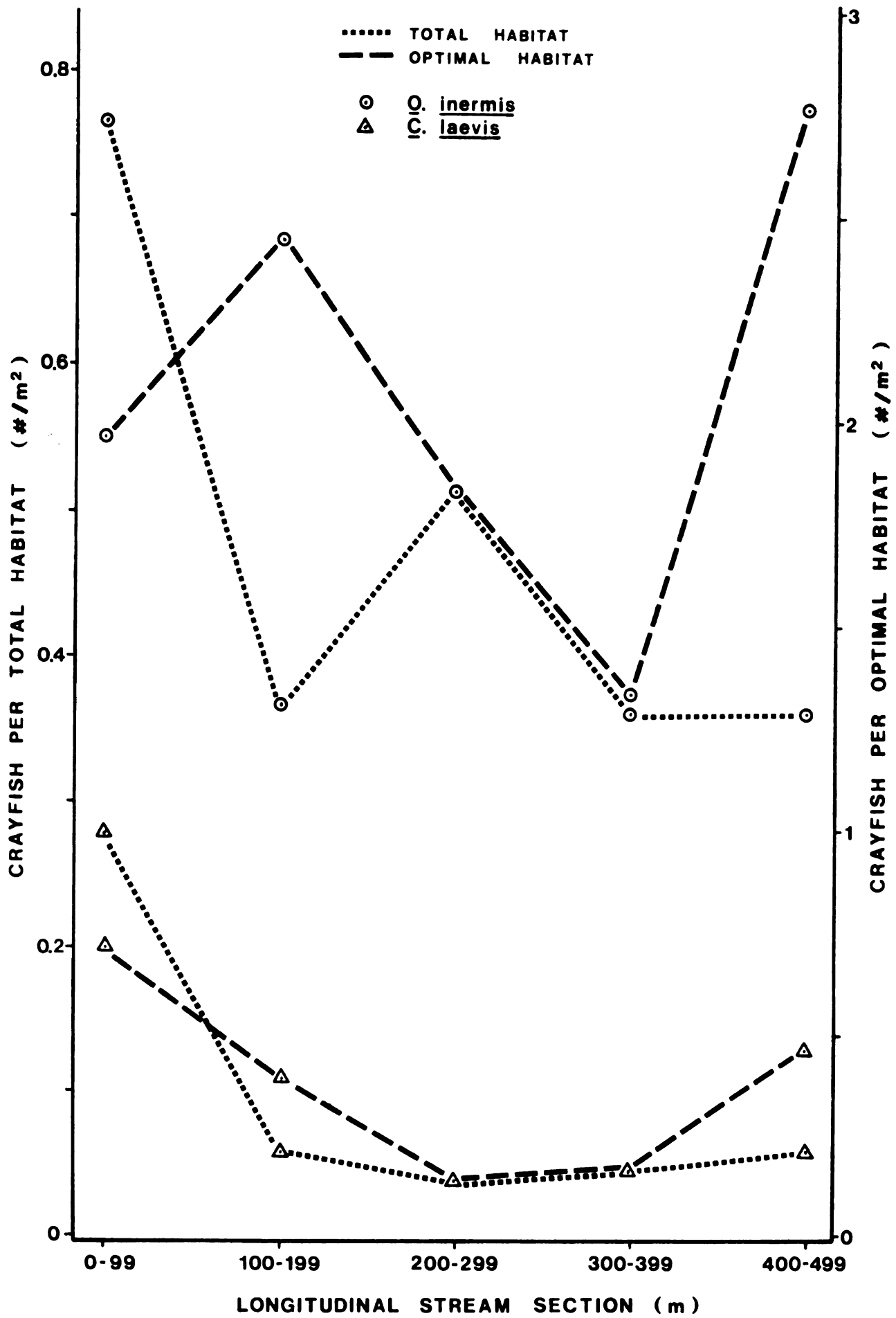


Figure 46

TABLE 31.--LONGITUDINAL VARIATIONS IN CRAYFISH NUMBERS

STREAM SECTION (meter location)	VARIABLE				
	1 MEAN NO. <i>C. laevis</i>	2 MEAN NO. <i>O. inermis</i>	3 <i>O. inermis</i> GROWTH*	4 NON-RIFFLE HABITAT (m)	5 FOOD LEVEL**
0-99	42	115	4.2	39	2.82
100-199	9	55	2.3	15	3.14
200-299	6	77	2.5	28	2.78
300-399	7	54	2.9	27	3.55
400-499	9	54	2.8	13	2.71
MEAN	15	71	2.9	24	3.00
STD. DEV.	15	26	0.7	11	0.35

*mean of annual length increments (mm) that were adjusted for variation in length-specific growth rates

** $r_1 + r_2 + r_3$, where

r = ratio of the measured food level in a stream section versus the mean for the study area

1 = microseston (cal/l)

2 = mud (cal/g)

3 = benthos (mg/m²)

SIMPLE AND (PARTIAL)* CORRELATION MATRICES

VARIABLE	1	2	3	4	5
1	..	0.90 (0.80)	0.94 (0.87)	0.71 (-0.66)	-0.30
2	0.90 (0.80)	..	0.84 (-0.54)	0.86 (0.80)	-0.43
3	0.94 (0.87)	0.84 (-0.54)	..	0.77 (0.64)	-0.19
4	0.71 (-0.66)	0.86 (0.80)	0.77 (0.64)	..	0.00
5	-0.30	-0.43	-0.19	0.00	..

*variable 5 not included

1

Figure 47. Inter-specific comparison of age pyramids, showing means and standard deviations derived from 4 seasonal population estimates.

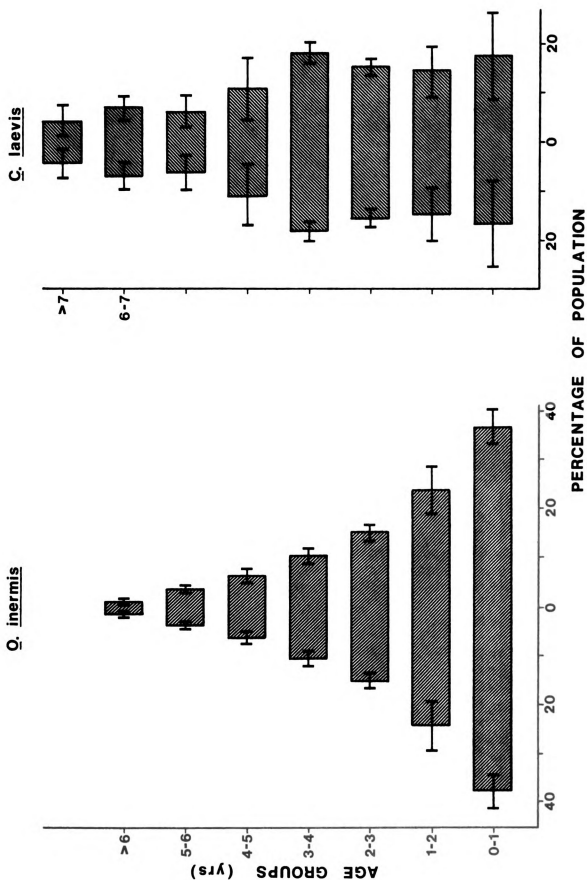


Figure 47

Because of the small number of stream sections studied, none of the partial correlations were significant (*t*-test), although some of the correlation coefficients were quite high. The density pattern seemed to result from movement into the census area, since densities were high in both sections adjacent to boundaries and lowest in the middle section. During the study, crayfish displacement was predominantly in the downstream direction; this movement corresponds quite well with the fact that the greatest crayfish density occurred in the section adjacent to the upstream boundary. This analysis was especially sensitive to such movement, because crayfish locations were determined from the initial capture sites.

Discussion

Tissue growth

Hypogean populations

The growth rate of *O. inermis* was quite low. The mean relative annual rate was 3.33 as compared to 4.64 for *C. laevis*. This was equivalent to a 9.34-fold difference in the absolute growth of the two species. Both crayfish were subject to the same environmental conditions and level of available food. *O. inermis*, with the troglobitic adaptations to the cave habitat, should be more efficient at exploiting the limited food supply than the troglophilic *C. laevis*. If this is true, then the observed growth rates must indicate that *O. inermis* possesses an inherently lower metabolism with restricted ingestion. Poulson (1963) reported a similar five-fold depression of the absolute growth rate in a troglobitic cave fish as compared to a troglophilic relative.

Both crayfish species showed a higher growth rate in the upstream section (0 to 99 meters). The longitudinal differences in the growth rates were apparently related to food quantity, although food quality may also have been of importance. The upstream section received water input through the breakdown at 0 meters and the Graveyard Waterfall Tributary at 71 meters. It also received water and vegetative matter from an intermittent stream flowing through the entrance during periods of flood. Except for a very small tributary at 285 meters, all food in the quantitative study area of the cave stream first passed through this upstream section. The food was depleted as it moved downstream through this section and the next three 100-meter sections. The last section (400-499 meters) received flow from the Blackdamp Tributary at 443 meters. There was a great difference in the amount of organic drift collected from weirs at 79 and 442 meters (see Table 7 above). Assuming that this drifting macroseston was gradually utilized as food as it passed downstream, it is surprising that the growth of *O. inermis* did not show a steady decline with downstream location. Instead, growth was not significantly different from 100 to 499 meters, although the sample mean was significantly higher for the upstream section. The probable explanation is that organic material, largely composed of leaves and twigs that gained access to the cave through the sinkhole entrance, was deposited in the extensive pools of this first section. These first pools apparently filtered out a large portion of the organic input. Any organic debris with specific gravities favoring passage through the first series of pools then passed through the cave or was evenly distributed throughout the remaining pools of the census area.

The *O. inermis* growth rate based on extended interval observations subsequent to the 1969-1970 study period was slightly, but not

significantly, less than that calculated from annual observations. If this decrease, which resulted in a reduction of approximately one mm in the theoretical maximum carapace length, was real, it might have resulted from a reduction in the food supply. This is a possibility because the entry of allochthonous material through the gate of the cave entrance was not strictly maintained during the latter portion of the 3.5-year observation period. Jegla *et al.* (1965) reported that populations of *O. inermis* from different caves differed by as much as 10 mm in maximum length (5 mm carapace length) and believed these variations might have been the result of different levels of food supply. In Shiloh Cave, *O. inermis* inhabiting the lower reach of the main stream attained a larger maximum size than crayfish in the census area. Of 69 crayfish examined from this downstream section, 7% were larger than the largest from the census area; the largest was a male with a carapace length of 32.0 mm found at 825 meters. The damming of the stream in this section causes the accumulation of water-logged detritus, which is an important part of the crayfish diet. Another explanation of the greater maximum length might be that these crayfish possessed increased longevity because they inhabited an area of the cave that received less human interference.

Comparison with epigean populations

From the results it is obvious that the growth rates of experimentally confined crayfish do not parallel natural growth rates. Young-of-the-year *C. laevis* maintained in the cave stream for a year showed a carapace length increase of only 1 mm, whereas information gained from the mark-recapture study indicates that unconstrained cave-inhabiting *C. laevis* probably increased 7.7 mm. In fact, the

experimental crayfish kept in the surface stream, where the growth rate of natural populations was greater than under cave conditions, only increased 6.8 mm in carapace length. These depressed growth rates were probably the result of confinement or the inaccessibility of food of large particle size, which, despite the net flow of substrate and detritus into the chamber, was excluded by the screen closure. Another indication of the unnaturalness of the experimental chambers was gained in a comparison study employing experimental young-of-the-year *O. inermis* in the cave habitat; a mortality of 100% occurred among the six crayfish during the course of a year. Despite lack of naturalness, however, the experimental results, which showed a much higher growth rate for *C. laevis* maintained in the surface stream, probably reflected real differences in the growth rates for crayfish inhabiting the two environments. This difference in growth rate occurred despite the fact that the cages placed in the cave stream were positioned downstream from the entrance sinkhole in a section where food, such as leaf detritus and benthos, was most abundant.

Differential growth rates were substantiated by size-frequency and recapture data for the surface *C. laevis* population, which had an absolute growth approximately 5.5 times greater than the cave population. This resulted in maturity during the third year instead of in the seventh as in the cave population.

The mean relative annual growth rate of 12.33 achieved by the epigean crayfish, *O. virilis*, studied by Momot (1967) was much greater than the hypogean crayfish; the growth rates of *C. laevis* and *O. inermis* were only 38% and 27%, respectively. Thus, despite winter temperatures in the cave stream that were more conducive to growth, the cave populations could not approach the annual growth rate of *O. virilis*, which

achieved rapid growth under the conditions of abundant food and elevated temperatures that prevailed during the spring and summer.

The retarded rate of growth of the cave population sheds some light on a general evolutionary theory developed by Heuts (1953) in his study of African cavefish. He believed that the paucity of food in caves caused a reduction in the bodily growth rate and that from this reduction stemmed all other changes, such as the regressive losses of eyes and pigmentation, and the reappearance of archaic features; these harmonic changes supposedly resulted from the effect of retarded body growth on the allometric growth patterns characteristic of various body systems. Data from the present study does not support this contention. The cave population of *C. laevis* had a much lower rate of body growth than the surface population, but they showed no indication of the morphological traits characteristic of troglobites. If the theory has any validity, it must depend upon genetic changes, such as those that have obviously occurred in the evolution of *O. inermis*, that reduce basal metabolism.

Banta (1907) stated that *C. laevis* inhabiting caves grew to a larger size than those living in epigean habitats. Maximum length information from the present study did not fully substantiate this conclusion (Table 32). The largest male *C. laevis* encountered, which had a carapace length of 58.5 mm (see Figure 43), inhabited Shiloh Cave; however, the largest female (57.0 mm) was found in the Speed Hollow surface stream.

More consistent results were obtained by taking the mean of the 10 largest individuals from each habitat. The hypogean males were smaller, but not to a significant degree, than those from either epigean location. In the case of the females, those from the cave

TABLE 32.--MAXIMUM LENGTHS ATTAINED BY *C. laevis* POPULATIONS LIVING IN DIFFERENT HABITATS; data based on sample of 230 individuals from each location

LOCATION	CARAPACE LENGTH (mm) OF LARGEST INDIVIDUAL		$\bar{x}$, (s) and t-TEST OF CARAPACE LENGTHS OF 10 LARGEST INDIVIDUALS *significant **highly significant	
	MALE	FEMALE	MALES	FEMALES
SHILOH SURFACE STREAM	50.5	49.5	47.4 (1.5) ↓ P>.50	48.2 (1.0) ↓ .01>P**
SHILOH CAVE STREAM	58.5	49.0	46.5 (5.3) ↓ P>.40	46.0 (1.5) ↓ .001>P**
SPEED HOLLOW SURFACE STREAM	50.5	57.0	47.9 (1.2) ↓ P>.02>P*	50.8 (2.9) ↓

were significantly smaller than those from surface locations. There was no difference in size between the sexes in the cave habitat, but females were larger than males in both surface streams, although the difference was significant in only the Speed Hollow location.

Two factors working in opposition were apparently influencing the maximum length attained; the growth rate favored greater maximum length in the surface habitat, whereas differences in longevity favored the cave habitat. From the results it appears that the growth rate was the more influential, although the data from individual and group analysis were not completely consistent. Different collection methods and epigeal-hypogean migration could have had confounding effects.

Molting

The annual cycles of reproduction and molting in the *O. inermis* of Shiloh Cave have been studied by Jegla (1966). Hobbs (1973) studied the molting of *O. inermis* from nearby caves. In general, information on molting gathered during the present study supports their conclusions. Jegla (1966) determined that there were two peak periods of molting for adults and large immatures of both sexes; the spring molt occurred in February and March, and the fall molt in August and September. In the present study, the molting frequency was greatest in the interim from July to November; this corresponded to the fall molt. The rate of molting was lower and approximately equivalent in the other two inter-census periods; this resulted from the March census occurring during the peak of the spring molt. The biannual molting pattern for young adults was evident, however, when individuals were observed throughout the year (Figure 37b).

This bimodal annual pattern of molting suggests a circannian rhythm synchronized by an exogenous factor similar to that operating in the reproductive cycle. However, this environmental cue did not have a pervasive or rigid temporal effect on the crayfish, because the molting pattern represented only changes in frequency at the population level and molting actually took place throughout the year. Whatever the environmental factor, it apparently initiates hormonal changes. The sinus gland, an important neurohemal organ located in the eyestalk, is involved in control of molting. Although the eye has undergone degeneration in troglobitic crayfish, the sinus gland in *O. inermis* is morphologically the same as that found in eyed crayfish (Jegla, 1964). Fingerman et al. (1964) have found a similar situation in the cave crayfish, *O. setosus*.

Many epigean species show a similar pattern of two annual molts for the adult males, but with only a single molt occurring in mature females; this divergent pattern is found, for example, in *O. virilis* (Momot, 1967), *O. propinquus* (Van Deventer, 1937), and *C. longulus* (Smart, 1962). The females of these species forego the second molt and subsequent body tissue growth; they instead funnel energy into developing oocytes. In *O. inermis*, however, most females failed to carry oocyte maturation to completion, and this resulted in a pattern of egg resorption and body growth that terminated with a second annual molt.

The two annual molts in mature male crayfish function not only in growth, but also in sequencing reproductive and nonreproductive sexual forms, which are usually synchronized to a fall breeding season. Creaser (1934) presented evidence to show that in *O. propinquus* growth does not always accompany molting that is associated with a

change of male sexual form. Males molting in response to the reproductive cycle, instead of growth, may explain the slight sexual differences in molting (males molted more frequently, but with a smaller growth increment) observed in *O. inermis* during the present study.

There was no clear seasonal variation in the molting frequency of juveniles. Molting, which occurred at a rate greater than that of young adults, was in response to endogenous growth stimuli. Unlike most epigean crayfish, growth occurred throughout the entire year. Older adults also lacked the biannual molting pattern characteristic of young adults. In this case, however, the molting frequency was reduced, usually to a single molt annually.

C. laevis faces unique problems because it is an epigean crayfish, from an evolutionary viewpoint, invading a hypogean habitat. The results from studies of other crayfish species suggest that this could pose special difficulties for initiating ecdysis. The strength of the tendency to molt increased with increasing length of photoperiod in the epigean crayfish, *O. virilis*; individuals maintained in constant darkness did not molt (Stephens, 1955). In *O. clypeatus*, Mobberly (1963) found molting peaks to be influenced by temperature and duration of light. Hypogean *C. laevis* live in an environment practically devoid of these exogenous cues necessary to initiate molting in most epigean crayfish, although temperature and other factors associated with flooding do undergo some variations.

In spite of these considerations, *C. laevis* inhabiting the cave stream were observed to have a molting pattern remarkably similar to *O. inermis*. The molting of juveniles, as in regeneration molting, was probably under endogenous control, but molting in adults required environmental cues to synchronize the population in a reproductive cycle.

The reproductive cycle appeared to be functioning in the cave *C. laevis*, so it seems logical that seasonal cues influenced molting, although this was not evident from the meager data collected. The data indicated that young adult *C. laevis* had a biannual molting pattern similar to that in *O. inermis*, but that they lacked any synchronized molting at the population level. It is possible that population turnover, by means of immigration and emigration, may perpetuate the reproductive cycle in the cave.

One question that should be addressed is whether the method of tagging had any effect on molting. This is a valid concern, because Stewart and Squires (1968) found that under certain adverse conditions tagging techniques, which were somewhat similar to the one employed in the present study, resulted in reduced molting rates in lobsters. Although there was no direct evidence to refute this possibility, the molting data had a logical pattern which showed no discernible evidence of interference.

Reproduction

The determination of spawning rates was based on the assumption that gravid crayfish were as prone to capture as other crayfish. Because of the long developmental periods characteristic of both species inhabiting the cave (about 109 days for *O. inermis*), there was a high probability of finding crayfish in berry. Several crayfish studies (Scudamore, 1948; Smart, 1962; Smith, 1953), however, have shown crayfish in berry to be secretive, but these were usually cases in which the species have a burrowing habit. Hobbs (1973) believes that both *O. inermis* and *C. laevis* have fossorial behavior, but this trait is probably not developed to the extent found in the species of the studies cited above. A study of *Orconectes virilis* by Momot (1967) showed that

females continued to feed while carrying eggs; it is reasonable to assume, therefore, that non-burrowing crayfish maintain normal activity patterns during the ovigerous period and that the low percentages of adult female *O. inermis* found to be reproducing annually were an accurate estimation. The cavefish, *Amblyopsis spelaea*, which inhabits neighboring caves, showed a similar reproductive pattern with only 10% of adult females breeding annually (Poulson, 1963).

O. inermis had an extremely low spawning rate, with less than 20% of adult females laying eggs annually. In contrast, at least 98% of mature females of the epigean crayfish, *Orconectes immunis*, produced eggs annually (Tack, 1941). The low fecundity of the *O. inermis* population probably resulted from the failure of females to obtain the critical threshold of energy necessary to maintain egg development. In the spring only 30% of the females showed evidence of oocyte enlargement and cement gland development, and of these about one-third failed to lay eggs. This restricted the population size and served as an adaptation to the low food level of the cave. There was no evidence, however, that the size of individual spawns had been reduced. The ovigerous cave crayfish had a mean of 13.0 eggs per wet gram of maternal tissue. In the epigean crayfish, *Orconectes virilis*, a value of 8.8 eggs per gram was derived from the data of Momot (1967), although the larger mature size of *O. virilis* may have been a confounding factor.

The low fecundity of the *O. inermis* population was accompanied by a retardation of the age of first reproduction and an increase in longevity. Epigean crayfish species commonly begin to spawn in the second and third years in contrast to the onset of reproduction during the fifth year in the cave crayfish, *O. inermis*. Longevity was likewise extended, but to what degree is unknown. Poulson (1963) has demonstrated

similar phenomena in related species of amblyopsid fish that show a sequence of adaptation to the cave habitat. He reported a 3.3-fold and 5.6-fold increase in age of first reproduction and longevity, respectively. Whereas decreased fecundity and increased age of initial reproduction would tend to depress the reproductive potential of the species, the extended reproductive period, which results from the increased longevity, would have the opposite effect.

One of the fundamental questions concerning the *C. laevis* crayfish is whether they have the capacity to reproduce in the cave habitat. With the notable exception of copulation, most aspects of the life cycle have been observed in this study. The presence and growth of all sizes, from newly hatched to sexually mature, have been demonstrated. The transformation of males from second to first form, and the carrying and hatching of eggs by females have been observed. An important consideration is whether females of the cave population could find and ingest sufficient food to promote growth of oocytes, or whether gravid females were recent immigrants from the epigeal population. In two of the five cases of egg-carrying females found in the cave, the crayfish were first captured before oviposition occurred. One of these was observed in February, and at that time cement glands were not developed. When this crayfish was recaptured five months later, the cement glands were developed, and oviposition of 70 eggs took place within 40 days. The development of pleopod eggs in a crayfish known to have resided in the cave for at least six months previous to oviposition is sufficient evidence to justify the assumption that *C. laevis* is a true eutroglophile, with the ability to complete its life cycle in the cave habitat. This assumption, however, does not rule out recruitment of females with developed ovarian or pleopod eggs from the epigeal population.

Comparison of the epigean and hypogean *C. laevis* showed some differences in ovigerous females. In the cave population only 25% of the potentially breeding females were in berry, whereas in surface populations the rate was 44%. There was also a difference in pleopod egg counts; the mean for cave crayfish was 97.7 ($s = 75.4$) and for surface crayfish 132.2 ($s = 68.5$), but the difference was not significant with the small samples obtained. A lower mean egg count was obtained from the cave population even though the mean carapace length of the ovigerous females from the epigean habitat was smaller (42.5 mm C L versus 43.3 mm C L). Variations in egg counts from both habitats were large; this may have been caused in part by migration, with a period of egg development being spent in the alternate habitat. The above information indicated that fecundity was reduced by a decrease in the percentage of females producing pleopod eggs and, possibly also, by a decrease in the number of pleopod eggs oviposited per female.

Retardation of the age of initial reproduction, as in *O. inermis*, was also characteristic of the cave-dwelling *C. laevis*. The surface populations probably reached sexual maturity in the third year. A member of the cave population required over six years to reach maturity. This is a long period of time, especially in light of the significant mortality factors of microsporidian infestation and spelunker traffic. For this reason, immigration is probably an important factor in maintaining the cave population, and the eutroglophilic nature of this crayfish, that is, the ability to complete its life cycle in the hypogean environment, is probably more relevant at the population level than at the level of the individual.

Cave streams have often been characterized as environments with little seasonal change. For example, they lack the change in

photoperiod characteristic of surface streams. Seasonal changes are limited to variations in water chemistry, pH, turbidity, organic content, flow, and temperature.

Temperature change has usually been considered quite small; Jegla (1966) reported an annual variation of only 1° C for Shiloh Cave. Temperature data from the present study, collected with a maximum-minimum thermometer, indicated an annual range of over 11° C; however, the temperature difference between summer and winter, during periods of normal seasonal flow, was only 1.5° C. Apparently, large temperature fluctuations were associated with sudden flooding and were of a transitory nature. A 3.6° C temperature drop associated with a winter flood was recorded by Scott (1909) in a neighboring cave.

It is uncertain whether temperature or another factor served as an environmental cue, but both crayfish species had similar annual reproductive cycles, releasing young during October and November. The existence of an annual reproductive cycle for *O. inermis* was also concluded by Jegla (1966); this was based on five ovigerous females found in Shiloh Cave over a five year period, all from 30 June to 21 August. Park, Roberts and Harris (1941) reported *Orconectes p. pellucidus* carrying eggs in June and September, which is consistent with the above data; based on the observations of a cave guide, however, they concluded that breeding periodicity was poorly developed. In Shiloh Cave, *O. inermis* carrying eggs were found over a 4.5-month time span, and those bearing young were found over a 2.5-month period; however, since developmental time was 3.5 months, it was impossible to ascertain the true extent of synchronization. It seems logical that, with the lack of such a precise exogenous cue as photoperiod, the annual synchrony of cave populations would also be less precise than surface populations.

Jegla and Poulson (1970) postulated that the *O. inermis* of Shiloh Cave have a circannian reproductive rhythm that is synchronized by the peak of annual surface run-off. They stated that egg-laying was triggered by this peak of surface run-off, but did not explain how the annual peak was recognized without the aid of retrospect.

The circannian reproductive rhythm in *C. laevis* is somewhat surprising when it is considered that most of the seasonal environmental cues are lacking in the hypogean habitat. Light, for example, has been shown to be important in controlling the reproductive cycle. In *O. virilis* kept under conditions of continuous darkness the oocytes underwent progressive maturation, but new oocyte formation and egg-laying were inhibited; light was necessary for cyclical activity (Stephens, 1952). Lowe (1961) found both temperature and duration of light influenced the reproductive cycle of *Cambarellus shufeldti*. In *O. rusticus* the cement glands of crayfish maintained in constant darkness developed at a slower rate than those of comparable animals exposed to a photoperiod (Stephens, 1953). In *C. laevis*, light, or the lack of it, was obviously not a factor imposing absolute control on the reproductive cycle. Apparently a different Zeitgeber functions in the cave, or the cave population is somehow synchronized with the surface population by means of the exchange of individuals between the two habitats.

The reproductive cycle is especially interesting because previous studies have often reported that crayfish of the cool water type, such as *C. bartoni* and some related species, often spawn throughout the year. This is refuted by McManus (1960), who believes that cases in which young are found attached to females in winter result from temperature-related arrested development. This belief in year-round spawning is also based on the fact that males of the copulatory form are present throughout the

year. This observation is supported by the present study; both the epigean and hypogean populations, although having a maximum proportion of copulatory males in the fall, had form I males present throughout the year. Although this may possibly suggest a lack of cyclic breeding activity, it does not indicate a lack of cyclic oviposition, since ovigerous females were only found during a restricted period of the year. Similar patterns of a cycling percentage of first form males and a definite spawning period have also been reported for *C. robustus* (McManus, 1961) and *C. bartoni* (Meredith and Schwartz, 1960). There is evidence to the contrary, however; in nearby Pless Cave, Hobbs (1973) found a *C. laevis* with young in April.

Mortality

General observations

The survivorship patterns based on both horizontal and vertical sampling data were strikingly similar in *O. inermis*. This indicates a high degree of temporal stability in recruitment and age-specific mortality. Aquatic cave communities generally have fairly low species diversity, and populational stability is contrary to what one would normally expect in this situation. Margalef (1969) has commented on this unusual combination, which is characteristic of both deep-sea and cave communities; he stated that, since diversity is not high, the extreme stability indicates that these ecosystems possess a high degree of organization. In caves, at least for troglobitic populations, stability probably results in large part from the shortness and inter-connectiveness of the food web. The cave crayfish feeds either directly on the allochthonous food base as a detritivore, or one step removed

as a predator of the benthos. This omnivorous nature provides trophic flexibility that enhances populational stability. Other important factors are probably the near constancy of the environment, and the low rate of successful invasion by members of other ecosystems.

A comparison of *C. laevis* survivorship patterns based on horizontal and vertical sampling indicate some temporal instability in population size and structure. Poulson (1965) stated that a low level of population regulation was characteristic of a troglophilic cavefish and believed that the fluctuations in population size were caused by an insufficient food supply. Troglophilic populations are generally not as efficiently adapted to utilizing the available hypogean food resources as are troglobites; consequently, food is more often insufficient for the troglophile, *C. laevis*, than it is for the troglobite, *O. inermis*. Temporal variations in food availability may have a profound effect on the hypogean population of *C. laevis*; these variations may result in changes to the mortality rate, the reproductive rate and the migration pattern between the epigean and hypogean populations. The operation of any of these factors would result in instability of the cave population.

In *O. inermis*, differential mortalities between the sexes were demonstrated. These generally favored the male sex — a result contrary to the often reported mass mortalities among males of other crayfish species. Size-specific differences in the mortality rates for each sex seemed in part to be correlated with the attainment of sexual maturity. The sexual patterns of mortalities and survivorships for *O. inermis* were very similar to the *O. virilis* population studied by Momot (1967), although the correspondence was best in terms of absolute rather than maturational age.

The annual actual mortality rates for *O. inermis* were quite low. The mean annual mortality rate was 0.443, as compared to the 0.722 value calculated by Momot (1967) for the *O. virilis* crayfish inhabiting a marl lake. Because of problems arising from migration, annual mortality of the cave *C. laevis* was not clearly defined, but it also appeared to be lower than that of the epigean crayfish, *O. virilis*. Since the mortality rate is the summation of many factors, such as environmental rigor, pollution, disease, starvation, predation, molting and physiological aging, no single factor is probably entirely responsible for the mortality differences between epigean and hypogean populations. An evaluation of the relative importance of the various factors for the two *Orconectes* populations will be made in the discussion of mortality factors.

Mortality factors

The various types of mortality to which the cave crayfish are subject will be discussed, and, where applicable, comparisons will be made with the *O. virilis* population of West Lost Lake. The ecology and population dynamics of this epigean crayfish are well known from the study of Momot (1967).

Physiological aging.— Aging is associated with rate of metabolism. The metabolic rate, as measured by rate of respiration (see the section on respiration below), was greatly reduced in *O. inermis* — the probable result of both restricted food intake and genetic control. Poulson (1964) reported that data on metabolic rates showed that metabolism was lower for troglobites than for epigean species. Aging is also temperature dependent. However, the mean annual temperature of West Lost Lake is probably not much different from that of the cave; consequently, temperature is probably not very significant in this respect.

The *C. laevis* populations are of special interest in respect to aging. Although both the cave and surface populations were exposed to nearly equivalent water temperatures and had equivalent basal metabolic rates, the cave population apparently had a much longer life span. The growth rate and the time required to reach sexual maturity may be factors involved in this difference in longevity.

Environmental stress. - On the other hand, the temperature variation (0.4 - 18° C) of West Lost Lake was much greater than the near constant temperature of the cave stream. This environmental stress, which results in severe overwinter mortality of the lake species, was one mortality factor not encountered by the cave crayfish. Light, itself, appears to be a form of stress. Stephens (1955) found that a group of *O. virilis* kept in constant darkness had a lower mortality rate than other groups subjected to various light regimes. Flooding appeared to be the major stress operating on the cave crayfish, but it is probably of insignificant effect.

Molting. - Mortality associated with molting is probably also diminished in cave crayfish as compared with their epigean counterparts. The molting period is subject to a high risk of death. This stems from both predatory and physiological factors. The lower food level in the cave stream depressed growth and reduced the molting frequency of juvenile stages; as a result, the importance of molting as an annual mortality factor was decreased.

Pollution. - Crayfish may be quite susceptible to certain kinds of pollution. Pollution of the Cheat River by acid mine wastes and the lumber industry have restricted the distribution and abundance of

C. bartoni and *O. obscurus* (Schwartz and Meredith, 1962). Although Duffield (1933) minimized the significance of pollution, he reported that Hofer (1909) had associated a crayfish epidemic with an intestinal bacterium that was propagated by river pollution.

The magnitude of pollution in the waters of Shiloh Cave is unknown, but is probably of a low level. Agricultural pesticides and organic matter from septic tanks are the principal types. Analysis of the organic content in waters of several neighboring caves indicated that Shiloh Cave was subject to comparatively little organic input. The clay soil of this region is not highly suited for the efficient functioning of septic tanks, and, judging from measurements of microseston energy contents and benthic standing crops, several nearby cave streams already seem to be experiencing a heavy load of organic pollution. Although Shiloh Cave is presently receiving only low levels of organic pollution, the number of septic tanks in the watershed is steadily increasing, and this additional organic input could create problems for the cave community in the future. It is assumed that pollution of the stream of Shiloh Cave is greater than the *O. virilis* lake habitat, which receives drainage primarily from wilderness areas. Pollution, however, does not appear to significantly affect either crayfish population.

Predation. - The crayfish that Momot (1967) studied suffered predation by trout, but to what extent was not reported. Van Deventer (1937) stated that fish predators exacted a fairly uniform (but unspecified) toll in all size classes of an *O. propinquus* population in an Illinois stream. Several species of fish, salamanders, frogs, turtles, and birds, and the water snake and raccoon were listed by Tack (1941) as crayfish predators. Poulson (1963) reported that the

cavefish, *Amblyopsis spelaea*, which inhabits neighboring caves, preyed on cave crayfish.

The cavefish did not inhabit Shiloh Cave and, with one exception, neither did any of the predators listed by Tack (1941). The lone exception was a small population of frogs, identified as *Rana palustris*, which inhabited the cave during cold weather. They were usually in a torpid state and were probably ineffective in prey capture in a lightless environment. Stomach analysis of one female frog with developing eggs gave evidence of a diet consisting only of vegetative matter. This observation, plus the fact that they were predominantly located near the spring entrance, negated any significant predation of the crayfish in the census area.

There is a probability, however, that *C. laevis* preyed on *O. inermis*. On nine occasions during the crayfish surveys, *C. laevis* and *O. inermis* were found occupying the same trap. Death resulted for *O. inermis* in 5 of the 6 cases in which the *C. laevis* crayfish were larger in size. In one case the two crayfish were of equal length, and the *O. inermis* crayfish was injured but not eaten. *O. inermis* and *C. laevis* cohabited the trap without injury to either in the two cases in which the *O. inermis* crayfish were larger in size. It must be emphasized, however, that the extremely small confines of the traps were highly unnatural conditions. The bait in the traps may have altered behavior additionally, since it was reported by Bovbjerg (1970) that the presence of animal food heightened aggressions in crayfish.

Other limited information concerning the interspecific relationship of the crayfish populations was gained from a feeding study carried out in 60 square meters of enclosed stream. This study employed mixed populations of crayfish of approximately equivalent body size.

Although the habitat was natural in this case, the crayfish were rigged with a food-study device that hampered escape behavior. During this study one case of predation of *O. inermis* by *C. laevis* was observed.

In the stream itself, *C. laevis* crayfish were observed eating *O. inermis* on only two occasions. It is uncertain, however, whether these were cases of predation or scavenging.

Eberly (1960) assumed that predation of the troglobitic crayfish by the troglophile occurred and considered it to be of pivotal importance in the equilibrium of the two populations. Information derived from the present study supports the occurrence of interspecific crayfish predation under unnatural conditions, but knowledge of the natural relationship is lacking. Hobbs (1973) stated that *C. laevis* and *O. inermis* in Mayfield's Cave, although frequently observed within 0.3 meters of one another, displayed no apparent concern for each other. This agrees with observations made in the present study. It is assumed that predation, if it does occur, is of minor proportions, otherwise the *O. inermis* population with its low reproductive rate would soon be decimated. The low mortality rate characteristic of the *O. inermis* population is strong argument against predation being a significant interaction. However, the observed fact that several nearby cave streams are inhabited only by *C. laevis* might possibly have resulted from predatory elimination of *O. inermis*.

Cannibalism was also observed in the traps. For those cases in which two individuals of the same species occupied the same trap, cannibalism or dismemberment occurred at a rate of 100% in *C. laevis* and 29% in *O. inermis*. It should be pointed out, however, that these observations once again pertain to a highly unnatural situation in which two crayfish were forced to share a very small space and in

which escape behavior was impossible. Therefore, as in interspecific crayfish predation, because of both the low density populations and the ability to carry out escape behavior that exist in the natural habitat, cannibalism is assumed to be a less important interaction than indicated by these observations. Poulson (1965), however, observed cannibalism in cavefish and believed it to be of importance in stabilizing their populations.

Human interference. - An important mortality factor in Shiloh Cave is the interference caused by human visitation. Interference takes several forms, such as specimen collecting, dumping carbide lamp residue into the stream, and stepping on crayfish. For instance, two crushed *C. laevis* crayfish were found after a party of three spelunkers were allowed to enter the cave. During the study spelunker traffic was curtailed, but elimination of this source of mortality was probably balanced by study-associated deaths, which totaled 12 *C. laevis* and 13 *O. inermis*.

Disease. - A few of the *O. inermis* displayed an aberrant condition in which the musculature had a white discoloration; this is symptomatic of a heavy infection by a microsporidian parasite. Sprague (1950) first reported the occurrence of this parasite in North American crayfish, and assumed that it caused the death of the host. In the present study all crayfish displaying this symptom were quite lethargic, and one held for observation died within 26 days. Individuals displaying symptoms were found throughout the year, and the disease seemed to be non-selective of size or sex. The percentage of the population heavily infested was never large and reached a high of 1.8% in the November census.

The first reported incidence of infestation of North American crayfish by microsporidian parasites occurred in *C. bartoni* inhabiting mountain streams along the Georgia-North Carolina border (Sprague, 1950). A similar infestation appeared to be prevalent in some populations of *C. laevis* investigated in the present study. The epigean population living in the Speed Hollow stream had a low incidence, reaching its highest level of 3% in July. *C. laevis* inhabiting the surface stream issuing from Shiloh Cave had a higher rate of infestation, with 11% of the population showing symptoms of the disease in July. In both populations an annual cycle of infestation seemed evident. The disease did not appear to be age or sex specific. *C. laevis* inhabiting Shiloh Cave during the year of study showed no symptoms of the disease.

The fact that the hypogean *C. laevis* were free of the parasite, in spite of the infested epigean population inhabiting the same stream, would seem to indicate a high degree of isolation. However, Duffield's (1933) discussion of the significance and characteristics of microsporidian epidemics in English crayfish populations suggests three other possible explanations: (1) crayfish epidemics are usually preceded by a period of extreme population abundance — a condition which did not occur in the subterranean habitat; (2) epidemics usually spread upstream; and (3) crayfish living in side streams and head waters often survive epidemics of the main stream. The validity of the second explanation was upheld by the observation of infected cave *C. laevis* at a later date, in July of 1970.

Starvation. - There is no evidence that starvation was an important mortality factor. It has been shown that food was extremely scarce, but the effect on the crayfish populations seemed to be only a

curtailment of certain energy-requiring functions, such as growth and reproduction. Inter-annual variation of the food input, in comparison to other ecosystems, is probably relatively slight; such variation could probably be accommodated by the above-mentioned mechanisms. Although *C. laevis* crayfish because of their higher energy requirements would probably be more prone to starvation, they also have the capability of emigrating to the epigean habitat.

Momot (1967) did not indicate that starvation was significant in the epigean lake habitat either. He found no difference in crayfish stomach contents between spring and fall.

Longevity

For both *O. inermis* and *C. laevis* the maximum age observed was in the range of 9 to 10 years. These life spans are much greater than those of epigean crayfish species reported in the literature, which range from one year for *Cambarellus shufeldti* (Penn, 1942) to five years for *Cambarus robustus* (McManus, 1961). *Orconectes virilis* has a three year life span (Momot, 1967). A study of the troglobitic crayfish, *O. australis* (formerly classified along with *O. inermis* as *O. pellucidus*), however, has provided evidence for longevity much greater than those estimated in the present study; based on the averaged observed growth rates, this species was calculated to have a life span of 176 years (Cooper, 1975).

The calculated life spans of the Shiloh Cave crayfish are thought to be fairly accurate, but it must be kept in mind that age determinations were made on only a few recaptured individuals. Larger samples might have extended the calculated longevity. Another consideration is that the 9-10 year figure represents crayfish that were still alive,

and is not directly a measure of longevity. On the other hand, several subsequent surveys of the crayfish populations in the cave have not resulted in any recaptures of marked crayfish. Questions remain concerning the relationship of longevity and sexual activity. Jegla (1965) stated that by the close of the third breeding period the lobes of the *O. inermis* were 71% degenerated and were probably incapable of producing more spermatogonia. In the present study, the male of this species was estimated to have a post-maturation life span of approximately 7 years. Thus, further research is indicated to resolve these separate findings.

With the exception of the data on *O. australis*, the calculated longevity for the Shiloh Cave crayfish agree quite well with other cavernicolous species. Ginet (1960) reported a troglobitic amphipod with a life span of approximately 6 years. Poulson (1963) reported a longevity in the cavefish, *Amblyopsis spelaea*, of 7 years — a 5.6-fold increase over the epigean relative, *Chologaster cornuta*. However, Poulson (personal communication) indicates that his longevity estimates, based on scale annuli, must be questioned; he cites another study that, based on growth between captures, suggests that this species lives 20 years.

Although there is great variation in the estimated life spans of those species that have been studied, it has been demonstrated that prolonged life cycles are characteristic of species that inhabit caves. In many cases this has been attributed to reduced metabolic rates, but evidence gained in the present study suggests that other factors may be involved. The longevity of hypogean *C. laevis* appeared to be roughly 2.5 times that of epigean members of the species, whereas measured respiration rates (see section on respiration below) were

not significantly different. In this troglophile, at least, longevity may be dependent upon the rate of body growth and attainment of sexual maturity.

Population size and structure

The *O. inermis* population underwent seasonal changes of sexual activity. In females this was evidenced by cyclic development of eggs and cement glands; in males there was a semi-annual change between functional and non-functional forms of the gonopods. A circannian reproductive cycle for *O. inermis* had previously been reported by Jegla and Poulson (1970). Results of this study agree, at least with respect to temporal phasing, with the conclusions of Jegla (1966), who studied this same population. Direct comparisons could not be drawn in the case of females, but in males there were differences in the seasonal proportions of the two sexual forms. In the present study the percentage (mean of age groups) of form I males was lower throughout the year; in the fall, 75% versus a range of 84 to 97% in Jegla's data, and in the summer, 7% versus a range of 27 to 50%.

The change in the percentage of males in the active sexual form may function as a population regulation mechanism. Form II males that ingested insufficient amounts of food to carry out the summer molt to the first form condition would not mate, and the decreased recruitment would adjust the population to the food supply. The winter molt to the inactive form would be less subject to this type of control, because of the more plentiful food supplied by winter floods. Therefore, a decreased food base would shift the proportion of the two sexual states toward the second form.

George (1976) suggested a mechanism in the salmonids of Lake George, in which males are the epideictic sex with responsibility for population density regulation. In the present study, however, both sexes are probably involved in control of population density. There are two reasons why this type of population regulation in males would probably not have the impact of a similar mechanism in females, which involves resorption of eggs when insufficient food is available (see section on reproduction above). First of all, the decrease in active form males is not necessarily accompanied by an equivalent decrease in mating, because those male crayfish that are first form may become more sexually active. Secondly, unlike the egg resorption mechanism in females, this feedback mechanism would have a lag period of a year between food deficiency and recruitment reduction.

In examining the survivorship curve (based on vertical sampling) of *O. inermis*, the consistent pattern (Figure 47) of the population decrease with age suggests extreme inter-annual stability. Either a constant rate of recruitment or a density-dependent mortality of young-of-the-year crayfish must be responsible. The influence of food availability on mating and egg development has been previously discussed. In either case, a finely-tuned feed-back system between environmental carrying capacity and the population is indicated. In addition, the survivorship curve suggests that the carrying capacity of the cave stream is stable from year to year. There is, however, some question about the degree of inter-annual stability in the food input; this study has indicated the important role of flooding, which appears to be quite variable in occurrence.

Young-of-the-year crayfish made up only 38.8% of the *O. inermis* population. By comparison, 67% of the epigeal crayfish population

studied by Momot (1967) were young-of-the-year. The low percentage for the cave crayfish indicates that the magnitude of both recruitment and mortality have been minimized in the dynamics of the population. The result is that very little energy is wasted in recruitment, and a high percentage of recruited individuals reach reproductive capacity.

In *C. laevis*, analysis of population structure was complicated by an additional factor. Extensive evidence points to exchange between the cave population and certain elements of the surface population. Changes in the size of the cave population corresponded to the mean direction of wandering, assuming movement between an upstream, sparse, cave population and a downstream, dense, surface population. Immigration of surface *C. laevis* into the cave is highly probable. Many investigators have observed the tendency of *Cambarus* species to enter springs. In addition, certain age (length) groups in the cave that were numerically larger than preceding age groups were equivalent in length to the high density young-of-the-year class inhabiting the surface stream (Figure 45). Studies of Bovbjerg (1956 and 1964) on dominance and dispersion in crayfish lead one to the conclusion that smaller sized individuals of a crowded population would most likely disperse. As a result, the preponderance of females in young-of-the-year surface crayfish apparently caused a shift in the sex ratio (favoring females) of equivalently sized cave crayfish. Emigration of *C. laevis* from the cave also occurred. This loss was illustrated by the one observed case of an individual moving between the two habitats — a female, initially 37.5 mm in carapace length, that was tagged in the cave near the entrance and was found fourteen months later in the surface stream. During the year of study, the cave *C. laevis* population, especially some of the older age groups, underwent a decline. During the same period, the mean

displacement for the population was downstream, and this suggests that emigration may have been a significant cause of the populational decrease.

Other observations indicated that migration patterns may be more complex, involving a significant level of movement between the census area and the cave upstream of the entrance breakdown. The nature of this section of the cave stream is unknown, but it is thought that it does not connect with any permanent body of surface water. One fact that suggests the significance of this upstream connection was evident from crayfish capture locations; over 57% of *C. laevis* were first encountered in the upstream 100-meter section of the census area, while only 12.7% were first encountered in the section adjacent to the downstream census boundary. This suggests the major influx into the census area from the upstream direction. However, the large number of initial captures in this reach of the stream may also have resulted from crayfish immigration from the surface stream. In response to positive rheotaxis they might have moved rapidly through the lower reaches of the census area until a barrier or stimulus that inhibited further upstream movement was encountered at the entrance breakdown. This section of cave stream near the entrance contained an exceptionally large proportion of the cave population, and, besides migration, the amount of available habitat, the amount of food and the presence of the twilight zone may have been factors leading to a high density population.

It is uncertain as to the relative importances of migration between either the census area and other reaches of the cave stream or the census area and the surface stream. There is no doubt, on the other hand, that the census area *C. laevis* population was subject to extensive

migration. This was illustrated by the high annual migration rates (up to 83%) that were determined by the decreasing percentage of tagged crayfish in the population (see section of activity above). The true degree of exchange between the epigean and hypogean populations was obscured by the small samples, the complex movement patterns of individuals, and the fact that movement patterns within the cave may have been different from those in the surface habitat. Despite these complications, the generalized pattern during the year of study seemed to be movement of smaller crayfish into the cave and larger ones out of the cave.

Although the epigean and hypogean populations of *C. laevis* superficially appeared to be similar, they were subject to vastly different growth rates, which imposed different length-age relationships in the two habitats. This relationship in the crayfish of the surface stream has been roughly estimated from size structure and limited growth data. Maturity was estimated to be reached at about two years, and they were judged to have a longevity of about four years. These estimates do not completely agree with the findings of Prins (1965), who studied *C. tenebrosus* in a Kentucky springbrook. *C. tenebrosus* and *C. laevis* are closely related and were formerly considered to be subspecies of *C. bartoni*; both species are also characterized by a troglomorphic habit. Prins stated that maturity was reached in 20 months and that their longevity was three years. However, *C. tenebrosus* attained a larger size in three years than *C. laevis* did in four. Differences in growth between these two species might be partly attributable to differences in temperature, although the stream inhabited by *C. tenebrosus* was only 1° C warmer. The epigean population of *C. laevis* was quite similar in age structure to the *C. robustus* population of a New York stream

(McManus, 1961). This population attains maturity after two full growing seasons and may live to be five years of age; growth, however, is slower with the smallest adults having a carapace length of 30 mm.

Because of the different growth rates in the epigean and hypogean populations of *C. laevis*, and, because movement between the two habitats has been demonstrated, it must be pointed out that aging based on carapace length was not accurate for all individuals. At any given time, certain members of the cave population may have experienced a history of growth in the surface stream. Although the population maintained an identity and could be characterized, there was an exchange of certain components with the surface population. Because individual histories could not be determined for a significant portion of the population, the aging procedure, based on individuals known to have been long-time residents of the cave stream, had to be applied to all individuals indiscriminantly. Thus, this aging convention was employed with the understanding that it was not accurate for all individuals.

It is difficult to compare the population levels of the Shiloh Cave crayfish with data from other cave studies; this is due primarily to the lack of comparable data. Cooper (1975) studied multi-species crayfish populations inhabiting the lentic waters of Shelta Cave. The ponded waters of this cave vary tremendously in area during the year. However, at low water, when the populations were most concentrated, the three most populous species had only 57% the population density of Shiloh Cave crayfish.

Hobbs (1973) studied Mayfield's Cave, which harbors the same two crayfish species as Shiloh Cave. He found the numerical relationship between the two species to be the same as in the present study, with *O. inermis* comprising 83% of the total population. Densities cannot

be accurately compared because information on the width of the stream of Mayfield's Cave is lacking. From personal observation of this stream, however, width, and consequently crayfish density, could be estimated; the density, like the relationship between species, appeared to be quite similar to that of Shiloh Cave.

The densities and relationship between these same two species in Pless Cave were quite different, however. Hobbs (1973) found that *O. inermis* made up 96% of the total population. Once again stream width was not stated, but stream flow data indicated that the stream was only slightly smaller than that of Shiloh Cave. Assuming the widths to be the same, the *C. laevis* density of Pless Cave was comparable, with 89% of the level of Shiloh Cave; the density of Pless Cave *O. inermis*, however, appeared to be 4.2 times greater. Hobbs made reference to sinkhole ponds which apparently communicate with the Pless Cave stream. This suggests that the food base of Pless Cave may be greater than that of Shiloh Cave, although there is no direct supportive data.

Biomass and Productivity

Results

Troglobite

Although there was a consistent decrease in the numerical densities of succeeding age groups, the biomass peaked in the four year old *O. inermis* (Figure 48). The standing crop of the census area was 0.81 dry kg/ha (4.71 wet kg/ha). The standing crop of the quantitative study area, which was bounded by the weir upstream and the Black Damp Tributary downstream, was less, with only 0.67 dry kg/ha. The ratio,

P/B, where P represents net production of body tissue and B is the standing crop biomass, was 0.53 (computed from the census area population). The turnover ratio obtained as equal to the life-cycle instantaneous growth rate was 5.8.

The annual production rate for the census area was 1.18 dry kg/ha. The production was composed of 36.5% body tissue, 62.8% molted skins and 0.7% pleopod eggs (Table 33). In the younger crayfish, net production of body tissue was slightly greater than production of molted skins, but molted skin production became predominant in older crayfish (Figure 49). Net production of body tissue reached a maximum in one and two year old crayfish.

Troglophile

The pattern of biomass distribution in the *C. laevis* population was quite different, with a general increase with succeeding age groups (Figure 48). The standing crop of the census area was 2.26 dry kg/ha (10.50 wet kg/ha). The standing crop of the quantitative study area was only 1.42 dry kg/ha.

The standing crop of *C. laevis* inhabiting the surface stream issuing from Shiloh Cave was estimated at 332 dry kg/ha (1538 wet kg/ha). This estimation was based on a fairly low recapture rate of 11.7%, and it might also have been influenced by crayfish movement, despite measures taken to eliminate this factor.

The hypogean P/B ratio, with P equivalent to body tissue production (molted skins not included), was 0.42 for the census area data. The turnover ratio, which was derived by summing instantaneous growth rates over the entire life cycle, was 8.0.

TABLE 33.--ANNUAL PRODUCTION IN DRY GRAMS OF THE CRAYFISH POPULATIONS INHABITING THE STUDY AREA (750 m²)

INITIAL AGE (YRS) GROUP OR STAGE	<i>O. inermis</i>			<i>C. laevis</i>		
	BODY TISSUES	MOLTED SKINS	PLEOPOD EGGS	BODY TISSUES	MOLTED SKINS	PLEOPOD EGGS
PLEOPOD EGGS	2.14	1.34	..	2.04	1.06	..
0-1	8.46	8.13	0	3.06	2.54	0
1-2	8.28	13.10	0	6.30	5.99	0
2-3	6.35	10.46	0	9.98	8.74	0
3-4	4.10	11.83	0	10.16	12.98	0
4-5	1.71	7.86	0.42	10.27	12.62	0
5-6	1.03	2.04	0.21	9.21	9.93	0
6-7	0.26	1.16	0	12.07	12.92	0
>7	-0.18	0.85	0	..	..	..
7-8	..	..	..	5.24	8.17	1.03
>8	..	..	..	2.77	9.54	0.38
TOTAL	32.15	55.44	0.63	71.11	84.50	1.41

Figure 48. Interspecific comparison of biomass and numerical densities.

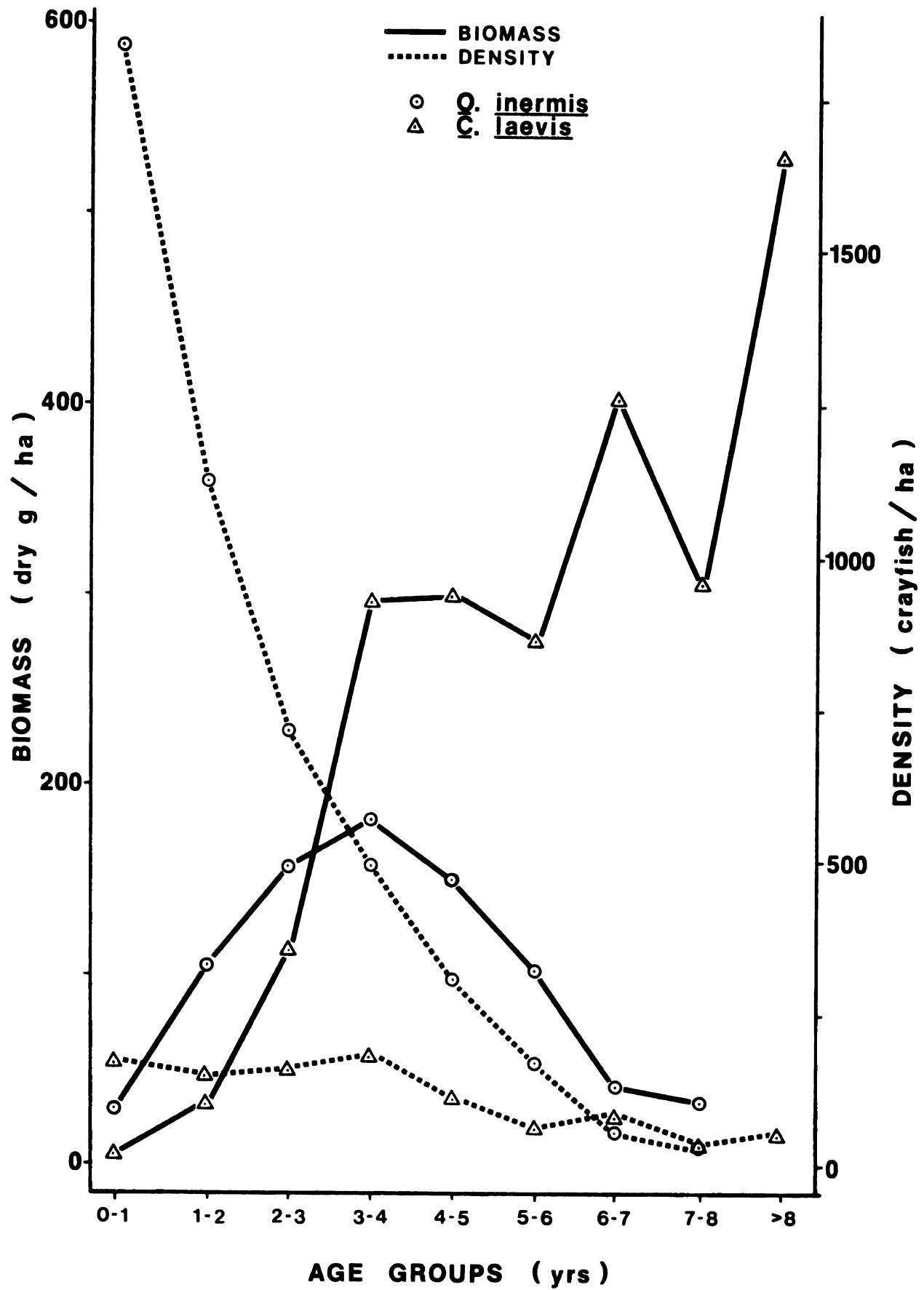


Figure 48

Figure 49. Interspecific comparison of the production rates of body tissues, molted skins, and eggs.

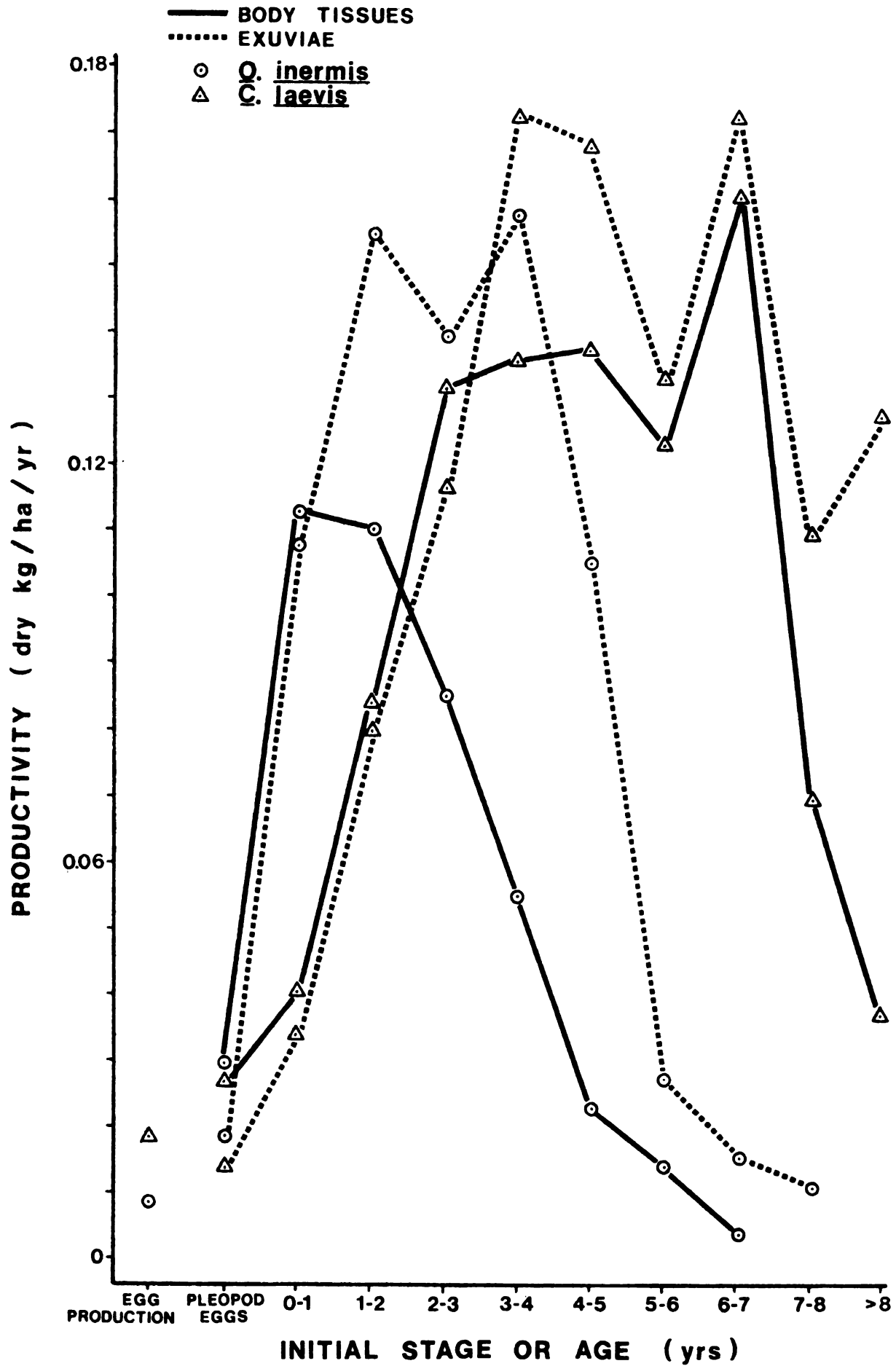


Figure 49

The annual production rate was 2.09 dry kg/ha for the census area (Table 33). The production consisted of 45.3% body tissues, 53.8% molted skins and 0.9% pleopod eggs. In contrast to the older stages, body tissue production was slightly greater than production of molted skins in younger crayfish (Figure 49). Age-specific body tissue production rates were generally high throughout the intermediate age groups.

Discussion

Despite a population density almost 5 times greater, the standing crop of *O. inermis* was less than 36% that of the cave *C. laevis*. This difference resulted from the disparity in body size of the two crayfish species.

Productivity was likewise less in *O. inermis*, but not to the same extent as the difference in standing crop; this was reflected in a larger P/B ratio for *O. inermis*. This greater ratio resulted despite a generally greater length-specific relative growth rate for *C. laevis*. The *C. laevis* population, however, possessed a size structure skewed toward the larger individuals with reduced growth rates, resulting in lower growth relative to biomass. This peculiar size structure apparently resulted from augmentation of the cave population by migrating epigean *C. laevis*. The P/B ratios of both species are within the range typical of animals requiring two or more years to complete their life histories (Mann, 1969). Turnover ratios expressed for the entire life cycles of the two cave crayfish are quite high compared to data presented by Waters (1969).

The populations of both species showed a similar pattern of production partitioning; body tissue production was slightly greater than production of molted skins in smaller crayfish, but there was a

reversal of this relationship in the larger crayfish. The decrease in the proportion of production funneled into body tissue with increasing body size was especially pronounced in *O. inermis*. Molted skins comprised the major portion of production in both cases, although *C. laevis* was more efficient overall in channeling production into body tissue.

This higher efficiency of body tissue production resulted even though the exoskeleton made up a greater percentage of body weight in *C. laevis*. For instance, in a 0.50 dry gram crayfish, *O. inermis* has a carapace length of 23.5 mm and the exoskeleton is 39.0% of the body weight, whereas *C. laevis* is 20.4 mm in carapace length with the exoskeleton making up 43.0% of body weight. Visual observation of the appearance and relative thickness of the exoskeleton leads one to expect an even greater difference, but the thinner, more elongate shape of *O. inermis* apparently increases the surface-volume ratio. The heavier exoskeleton of *C. laevis* was more than compensated by a greater mean relative growth increment per molt — 0.60 for *C. laevis* versus 0.56 for *O. inermis*. Molted skins represent a sizeable loss of energy and minerals from the population. This loss is minimized by mobilization of a portion of the carbohydrates, proteins, and minerals from the exoskeleton prior to molting. Schurr and Stamper (1962), for example, reported a 20-30% withdrawal of calcium into body reservoirs.

The extremely low standing crops of the cave populations are evident when they are compared to the standing crop of the epigean *C. laevis* population. The biomass per unit area for the cave population of *C. laevis* was only 0.7% of the level for the surface population. The estimated standing crop of the surface population was extremely large, and it is possible that crayfish movement between various sections of the surface stream, despite erected barriers, may have

interfered with the census and resulted in an overestimation. It is my belief, however, that the population estimate is reasonably accurate. Food items, especially detritus (70.9 ash-free grams per m^2) and amphipods (4.4 dry grams per m^2), were quite abundant and could reasonably support a large stable population. Poulson (1965) reported that the population density of a troglophilic amblyopsid cavefish was 240 times greater in a spring than in an associated cave. The population might also have been inflated beyond its stable level by seasonal movement of *C. laevis* into these headwaters below the springs of Shiloh Cave.

Very little literature exists on crayfish production. Momot (1967, errata) studied *O. virilis* inhabiting a marl lake and determined their annual production to be 100.4 wet kg/ha. The production of both cave species together was only 6.9% of this value. The productivity of the river-dwelling *O. propinquus* was reported by Vannote (1963) to be 415 wet kg/ha/yr — a rate 60 times greater than the combined production rates of the two species of Shiloh Cave crayfish.

More information is available on the standing crops of crayfish occupying various habitats (Table 34). Of all these studies, none reported standing crops as low as the 15.2 wet kg/ha that was found for the combined cave populations of the present study. Only one station on a biologically unproductive epigean stream with shale and sandstone bedrock approached the low standing crop of Shiloh Cave (Slack, 1955b). On the other hand, the 1538 wet kg/ha biomass density of the epigean *C. laevis* population was extremely high when compared to other studies. Only a fish-farm pond studied by Langlois (1935) showed a higher standing crop of crayfish. Unfortunately, no information on standing crop or productivity of *Cambarus* species was found in the literature.

TABLE 34.--COMPARISON OF CRAYFISH STANDING CROPS FOUND IN THE LITERATURE WITH DATA FROM THE PRESENT STUDY

STANDING CROP (wet kg/ha)	SPECIES	HABITAT	LITERATURE SOURCE
17 - 246	<i>O. propinquus</i>	Indiana streams	Slack, 1955b
430	<i>O. propinquus</i>	enriched warm-water Michigan river	Vannote, 1963
30 - 730	unidentified	Ohio stream	Wickliff, 1940
56 - 1345	<i>O. immunis</i>	Michigan ponds	Goellner, 1943
52 - 286	<i>O. immunis</i>	New York fish-farm ponds	Tack, 1941
772 - 909	<i>O. immunis</i>	Michigan pond	Lydell, 1938
92 - 120	<i>O. virilis</i>	Michigan marl lake	Momot, 1967
226 (estimated)	<i>O. virilis</i>	Massachusetts pond	Camougis and Hichar, 1959
751 - 1743	<i>O. rusticus</i>	Ohio fish-farm ponds	Langlois, 1935
5	<i>O. inermis</i>	Shiloh Cave stream	present study
11	<i>C. laevis</i>	Shiloh Cave stream	present study

Respiration

Introduction

The respiratory rate of crayfish is modified by many factors. In the cave environment several of these factors, such as temperature, oxygen tension, diet and activity, are essentially of a constant or characteristic nature. Because the primary purpose of these respiratory rate experiments was to determine naturally occurring respiratory rates, experimental conditions were kept as close to those of the field as possible.

Most determinations were conducted in the field using cave water and utilizing the cave stream as a temperature bath (12.5° C). The cave stream is nearly always saturated with oxygen, and, although oxygen tension decreased in the respiration chambers during the experiment, the results were adjusted to saturation levels. Crayfish used in the experiments were captured and immediately placed for one day in a respiratory chamber with cave mud and detritus as a food source. This allowed the crayfish to adjust to the experimental conditions and to still maintain a fairly normal diet until the commencement of the respiratory experiments. The most unnatural aspect of the experiment was the confinement imposed by the chambers, although they were sufficiently large for the crayfish to move about. The degree of activity modification is uncertain, but the crayfish in the chambers were observed to be generally quiescent with only occasional periods of sustained movement. However, there is good evidence from other aspects of the study that the measured respiratory rates grossly under-estimated naturally occurring respiration. It is thought that

natural activity was restricted in the chambers and that the measured respiratory rates represent near basal levels.

Other factors suspected of possibly affecting the respiratory rate, such as species, habitat, body weight, sex, season and time of day, were of variable nature, and the experiments were designed to investigate this variable aspect of respiration.

Results

Troglobite

The rate of oxygen consumption was found to vary with oxygen tension (Table 35). There was no evidence that these crayfish were able to carry out respiratory regulation. Although low oxygen tensions are probably never experienced in their natural environment, these crayfish displayed the capacity to survive very low oxygen levels; they usually survived oxygen tensions as low as 0.20 cc/l, although one death did occur.

Respiration also varied with body weight. Oxygen consumption was proportional to the 0.72 power of the wet body weight (Figure 50). This relationship resulted from an inverse linear regression between the log of body weight and oxygen consumed per unit weight (Table 35). At a temperature of 12.5° C and an oxygen tension of 7.50 cc/l, which is the saturation point, oxygen consumption ranged from an extrapolated high of 0.079 cc/wet g/hr for a newly hatched crayfish of 5.0 mm carapace length, to a low of 0.018 cc/wet g/hr for the largest *O. inermis* encountered during the study (32.0 mm).

Sexual and seasonal variations in oxygen consumption were statistically tested after adjusting respiratory rates for body weight

TABLE 35.--THE RELATIONSHIP OF OXYGEN TENSION OR BODY WEIGHT TO THE OXYGEN CONSUMPTION OF CRAYFISH AT 12.5° C

CRAYFISH SPECIES	REGRESSION OF OXYGEN CONSUMPTION ON OXYGEN TENSION, where Y = oxygen consumption cc/wet g/hr X = oxygen tension in cc/l	REGRESSION OF OXYGEN CONSUMPTION PER UNIT WEIGHT ON BODY WEIGHT, AT AN OXYGEN TENSION OF 7.5 cc/l (SATURATED), where Y = oxygen consumption in cc/wet g/hr X = body weight in wet g
<i>O. inermis</i>	log Y = 0.0701 X - 2.253 $r^2 = 0.447$ n = 88	Y = 0.0382 - 0.0231 log X $r^2 = 0.425$ n = 45
<i>C. laevis</i>	log Y = 0.3118 log X - 1.933 $r^2 = 0.281$ n = 70	Y = 0.0846 - 0.0352 log X $r^2 = 0.613$ n = 58

Figure 50. Interspecific comparison of respiration over the observed size range, at 12.5° C and oxygen saturation.

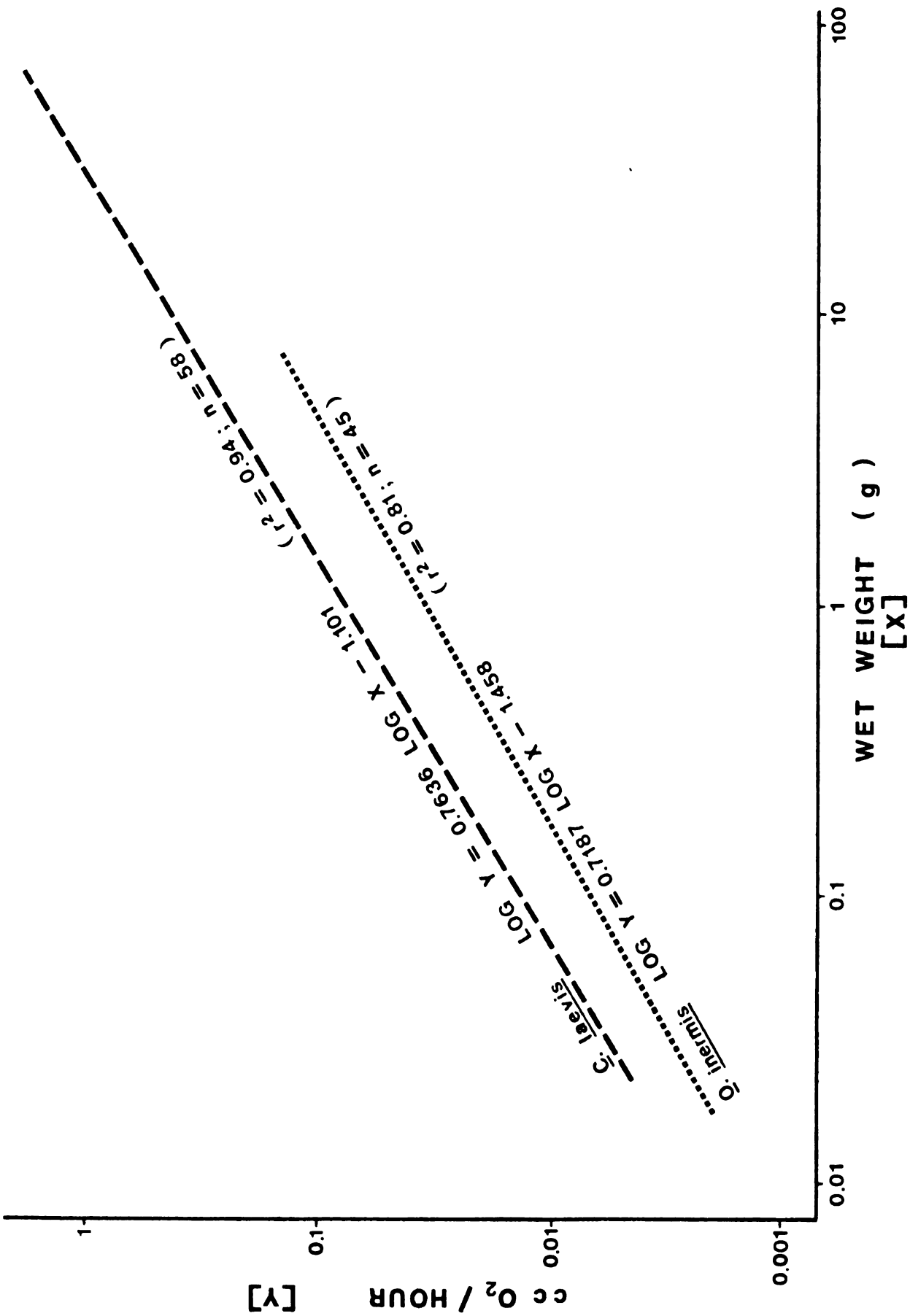


Figure 50

and oxygen tension (Table 36). Neither sexual nor seasonal variations were significantly different.

Troglophile

The rate of oxygen consumption varied with oxygen tension, although the log-log linear relationship indicated the probability that respiratory regulation occurred at higher oxygen tensions (Table 35). These crayfish survived oxygen tensions as low as 0.20 cc/l, although temporary paralysis occurred in one case.

Respiration also varied with body weight, with an inverse linear regression between the log of body weight and oxygen consumed per unit weight (Table 35); this resulted in oxygen consumption being proportional to the 0.76 power of wet body weight (Figure 50). In 12.5° C, oxygen-saturated water, respiration varied from 0.143 to 0.020 cc/wet g/hr for extrapolated weights ranging from hatching to the largest (58.0 mm C L) individual encountered.

Statistical testing of respiratory rates, adjusted for oxygen tension and body weight, indicated no significant differences between the two sexes, or between the epigean and hypogean populations (Table 36). Seasonal differences were significant for both sexes, with the respiratory rates greater in May than in November. However, the seasonal factor was probably confounded by the change of the test site from the field to the laboratory; for this reason, the source of this variation can not be identified.

The relatively high respiratory rate of *C. laevis* permitted sampling intervals which allowed the determination of diurnal variation in oxygen consumption. The three 8-hour periods were significantly different, with the 1600-2400 period having a higher respiratory rate than the other two periods of the day.

TABLE 36.--INTERSPECIFIC, SEXUAL, TEMPORAL AND ENVIRONMENTAL COMPARISONS OF CRAYFISH OXYGEN CONSUMPTION RATES; data stated as cc/wet g/hr, adjusted to 7.5 cc O₂/l (saturated) and 3.0 wet g body weight; tests conducted at 12.5° C

SPECIES	SEX	HABITAT	SEASON*	DIURNAL PERIOD	$\bar{x}$	s	n	STATISTICAL TEST GROUP								
								1	2	3	4	5	6	7	8	9
<i>O. inermis</i>	Females	Cave	Spring	24 hours	0.0267	0.0129	18	x	x		x					
<i>O. inermis</i>	Males	Cave	Spring	24 hours	.0262	.0072	19	x	x			x				
<i>O. inermis</i>	Females	Cave	Fall	24 hours	.0244	.0096	34				x					
<i>O. inermis</i>	Males	Cave	Fall	24 hours	.0251	.0066	21					x				
<i>C. laevis</i>	Females	Cave	Spring	24 hours	.0663	.0140	23	x		x			x		x	
<i>C. laevis</i>	Males	Cave	Spring	24 hours	.0691	.0221	17	x		x				x	x	
<i>C. laevis</i>	Females	Cave	Fall	24 hours	.0446	.0132	19						x			
<i>C. laevis</i>	Males	Cave	Fall	24 hours	.0524	.0121	17							x		
<i>C. laevis</i>	Females	Epigeon	Spring	24 hours	.0710	.0210	8								x	
<i>C. laevis</i>	Males	Epigeon	Spring	24 hours	.0662	.0187	10								x	
<i>C. laevis</i>	Both	Cave	Fall	2400-0800	.0504	.0106	25									x
<i>C. laevis</i>	Both	Cave	Fall	0800-1600	.0505	.0114	7									x
<i>C. laevis</i>	Both	Cave	Fall	1600-2400	.0613	.0064	9									x

*Spring tests performed in field; Fall tests performed in laboratory.

VARIABLE TESTED	TEST GROUP	STATISTICAL TEST	VALUE	d f	PROBABILITY	DIFFERENCE BETWEEN MEANS	
						S = significant	ns = not significant
Species	1	t	12.52	83	.001>P		S
Sex (<i>O. inermis</i>)	2	t	0.14	35	P>.50		ns
Sex (<i>C. laevis</i>)	3	t	0.49	38	P>.50		ns
Season (<i>O. inermis</i> , females)	4	t	0.73	50	.50>P>.40		ns
Season (<i>O. inermis</i> , males)	5	t	0.51	38	P>.50		ns
Season (<i>C. laevis</i> , females)	6	t	5.13	40	.001>P		S
Season (<i>C. laevis</i> , males)	7	t	2.73	32	.02>P>.01		S
Habitat	8	t	0.16	56	P>.50		ns
Diurnal period	9	A.O.V.	4.18	2/38	.025>P>.010		S

Discussion

The respiratory rates of the two crayfish species were found to be inherently different. The rate of oxygen consumption per unit body weight of *C. laevis* averaged 2.3 times that of *O. inermis* under identical conditions. Similar differences between the respiration of epigean and hypogean crayfish were reported by Burbanck, Edwards and Burbanck (1948). They found that the cave crayfish, *Cambarus setosus*, survived 3.3 times longer in sealed chambers than did the surface crayfish, *Orconectes rusticus*, and, since the oxygen tension was reduced to about the same level by both species, they concluded that the cave crayfish had a lower metabolic rate. Jegla (1964) found that the troglphilic crayfish, *C. bartoni*, had a rate of oxygen uptake from 2.5 to 3.0 times higher than that of the troglobitic *O. inermis*. On the other hand, Eberly (1960), using these same two species, concluded that the oxygen consumption per unit of body weight was higher in the troglobite; his results, however, were based on a total of only four experimental animals.

Although it is difficult to compare respiratory rates from different studies because of differing experimental conditions, it appears that the oxygen consumption rate of the troglphilic crayfish, *C. laevis*, used in the present study, is similar to most crayfish respiratory values found in the literature. The 2.3-fold difference in respiration rates between the troglphilic and troglobitic crayfish must, therefore, result from a depressed metabolic rate in *O. inermis*. Consequently, the evolution of this crayfish to its present troglobitic status must have included not only morphological change, but also modification of its metabolic rate as an adjustment to the food-poor nature of the cave. These crayfish were observably less active, which would account for a

reduction in metabolism, but the results of the respiratory experiments, in which locomotive activity was largely restricted, indicated that a reduction of the basal metabolism rate is also involved. The inter-specific difference in respiratory rates observed in this study probably underestimates the difference that occurs under natural conditions in the cave stream, where the crayfish have freedom of movement.

The above discussion concerned respiratory rate per unit weight. When considered at the level of the organism, the metabolic differences between the species are of even greater significance. This is due to the greater size attained by *C. laevis*, which, together with its higher metabolism, results in a greater impact on food resources. That this greater energy demand of *C. laevis* is a disadvantage in its interactions with *O. inermis* is supported by the observations of Eberly (1960), who has frequently observed *C. bartoni* that have died of apparent starvation in food-deficient cave pools. However, mortality resulting from starvation has never been observed in the present study of Shiloh Cave. Wiens and Armitage (1961) reported a similar relationship between two species of epigean *Orconectes*, but concluded that the larger-sized species with a higher metabolic rate probably had an adaptive advantage. These contradictory conclusions may both be valid, as it is probable that the adaptive fitness of a feeding strategy varies with the quantity and quality of the food base.

Although the surface and cave habitats maintain essentially identical temperatures, they differ in the nature of their food resources and in their light regime. Both of these factors might be suspected of influencing crayfish metabolism, especially by modifying activity patterns. The respiratory results, which measured basal metabolism but underestimated respiration associated with digestion

and activity, did not differ significantly between the hypogean and epigean populations of *C. laevis*. However, because the experimental chambers modified normal activity, it is not known whether the actual respiratory rates of unconfined crayfish differ between the two habitats. It is assumed that the level of food-search activity is either maintained or increased in the food-poor cave habitat; in either case, the respiratory fraction of the assimilated energy increases, as both ingestion and growth rates are known to be lower in the cave.

The relationship of sex and body weight to respiratory rate was in general agreement with results reported in the literature. Sex was found to have no significant effect on the metabolic rate of either species. This conclusion is in agreement with the results of Helff (1928), who studied the respiration of *Orconectes immunis*, and Thomas (1954), who studied the lobster, *Homarus vulgaris*. The respiration rate per unit body weight varied inversely with body weight, and this relationship is of almost universal occurrence in the literature. The organismic oxygen consumption was proportional to the 0.72 power of the wet body weight in *O. inermis* and to the 0.76 power in *C. laevis*. These values fall within the 0.7-0.8 range which characterizes a great variety of animal species (Florey, 1966).

Because of the lack of annual temperature variation, temporal cycling of the respiratory rate was not rigorously studied. There was an indication, however, that seasonal modification may occur, at least in *C. laevis*, which underwent a significant decrease in oxygen consumption between May and November. Seasonal data for both species may have been confounded, however, by an accompanying change in the test site from the cave to the laboratory; although diet, temperature, and water chemistry were maintained as close to cave conditions as possible, there was an

unavoidable time delay between capture and testing. Seasonal change in the oxygen consumption of *O. inermis* was found by Jegla (1964), who noted an increase in the fall respiratory rate over summer values; he attributed this change to increased reproductive activity during the fall months. If this increase in respiration is applicable to the present study, then the failure to detect it may have resulted from an equivalent decrease associated with the change in the test site. The molting rate is also seasonably variable, and there is contradictory literature concerning its effect on respiration. Vannote (1963) found the oxygen consumption of an individual *Orconectes propinquus* to be significantly increased for a 13-hour period during and following molting, whereas Helff (1928) observed 8 cases of molting in *O. immunis* and found no marked changes in oxygen consumption. Thus, there is inconclusive evidence that molting and reproductive activity may result in seasonal changes in respiration. Nevertheless, the seasonal respiratory pattern of hypogean crayfish possesses a stability not usually found in comparable epigean populations; this stability results from the small annual temperature fluctuations of the cave stream.

Diurnal rhythms of respiration have been established in epigean crayfish. Wiens and Armitage (1961) reported peaks of oxygen consumption occurring at dawn and dusk periods for both *Orconectes immunis* and *O. nais*. This respiratory rhythm is apparently correlated with activity patterns. The existence of similar rhythms in cave populations seems questionable from both mechanistic and ecological aspects, and several papers have been addressed to this problem. In the present study, diurnal variation of oxygen consumption was investigated in the hypogean crayfish, *C. laevis*, and it was found that the 1600-2400 period was associated with significantly higher respiration. This respiratory

rhythm does not coincide with information on activity patterns derived from visual captures (see section on circadian rhythm above), which suggested that activity was maximal around 1500. It does, however, agree with the conclusion of Brown (1961) that the troglobitic crayfish, *Orconectes pellucidus*, possesses a circadian rhythm with maximum activity around 1900; it must be pointed out, however, that the conclusions of Brown have been widely questioned in the literature. Jegla and Poulson (1968) also found evidence of a circadian rhythm of activity and oxygen consumption in *O. pellucidus*; they concluded, however, that the rhythm was not as well developed as in epigean crayfish kept in constant darkness and that the rhythm patterns of individual crayfish were not synchronized at the population level. Based on studies to date, which have provided conflicting information and have lacked either sufficient data or rigorous experimental procedures, very little can be concluded concerning circadian rhythms of hypogean crayfish.

CHAPTER VII

ENERGY AND PROTEIN BUDGETS — AN INTEGRATION

Description of Inter-relationships

In this chapter, previously discussed aspects of crayfish ecology are inter-related in terms of energy or crude protein found in both standing crops and flow pathways. The computation of protein and energy partitioning is based in large part on values derived in previous chapters that are here adjusted to express the annual rates for the quantitative study area (548 square meters). In cases where a relationship was found to exist between a factor and the body size of the crayfish, expressed values are based on the structure of the crayfish population. The energy and crude protein contents of various components not previously tabulated are summarized in Table 37.

The flow pattern for energy and crude protein is diagrammed in Figure 51. The input of particulate matter, which was divided into microseston and macroseston portions, either flowed through or was retained in the quantitative study area of the cave stream. The fraction retained either became mixed into the substrate or was consumed by the biota, of which the smaller-sized benthos generally grazed the microfloral film covering both the microseston and macroseston; the crayfish, on the other hand, probably ingested only the larger particles, the macroseston, but consumed them in their entirety. The seston incorporated into the substrate was utilized by those benthos with

TABLE 37.--ENERGY AND CRUDE PROTEIN CONTENTS OF CAVE CRAYFISH AND OTHER BENTHOS

BENTHIC GROUP	TISSUE	TAXON	TISSUE CONTENT					
			ENERGY (g cal/mg)			% CRUDE PROTEIN		
			$\bar{x}$	s	n	$\bar{x}$	s	n
Crayfish	Body	<i>O. inermis</i>	3.50	0.23	5	47.2	4.6	5
Crayfish	Body	<i>C. laevis</i>	3.62	.67	4	44.0	3.1	4
Crayfish	Exuviae	<i>O. inermis</i>	1.32	.35	5	17.8	4.2	3
Crayfish	Exuviae	<i>C. laevis</i>	1.15	.13	4	17.7	2.1	3
Crayfish	Eggs	<i>O. inermis</i>	5.88	.14	2	54.4	3.1	2
Crayfish	Eggs	<i>C. laevis</i>	6.51	.03	2	45.2	1.1	2
Amphipod	Body	<i>Crangonyx</i> sp.	4.32	.24	4	54.4	4.9	5
Isopod	Body	<i>Asellus</i> sp.	3.11	.47	2	39.3	2.9	4
Oligochaete	Body	Spp.	2.15	.08	2	26.7	1.2	2

Figure 51. Annual flow of energy and crude protein through the quantitative study area of 548 m²; respiration values adjusted by the difference in laboratory and field fecal rates.

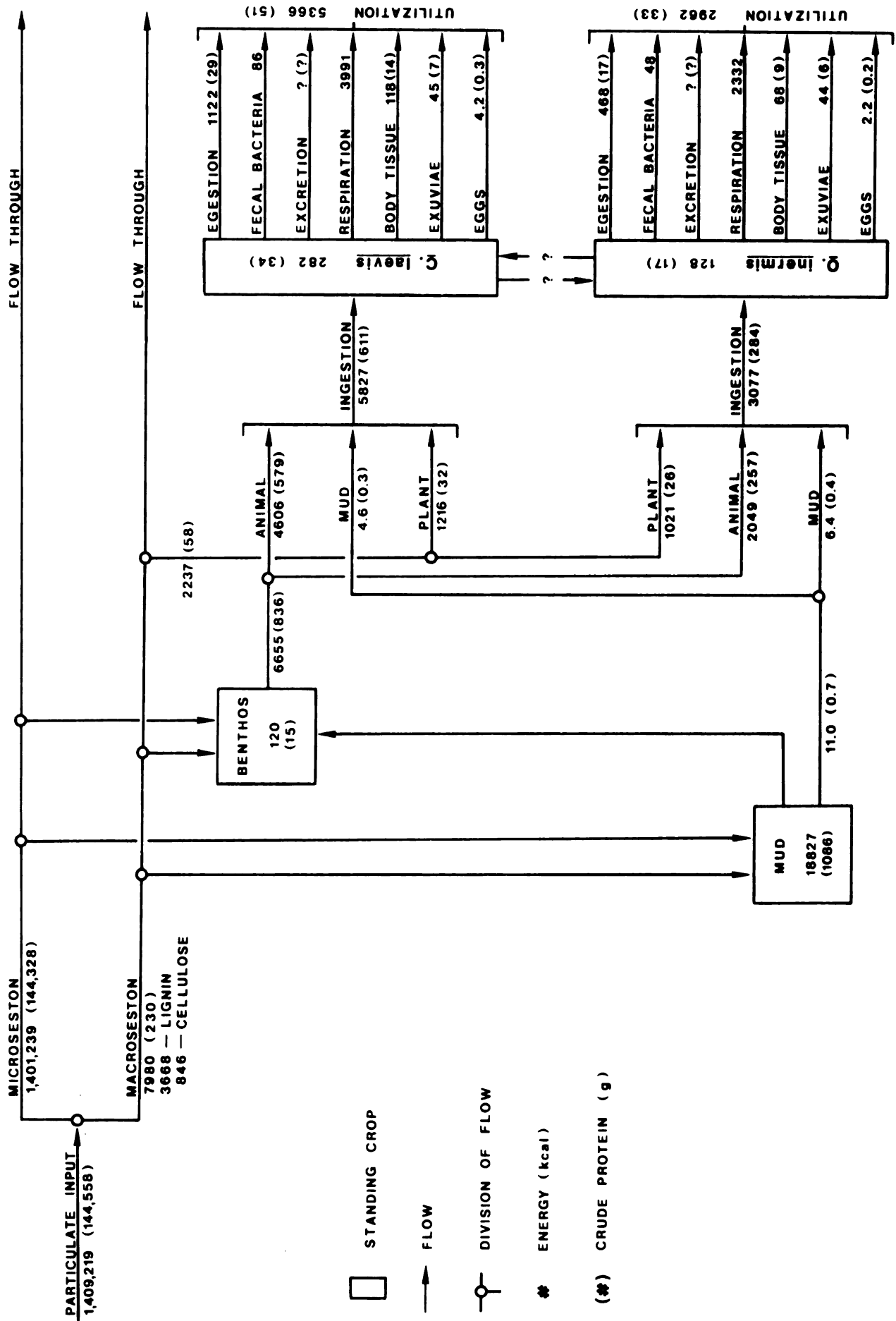


Figure 51

limivorous habits and also to a slight extent by the crayfish. The benthos, in turn, was ingested by the crayfish and, together with possible cannibalism and interspecific crayfish predation, composed the animal component of the crayfish diet. Quantitatively, mud was consumed at an insignificant rate, and animal material was the major dietary component of both crayfish populations. The calculated ingestion rate was reduced to correct for a 4% error in weight assimilation, as discussed in a previous chapter.

Measurement of energy pathways within the crayfish accounted for a large portion of ingested energy — 96.3% in *O. inermis* and 92.1% in *C. laevis*. These results required, however, that the laboratory measured respiratory rate be multiplied by the difference between the respiratory rate in the laboratory and field for both crayfish; this factor was estimated from the difference between the laboratory and field fecal rates (see section on diet analysis above). Justification for this procedure is founded in a similar situation reported by Odum (1962); based on the fact that the excretion rate paralleled oxygen consumption, he monitored excretion rates of a snail in the two situations and concluded that the snail was more active in the field than in the laboratory.

The excretion of nitrogenous waste products was not measured, but it is assumed that this metabolic pathway accounts for only a very limited fraction of the total energy flow. Crustacea are primarily ammonotelic (Meglitsch, 1972), and ammonia is the end product of the wet oxidation technique used for energy measurements; thus, unmeasured excretion energy is derived only from small amounts of low-energy urea and uric acid that are produced. Its calculation, however, would have improved the balance between energy influx and efflux. Unlike energy,

measured protein usage accounted for only a small part of the crude protein ingested by the crayfish; this is attributable to the apparently large quantities of excreted crude protein.

Because of the method used to measure egestion, bacteria mixed with the feces partially processed the fecal substrate, and this rate, as measured under experimental conditions, is expressed separately in Figure 51; for the calculation of assimilation, however, it is combined with egestion to express the total non-assimilated rate. Bacterial decomposition of fecal matter is a natural process, which after the two day duration of the experiment, had already occurred to an appreciable extent. The fecal decomposition results, however, are in disagreement with those of Davies (1964), who in a similar experiment, found no significant bacterial processing of goldfish feces after 23 days.

The remaining energy pathways within the crayfish are concerned with production; they involve energy storage, either in the form of body tissues, exoskeleton material lost during ecdysis (exuviae), or structures, such as eggs, that are associated with reproduction. Their quantitative relationships were, in respective order, 70.6%, 26.9% and 2.5% for *C. laevis*, and 59.9%, 38.5% and 1.9% for *O. inermis*.

Energy efficiencies for the crayfish species are shown in Table 38. Those efficiency ratios involving respiration should be regarded as estimates, because of the indirect method used to arrive at the respiration rates in the field. The net growth efficiency [production/(assimilation)(100)] values of 4.5% for *O. inermis* and 3.6% for *C. laevis* are quite low, whereas the assimilation efficiency [assimilation/(ingestion)(100)] values of 83.2% for *O. inermis* and 79.3% for *C. laevis* are fairly high.

TABLE 38.--ENERGY EFFICIENCIES AND RATES FOR HYPOGEAN CRAYFISH POPULATIONS

SPECIES	ENERGY EFFICIENCY (%)			ENERGY RATE (kcal/m ² /yr)	
	P/A	R/A*	R/I*	A/I	PRODUCTION RESPIRATION*
<i>O. inermis</i>	4.5	91.1	75.8	83.2	0.21 4.26
<i>C. laevis</i>	3.6	86.4	68.5	79.3	0.31 7.28

P = production of body tissue, exuviae and eggs

R = respiration*

A = assimilation

I = ingestion

*value based on a laboratory-derived respiration rate, adjusted by a conversion factor equivalent to the relative difference between laboratory and field fecal rates.

Discussion of the Pattern

Mud was ingested in only small amounts by both crayfish species. Although a large absolute quantity of energy is stored in the mud substrate, organic matter is present in only very low concentrations and cannot be efficiently assimilated. Mud, however, may furnish minerals or vitamins that are an essential part of the crayfish diet, but that require only a low rate of ingestion. It is also a possibility that mud is accidentally ingested, in whole or in part, in conjunction with the eating of other food items that are associated with the substrate. Nevertheless, crayfish maintained in the laboratory on a diet of mud alone ingested this dietary component at a rate similar to that observed in the field.

Plant material made up a significant part of the diet and, as discussed in a previous chapter, the cave-dwelling *C. laevis* has shifted its diet from animals to plants in relation to the surface-dwelling population. This shift towards a dietary dependence on a lower trophic level apparently is advantageous in the food-poor cave environment, and the troglobite, *O. inermis*, has an even greater dietary dependence on plant detritus than does the troglophile, *C. laevis*. This shift to a larger food base is of limited extent, however; this is probably because it is accompanied by a decreased assimilation efficiency. Unlike other members of the benthos which are of small enough size to graze the microfloral film, crayfish, in addition, ingest the plant particles which are composed mostly of indigestible cellulose and lignin. Of the total caloric content of the macroseston plant material, 57% is derived from cellulose and lignin, and an unknown fraction of the remainder may be rendered unavailable by the structural organization

of the cellulose and lignin. This unavailability may apply to the crude protein contained in the plant material, which, at least in its total amount, appeared sufficient to satisfy the needs of the crayfish.

It is assumed that, because of the small particle sizes of the microseston, plant detritus ingested by the crayfish is derived principally from the macroseston. The crayfish, *O. inermis* and *C. laevis*, ingested 13% and 15% of the total available macroseston, respectively, and this is much greater foraging impact than indicated by other studies, such as the 5% level of kelp-feeding isopods (Hayes, 1974), and the low utilizations indicated for a salt marsh herbivore and detritivore (Odum and Smalley, 1959). Lee and Inman (1975) suggested that the fact that herbivores consume only a low percentage of available net primary production reflects their role as an ecosystem regulator. The detritus-based trophic ecology of the cave, however, lacks the structure necessary for such a control function, since the foraging activity of the crayfish has no effect on future food influx.

The rate of plant consumption for the combined crayfish populations was almost twice as great as the calculated macroseston retention rate, and this does not even take into account macroseston channeled through the benthos or mud substrate. It seems obvious that the macroseston retention rate, which was calculated during base stream flow, was a gross under-estimation, since it was inadequate to supply the needs of the crayfish populations. This implies that macroseston washed into the cave during periods of flood was retained in large quantities and that this stored food base was utilized throughout the year in conjunction with current seston inputs. The calculated retention rate measured only that portion of utilized energy based on macroseston retained during periods of low discharge and did not measure the stored

macroseston utilized by crayfish. That fraction of the macroseston that did not go into storage or that was not consumed by the biota flowed through the quantitative study area to downstream areas of the cave and surface stream. The retention rate calculated for the microseston may also have been in error, but flooding probably did not lead to the degree of storage that is suspected in the macroseston.

Animals were the most important food item in the diets of both crayfish, especially *C. laevis*. This food is easily digested and contains high levels of crude protein that, in comparison to plant material, probably has an amino acid content more in balance with the needs of the crayfish. Although part of this animal dietary component was possibly obtained by inter- and intra-specific predation on crayfish, the bulk was derived from the benthos. The animal component of the diet remains important, not only because of its assimilable nature, but also because it allows the crayfish to utilize, at least indirectly, the energy of the microseston, which is fed on by the benthos.

Although both the benthos and crayfish depended upon the macroseston as a dietary component, competition seemed to be tempered by interactions that suggest some degree of protocoooperation. To a large extent the diet of the benthos is obtained by grazing the microflora, which is renewable. On the other hand, most of the crayfish-processed macroseston, because of its high content of cellulose and lignin, is recycled to the substrate where it is available to the benthos of coprophagous habit. Nevertheless, predation on the benthos not only provides a ready source of energy and crude protein, but also increases the quantity of macroseston available to the crayfish. Although both the benthos and the crayfish utilize the macroseston, the microseston is probably only significantly utilized by the benthos.

For the benthos the calculated yield/biomass ratio, where yield is based on the animal component of the crayfish diet, was 55. This value is actually too high, because the yield does not take into account either cannibalism or predation on crayfish. In addition, the benthic biomass was under-estimated. This was due to certain components of the benthos that were not quantitatively sampled because they were either too small in body size (e.g., copepods, ostracods and nematodes) or too deep in the substrate. Waters (1969) stated that turnover ratios calculated by the predation method, as in the present case, are invariably high. Hynes (1970) suggested that a P/B value of 10 is probably characteristic for populations dominated by arthropods.

Although *O. inermis* comprised only 32% of the total crayfish biomass, this troglobite had a slightly disproportionate impact on the ecosystem by ingesting 35% of the energy flowing through the crayfish populations. This resulted despite the fact that, in considering crayfish of equivalent body size, *C. laevis* experiences the greatest energy flow; at the population level, however, the mean smaller body size of *O. inermis* favors energy flow through this species due to the inverse correlation between ingestion and body size.

The growth efficiencies for both crayfish were extremely low. This resulted from the fact that a very high percentage of assimilated energy is used up in respiration, which is apparently associated with foraging activity. The relationship between net production and maintenance metabolism indicated an efficiency much lower than the regression for poikilotherms formulated by Engelmann (1966). In a study of three arthropods, Van Hook (1971) found in all cases that growth efficiencies were higher and respiration efficiencies lower than in the present study. Thus, in this ecosystem where energy is extremely

limited, growth is not only low in an absolute sense, but also receives a lesser share of energy flowing through the population. *O. inermis*, the troglobite, attained a higher growth rate than the troglophile, *C. laevis*. The troglobite has achieved this increased efficiency by decreasing locomotive activity.

O. inermis also had a higher assimilation efficiency than *C. laevis*, and this resulted in spite of the fact that *O. inermis* consumed a higher percentage of highly indigestible plant material. Both crayfish had high assimilation efficiencies. Odum and Smalley (1959) believe a high assimilation efficiency would benefit a population whose food is produced at a low, but continuous rate; this they concluded from a study of a slow-growing, long-lived, detritus-feeding snail population, in which they found that the snails assimilated a considerable portion of ingested food, but used a relatively small part of the assimilated matter for growth. Consequently, there is not only ecological and population dynamic similarities between the snail and crayfish, but also similarities in their patterns of energy flow. Welch (1968) believes that net growth efficiency is negatively correlated with assimilation efficiency; he suggests that a decrease in net growth efficiency and an increase in assimilation efficiency is in response to a depressed ingestion rate. For the cave-inhabiting crayfish, net growth efficiency, when plotted against assimilation efficiency, falls far below the regression relationship presented by Welch (1968); this again demonstrates the dominance of respiratory energy flow over growth pathways for these cave populations.

As discussed in a previous chapter, crayfish maintained in the laboratory with abundant food had a much lower ingestion rate than crayfish inhabiting the food-poor cave. This foraging response to

a change in food abundance characterizes the strategy of a time minimizer, as opposed to an energy maximizer (Rapport and Turner, 1975). This strategy leads to a more efficient utilization of resources, because a minimum quantity of resources is used for maintenance and self-replacement. This is contrary to the strategy of an energy maximizer, which favors a higher feeding rate in order to maximize the energy surplus available for reproduction. This implies that the cave-inhabiting crayfish, as time minimizers, target only a certain limited amount of energy for reproduction.

The ability to govern reproduction would certainly be an asset to a population living in an ecosystem where energy is extremely limited. In fact, only a small percentage of adult female crayfish produced eggs, and these were apparently the individuals that were able to secure energy sufficient to carry out the high energetic cost of egg production. In comparison to epigean crayfish, reproduction was drastically reduced, and the low percentage of storage energy diverted to egg production (1.9% for *O. inermis* and 2.5% for *C. laevis*) suggests that production energy in cave-inhabiting crayfish is preferentially funneled into tissue growth and exuviae production. Unfortunately, comparable energetic data is not available for epigean crayfish to substantiate this opinion. The strategy of a time minimizer has the effect of minimizing depletion of the food base by decreasing both the ingestion rate and the population size. This trophic strategy is well-suited to the cave ecosystem, and its operation seems to be substantiated by the study results.

Although it was initially assumed that protein might be a critical factor, the study revealed that protein was available in surplus quantities. Microflora processing the protein-poor seston

input apparently augment the supply by incorporating and converting inorganic nitrogen dissolved in the water. The low energy influx seems instead to be the most critical factor in the trophic ecology of the hypogean crayfish.

Budget Changes Associated with Adaptation to the Cave Habitat

The decreased energy flow is important, not only in itself, but also in the changes in energy partitioning that have resulted. These changes are shown in Figure 52, which indicates sequential partitioning modifications associated with epigeal-hypogean and troglophilic-troglobitic transformations. Because of a lack of literature on the energetics of epigean crayfish, the proposed energy scheme is based in part on data from other animal groups and on certain suppositions, although the limited study of the epigean *C. laevis* population has provided some data. Modifications occurring in the energy flow of the hypogean population of the troglophile are assumed to be of a physiological nature, whereas in the troglobite genetic factors are assumed to be involved.

In this scheme a significantly decreased food base is thought to trigger a sequence of energy flow modifications. Initially, the scarcity of food depresses the ingestion rate. In *C. laevis*, the troglophile, the reduction is modest, but in *O. inermis*, the troglobite, there is a low rate that represents a major decline from epigean levels. The depression of the ingestion rate is much greater in the troglobite, even though evolutionary modifications have increased its food-detecting capabilities (Packard, 1888). The decreased rates result from a lower level of available food, and, at least in the troglobite, the decrease probably also involves a shift in trophic strategy from energy

Figure 52. Energy flow through hypogean crayfish, showing sequential partitioning modifications that result from the decreased food base of the cave. Compartment arrows indicate changes from the pattern in epigean *C. laevis* in the relative amounts of energy entering different compartments at each processing level. Arrow direction (up = increase, and down = decrease) indicates direction of change, and arrow size (large = greater, and small = lesser) indicates degree of change, relative to the other hypogean species. The energy flow pathway between succeeding processing levels that is favored by partitioning changes associated with either epigeal-hypogean (*C. laevis*) or troglophilic-troglobitic (*O. inermis*) transformations is indicated.

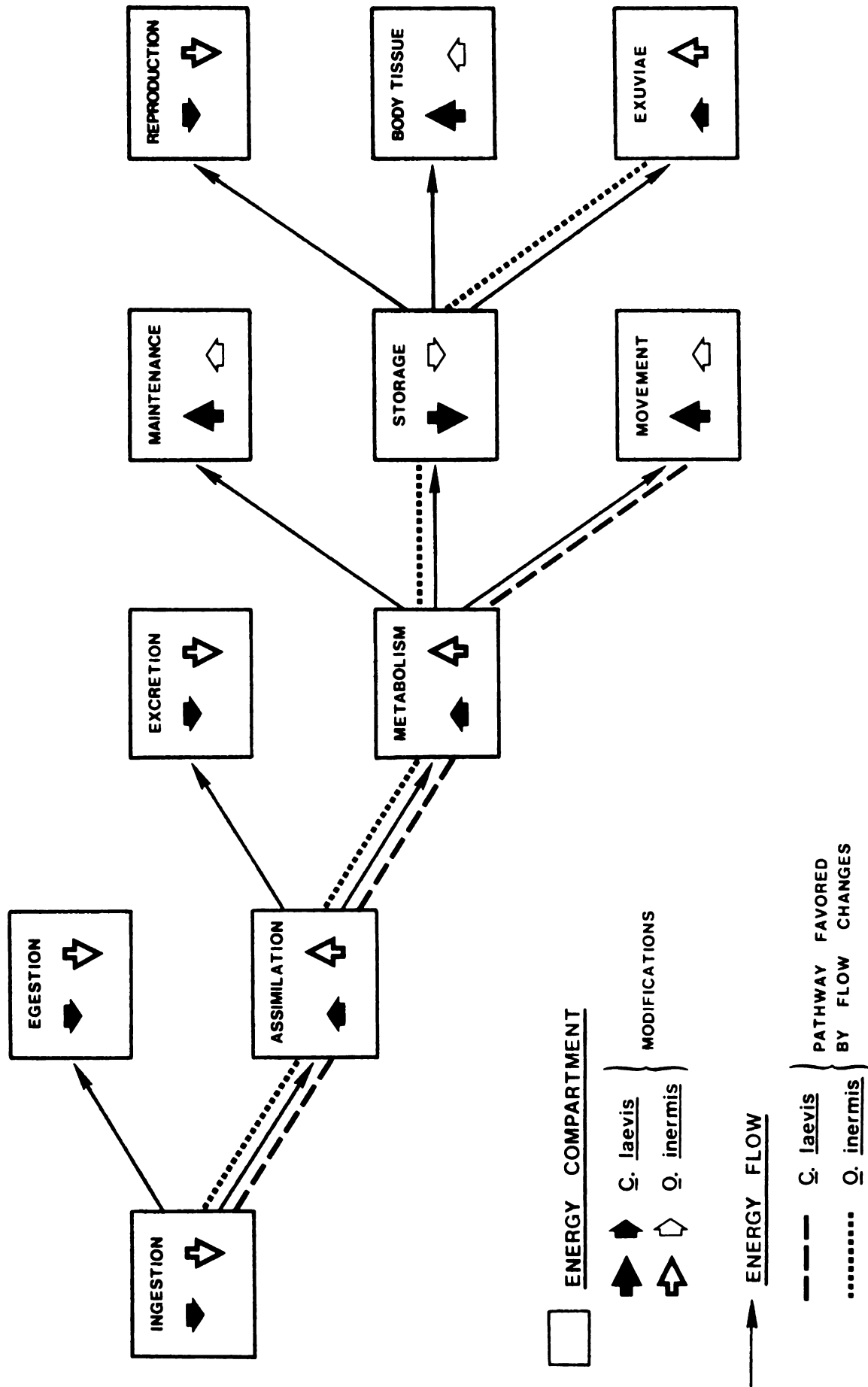


Figure 52

maximization, or perhaps even power maximization (Odum and Pinkerton, 1955), to time minimization. Odum and Pinkerton stated that when food is supplied at constant and minimal rates it might be advantageous for an organism to be metabolically slower, but more efficient.

Ingested energy is either assimilated or egested. Both cave-inhabiting crayfish have probably shifted energy flow into the assimilated energy compartment. Increased assimilation efficiency in the troglophile would result from slower passage of food through the digestive tract, whereas in the troglobite even greater efficiency may be explained by the same mechanism, and, perhaps also, by changes in basic digestive capabilities. In considering their natural diets, however, a net increase in assimilation is uncertain because of the dietary shift in the cave-inhabiting crayfish to a higher proportion of relatively indigestible plant material.

Assimilated energy is either excreted or metabolized, but, since excretion was not studied, only a few general assumptions can be drawn. Hypogean *C. laevis* consumes a higher percentage of relatively protein-poor plant detritus in its diet than does the epigean crayfish; this suggests that the cave-inhabiting troglophile shifts energy flow away from the excretion pathway. The troglobite not only extends this dietary trend, but also contains a higher percentage of protein in its body tissues (see Table 37 above); both of these characteristics would tend to shift energy flow even further away from excretion.

Metabolizable energy is distributed to either maintenance, muscular work (movement) or storage (body tissue, exuviae and reproductive components). Although maintenance should be the same for the two *C. laevis* populations, movement associated with foraging is probably more extensive in the cave. This additional energy expenditure,

coupled with decreased energy influx, shifts energy flow away from the storage compartment; the P/A ratio, which was extremely low, supports this conclusion. Maintenance, although unchanged, is relatively greater, and movement, which has probably become more extensive, increases its share of energy to an even greater degree. The low P/A ratio indicates that the troglobite has probably also shifted energy flow away from storage, but not to the extent of the troglophile. This was accomplished by reducing both maintenance and movement costs. Evolutionary improvements in sensory systems permit the troglobite to reduce locomotive energy costs of foraging. Nevertheless, it is thought that energy flow through the troglobite, in comparison to epigean crayfish, still favors movement. Consequently, it might seem surprising that this crayfish, when compared to surface-living species, appears lethargic. This behavior results from the depressed energy flow, which causes a reduction in the absolute amount of energy entering this relatively important pathway. Both maintenance and movement show increases relative to storage, although both are less in absolute value than in epigean crayfish.

Storage energy is partitioned into the production of either body tissue, exuviae or reproductive components (eggs). Based on the low percentage of storage energy that was utilized for reproduction, it seems highly probable that the hypogean troglophile diverts energy away from reproductive functions; this is funneled instead into both body tissue and exuviae production. Exuviae energy losses are relatively greater in the cave because of seasonal molting associated with reproduction, even though reduced growth rates may make molting unnecessary from a growth standpoint. The troglobite even further reduces flow along the reproduction pathway, and the energy instead is used in

body tissue production and, more importantly, in exuviae production.

Molting is relatively more costly in the troglobite, despite the fact that its exoskeleton comprises a lower percentage of total body weight than is the case in *C. laevis*. The increased molting cost is due to the smaller relative growth increment per molt that occurs in *O. inermis*.

CHAPTER VIII

GENERAL DISCUSSION

The various shifts in energy flow have led to characteristic patterns in the troglophilic and troglobitic crayfish. In comparison to its surface relatives, the hypogean troglophile has an energy flow pattern that funnels, at the expense of growth and reproductive functions, a greater proportion of ingested energy into movement. This crayfish, in the food-poor cave habitat, directs a disproportionate amount of its energy into food-search activity. *C. laevis* is a crayfish with generalized capabilities that permit it to survive in either an epigean or a hypogean habitat. As such, it can be described as merely adequate in its ability to cope with the trophic rigors of the cave, and its physiological adjustments center on trying to overcome the deficient energy supplies. Genetic changes, which would enhance its ability to compete with the troglobite, are prevented by the gene flow between the epigean and hypogean habitats.

The troglobite, on the other hand, has undergone evolutionary changes that have helped it adapt to the hypogean ecosystem. These changes include such things as elimination of the unnecessary energy costs of eyes and pigment, increased foraging efficiency that incorporates improved sensory perception with reduced random movement, reduction of body size, and increased metabolic efficiencies. These changes, which are principally of a genetic nature, have produced a specialized organism that is well adapted to the trophic characteristics of the cave stream.

Instead of expending an inordinate amount of energy in procuring food, the troglobite, with its reduced energy needs, can direct energy flow into storage functions, such as growth and, especially, exuviae production, which has become relatively more costly as a result of the evolution to a troglobitic condition.

The end result is that the troglobite can support its biomass with less impact on scarce food resources than can the troglophile, and, in so doing, it contributes to the stability of the hypogean ecosystem. The troglophile, on the other hand, exploits both epigean and hypogean habitats, and, if its impact exhausts the food base of the cave stream, it can continue to survive in the surface stream where the bulk of the population resides. Based on the occurrence of these two crayfish species co-existing in several southern Indiana caves, both of these contrasting strategies seem to be successful and compatible — at least for the short-term.

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