LIFE HISTORY RESPONSE OF THE CRAYFISH ORCONECTES VIRILIS (Hagen) TO ACIDIFICATION IN THE EXPERIMENTAL LAKES AREA, NORTHWESTERN ONTARIO : A LABORATORY AND FIELD STUDY

by

ROBERT LAWRENCE FRANCE

A thesis
presented to the University of Manitoba
in partial fulfillment of the
requirements for the degree of
Master of Science
in
Zoology

Winnipeg, Manitoba, 1983

LIFE HISTORY RESPONSE OF THE CRAYFISH <u>ORCONECTES</u> <u>VIRILIS</u> (Hagen) TO ACIDIFICATION IN THE EXPERIMENTAL LAKES AREA, NORTHWESTERN ONTARIO

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BARTLETT'S ALGORITHM

- A cry for sanity in our silent night of madness -

"Very well, Planchet, that is all right. Now, then, I pass to what concerns me - my supper?"
"Ready. A smoking roast joint, white wine, crayfish, and fresh gathered cherries. All ready, (D'Artagnan) may master".
- Alexander Dumas, The Vicomte de Bragelonne

A lake is the landscape's most beautiful and expressive feature. It is earth's eye, looking into which the beholder measures the depth of his own nature.
- Henry David Thoreau

That land is a community is the basic concept of ecology, but that land is to be loved and respected is an extension of ethics.
- Aldo Leopold

The most important function of the wilderness for modern man is the opportunity of glimpsing for a moment what harmony really means.
- Sigurd Olson, Reflections from the North Country

Nature was created for man to exploit.
- Lenin

The 'control of nature' is a phrase conceived in arrogance ... when it was supposed that nature existed for the convience of man.
- Rachel Carson

"Any fool can destroy trees".
- John Muir

"Ninety percent of all pollution is caused by trees and flowers".
- Ronald Reagan

"Blaming Ohio for acid rain is like blaming Florida for hurricanes".
- Ohio State Governor

"We will never really become serious about air pollution until it begins to interfere with our television reception"
- Pessimistic delegate at Action Seminar on Acid Precipitation, Toronto 1979.

"I am pessimistic about the human race because it is too ingenious for its own good. Our approach to nature is to beat it into submission".
- E.B. White <u>in</u> Silent Spring

Anyone who has considerably meditated on man, by profession or vocation, is led to feel nostalgia for the primates ... I sometimes think of what future historians will say of us. A single sentence will suffice for modern man: he fornicated and read the papers ...
- Albert Camus, The Fall

Nature has no voice of its own, so some of us must try to speak for it.
- Hugh Cochrane, environmental journalist

There are some who can live without wild things, and some who cannot. These essays are the delights and dilemmas of one who cannot.
- Aldo Leopold, A Sand County Almanac

One could do worse than be a swinger of birches.
- Robert Frost

To my parents, who supplied me with a house full of books in which to grow up in.....and to the memory of Jared, friend and climber.

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No thesis or any piece of scientific research conducted at ELA is the sole product of a single author. Dave Schindler has attracted and held together a multidimensional group of closely working individuals at the field station that provides a research and learning ambiance that is truly unique in the scientific world. The finished piece of research from this institution represents an integration of ideas, advice and criticisms collected from a multitude of often informal 'bull sessions' with ELA personnel whilst over meals, on canoe trips, at parties, or back in Winnipeg at the Institute. It is with the greatest appreciation that I thank Dave for allowing me the opportunity to be a part of the atmosphere, both scientific and social, that comprises the magic of what ELA represents. Much of my outlooks and philosophies towards limnology and environmental science in general I owe to 'falling under Dave's wing' at an early influencial period of my career.

Several persons involved with the ELA project deserve special mention for the key roles they played in the production of this thesis. Ian Davies got me started in the 'business' and provided equipment, advice, encouragement and many hours of general crayfish conversation. Ken Mills provided me with a strong background in fishery statistics and was truly 'the grad student's best friend' in his diligent day-to-day handling of cheques, purchasing, funding etc. Jack 'the big-K' Klaverkamp introduced and instilled in me an attitude and philosophy toward the fledgling science of ecotoxicology that will always remain a directive force. Diane Malley helped to smooth the edges in the often traumatic introduction into the world of physiology for a born and raised ecologist. Ray Hesslien was always willing to take the time to explain to a slow biologist the enlightening world of geolimnology/aquatic chemistry. Paul Campbell, as limnology section head, was an easy and understanding person who would never begrudge time spent with those of us on the bottom of the totem - i.e. graduate students. L. Graham at U of M, identified Thelohania. T. Dick, W. Nero and K. Mills aided in the photography. Without the influence of my fellow graduate students - Peter, Woody, Dave and Goeff, this thesis would no doubt have been finished much sooner but with also much less enjoyment along the way. Our scientific discussions, often in the midst of a pubinduced nirvanic bliss, always supplied refreshing insights into environmental research directions. Peter also provided many hours of time and much effort with the Scuba collections. I thank my committee (D. Schindler, G. Robinson, L. Graham and J. Klaverkamp) for their helpful suggestions. Donna Laronqee typed the manuscript.

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available to provide me with an often much needed alternative to science. Finally I would like to thank my family for being understanding and accomodating the idiosyncracies peculiar to a person involved with producing a graduate thesis.

FORWARD

This thesis has been organized in a format to better facilitate its conversion into manuscripts suitable for journal publication. Following the general introduction, four papers appear: the first, a field study comparing the life history aspects of _O. virilis_ in response to lake acidification, the second on the effects of experimental acidification on the life cycle of this crayfish in the laboratory, the third on the acclimatization and avoidance reactions of _O. virilis_ to laboratory acidification, and the fourth (appendix) dealing with growth and population regulation of _O. virilis_ in the ELA region.

ABSTRACT

Life history characteristics of the crayfish Orconectes virilis were examined during 1979-81 in four small oligotrophic Canadian Shield basins in the Experimental Lakes Area, northwestern Ontario. One of these lakes, Lake 223 (L223), has been undergoing experimental acidification since 1976 to simulate the effects of acid precipitation. Carapace rigidity and Ca^{++} content were significantly lower in L223 crayfish, suggesting an inhibition of postmolt exoskeleton calcification. Growth in L223 has not been affected by acidification to pH 5.35. Egg resorption, fertilization and rate of development were not significantly affected by lake acidification. Incomplete hardening of the glair-cement compound forming the egg capsule membrane and stalk has resulted in a failure of secure pleopod egg attachment causing the L223 population to suffer recruitment failure. On an egg production basis, the percent reproductive failure in the control populations averaged 3.2 ± 1.8 (95% C.I.) while levels of 18.7, 36.2 and 29.4% were obtained during 1979-81 in L223 at pH 5.05 - 5.60. Lake 223 crayfish have responded to acidification-caused remobilization of trace metals with an increased bioaccumulation of Mn and possibly Hg. The level of microsporidian Thelohania sp. infection in the L223 population was 1.7% during 1979 and increased to 6.5% during 1980 with a constant incidence of <1% in the control lakes. The loss of crayfish populations exposed to lake acidification may be brought about by reproductive impairment and possibly increased susceptibility to parasitic infection long before lake water is acid enough to be directly toxic to crayfish stocks.

The life cycle response of O. virilis to experimental acidification was investigated in the laboratory. Development from a 2 week old hatchling stage to a 2 1/2 month old juvenile stage results in a seven-fold increase in acid resistance; continued maturation to a 2-3 year adult results in a further twenty-fold increase in resistance. Comparison of laboratory survival results and life history information with data from the literature concerning the magnitude and duration of chemical fluctuations, indicates that O. virilis populations are not likely to decline as a result of severe episodic and pulses. Nevertheless, gradual acidification of any lake to an average annual pH below 5.5 could result in eventual population extinction due to mortality of the young life stage. Juvenile crayfish can recover following exposure to acid stress. A significant decrease in percent time spent aerating the eggs with the pleopods was obtained, but this only occurred at pH levels well below those previously known to cause egg mortality in L223. Consequently, low pH will directly affect crayfish recruitment long before modifications of maternal behavior become important.

Acclimatization to and avoidance of low pH by O. virilis were tested and compared for animals from populations inhabiting a control lake and the experimentally acidified L223. Acclimatization of adult crayfish to acidic water as measured by resistance to low pH during month-long exposures, has not occurred for the L223 population. Adult and yearling crayfish from the control population avoided water of pH 4.5 and below that would prove lethal, but demonstrated no discrimination above pH 5.0 at values toward which this species has been shown to experience serials reproductive difficulties. Crayfish from L223 had a noticably reduced

avoidance response (i.e. individuals developed a sensory adaptation to lowered pH), significant at only pH 4.0, with no avoidance occurring above pH 4.5. The long term survival of *O. virilis* populations in lakes receiving acidic snowmelt seems doubtful if based solely upon an implied potential ability for acclimization or pH-induced behavioral modification of distribution.

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CHAPTER I

GENERAL INTRODUCTION

1.1 ACID PRECIPITATION

Anthropogenic emissions of SO_2 from fossil fuel burning and metal smelting pose a global problem and have led to increases in the acidity of precipitation in Scandinavia (Oden 1976), the northeastern United States (Cogbill and Likens 1974), and eastern Canada (Summers and Whelpdale 1976; Dillon et al 1978). Consequently, this has led to the acidification of many softwaters occupying those geologically acid sensitive areas with little calcareous overburden to serve as a mitigative buffering force (Beamish and Harvey 1972; Dickson 1975; Watt et al 1979; Jeffries et al. 1979). Lakes and rivers in north-temperate climates are subject to the accumulation of acidic snow throughout the winter and its subsequent melting in the spring (Hagen and Langeland 1973; Hendrey et al. 1980). Laboratory and field experiments reveal 50-80% of the pollutant load is released with the first 30% of meltwater (Johannassen and Henrickson 1978). Because the soil may still be partially frozen, much of this melt can quickly run off to the lakes and rivers with little neutralization in the watershed (Haapala et al. 1975; Henricksen and Wright 1977). Typically, between 36-77% of the annual export of H^+ from the watersheds over much of south-central Ontario occurs during this short period (Jeffries et al. 1979). The resulting rapid and severe pH decreases in receiving waters can have profound effects upon their inhabitants (Hultberg 1976; Leivestad and Muniz 1976; Groterud 1976).

Considerable study has been devoted to the investigation of the often irreversible damage inflicted upon the resident biota of culturally acidified waters, as reviewed by Hendrey et al (1976), Leivestad et al.

(1976), Wright et al (1976), and Almer et al (1978). Although numerous studies have been performed concerned with the ecological effects of acidification upon fish populations or the physiological/toxiocological laboratory reactions of fish to low pH, as reviewed in Fromm (1980), Daye (1980), and Spry et al (1981), little work has been undertaken on the response of invertebrates to acid pollution.

1.2 CRAYFISH ECOLOGY

Crayfish (Decapoda, family Astacidae) are the largest and longest-lived members of the freshwater Crustacea. Of the 150 species that inhabit North America, 9 are important members of the freshwater biota in Ontario waters (Crocker and Barr 1968; Berrill 1978). Almost every type of freshwater habitat can be utilized by one or more crayfish species. Because of their large size, and in many cases, numerical abundance, crayfish may dominate the biomass and annual production of benthic communities.

Due to their omnivorous habits, crayfish serve as a key energy transformer between various trophic links, making major sources of energy such as detritus and decaying plants and animals available to higher vertebrates, thus helping to maintain an efficient energy flow throughout the system (Momot et al. 1978; Crocker and Barr 1968). Their functional trophic importance is even further accentuated in low nutrient oligotrophic lakes that strongly rely upon recycling of organic matter for energy sources (Momot et al 1978).

Crayfish play an important multifaceted role in the general ecology of the aufwuchs community. Through ingestion of slowly decomposing materials such as wood or pine needles, crayfish structurally and chemically transform it into a usable form for other decomposers (Momot et al. 1978). Studies suggest that crayfish grazing can strongly influence the abundance and diversity of aquatic vegetation and regulate littoral primary production (Abrahamson 1966; Flint and Goldman 1977). Predation by crayfish can significantly alter the relative abundances of gastropods, leeches, tadpoles, and many aquatic insects (Lorman and Magnuson 1978). In this way crayfish not only contribute to the overall stability of their prey communities but also regulate the benthic faunal production available to fish (Momot et al. 1978). A large proportion of crayfish production may be recycled back into the population through cannibalism.

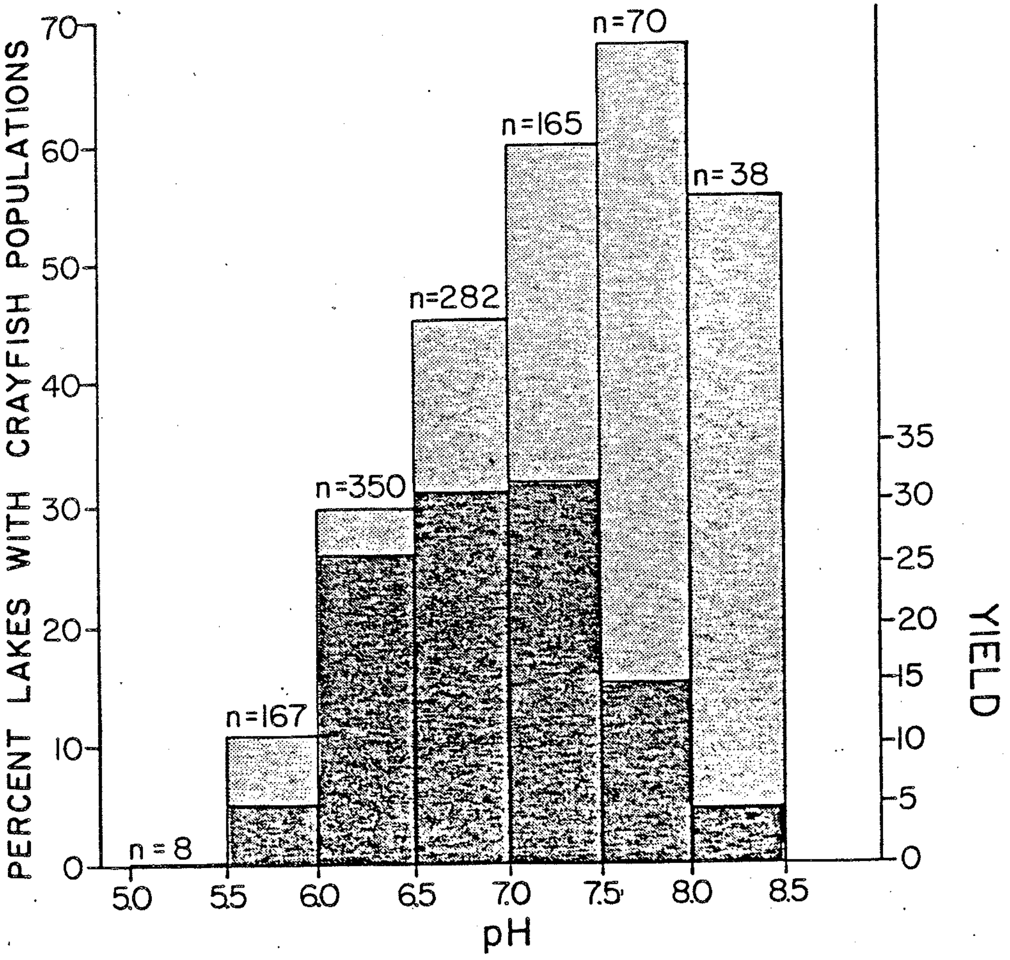
Predation of crayfish by at least 46 species of fishes, 10 species of amphibians, 20 species of reptiles, 38 species of birds, and 6 mammal species have been documented (Penn _in_ Crocker and Barr 1968). Crayfish are part of the diets of many fish species: perch and shag (Scott and Duncan 1967; Reid 1972); brook trout (Gowing and Momot 1979); smallmouth bass (Rickett 1977) lake trout and rainbow trout (Flint 1975b); and other important Canadian Shield game species such as rock bass, pickerel, sunfish, and especially northern pike (Penn _in_ Crocker and Barr 1968). Young crayfish in turn function as an important food supply to larger benthic invertebrates (Momot et al. 1978).

1.3 RELEVANCE OF STUDY AND STRATEGY EMPLOYED

Evidence suggests that crustaceans are among the most sensitive organisms to lake acidification and a full understanding of the complex relationships between their biology and depressed pH is therefore of paramount importance. Previous studies have been largely survey-oriented and show that the distribution of many crustaceans is curtailed below pH 6.0 (Okland 1980; Raddum 1980; Okland and Okland 1980) or that communities experience progressive alterations in diversity with increasing water acidity (Sprules 1975; Roff and Kwiatkowski 1977; Fryer 1980). Inherent difficulties exist in establishing causal relationships between pH and survey-based data. Only a few investigations have monitored the response of crustaceans to acidification over time within a particular affected basin, similar to the study conducted by Beamish and his colleagues with fish (Beamish et al. 1975). These studies have been restricted to dealing with only the planktonic Crustacea (Yan and Strus 1980; Malley et al. 1981; Nero 1981). Crustacea/acid laboratory experiments have been conducted on pH tolerances (Davies and Ozburn 1969; Borgstrom and Hendrey 1976) and mechanisms of ionic disturbance (Potts and Fryer 1979).

The acidification of softwater lakes and rivers in Scandinavia and North America poses a serious threat to the long-term survival of crayfish populations. Svardson's (1974) survey of Astacus astacus distribution in relation to the pH of Swedish waters (Fig. 1) suggests that crayfish may serve as an important indicator of the early stages of lake acidification at pH values even above 6.0. There are numerous citings of A. astacus populations that have suffered from increasing acidity over recent years (Abrahamson 1972a; Hultberg 1976; Almer et al. 1978; Furst 1977b; Appelberg 1980). Despite the generally accepted belief that

Figure 1: Distribution of *Astacus astacus* in 1,080 Swedish lakes. Yield (dark shading) represents number of crayfish per hectare from survey of 113 lakes. Data from Svardson (1974).


PERCENT LAKES WITH CRAYFISH POPULATIONS
70
60
50
40
30
20
10
0
n = 8
n=167
n=350
n=282
n=165
n=70
n=38
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
pH
35
30
25
20
15
10
5
0
YIELD

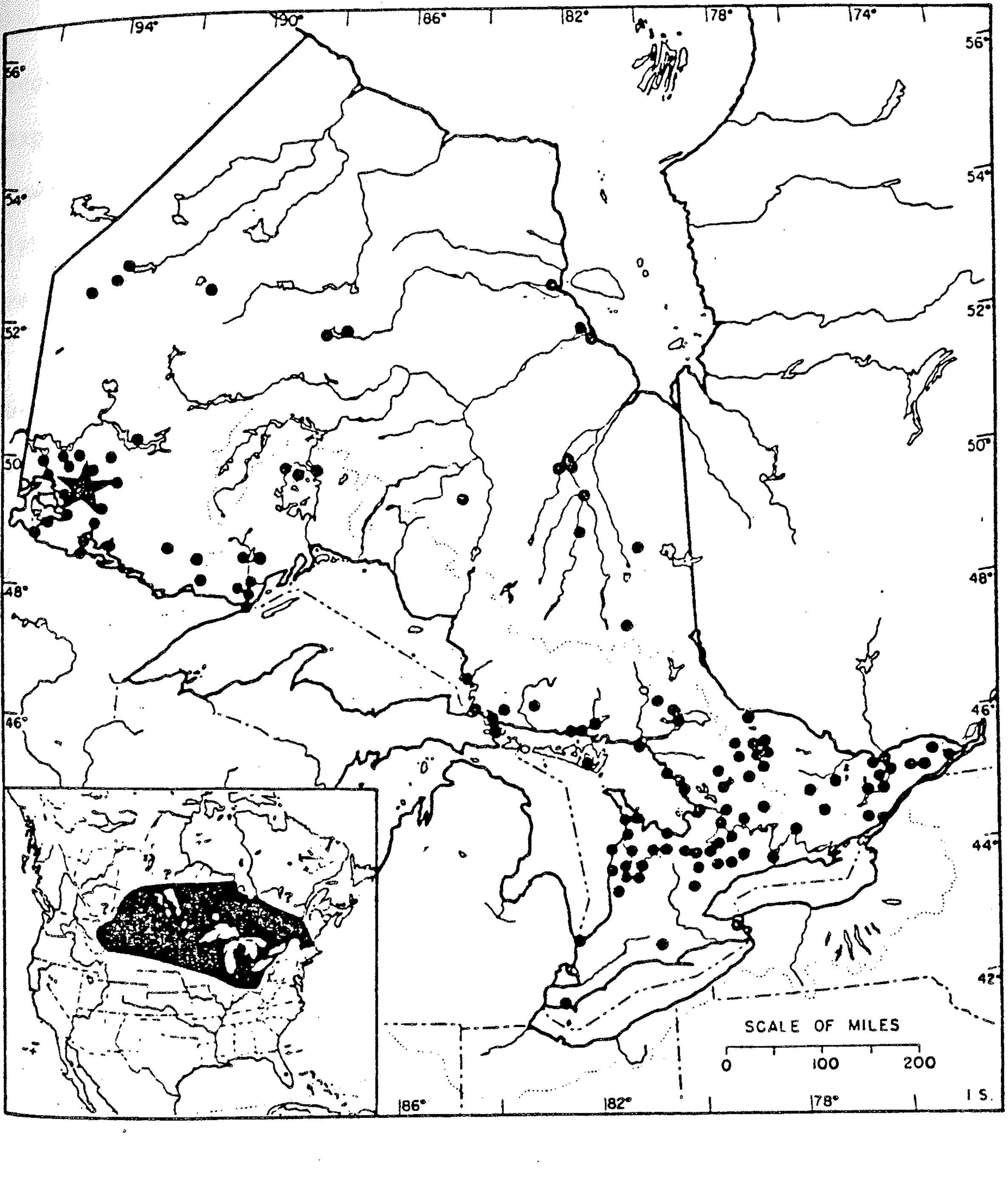
crayfish disappear below pH 6.0 (for example, several Swedish tourist information brochures on acid rain) the actual mechanisms of population decline have never been investigated.

The pH tolerances of a Tasmanian crayfish native to acid waters (Newcombe 1975), a British species (Jay and Holdich 1977), and the North American species *Orconectes rusticus* (Baker unpub.) have been determined by short-term laboratory experiments. The physiological responses of *Procambarus clarkii* and *O. rusticus* subjected to acidic water have been monitored (McMahon and Morgan 1981). There is a paucity of data on the sublethal effects of acid stress on crayfish or any crustacean. Furst (1977a) experimentally found a lower limit of pH 5.6 for the successful reproduction of *A. astacus*. Appelberg (1981) developed a sublethal test involving the effects of acid on oxygen uptake by *A. astacus* eggs. Malley (1980) and Appelberg (1980) determined that low pH can exert sublethal effects upon crayfish molting and postmolt calcification. It is not known if the results of these studies can be extrapolated to natural populations.

On an economic basis, the crayfish *A. astacus* is considered to be the single most important inhabitant of many Scandinavian lakes and rivers, supporting a multi-million dollar fishery (Svardson 1949; Abrahamsson 1972b). Svardson (1974) has stated that, "it is justifiable to conclude that many holders of fishery rights, who still fish crayfish in their lakes, have lost by now a part of the yield because of recorded acidification." Since the demise of many *A. astacus* populations during the last 25 years due to the crayfish plague *Aphanomyces astaci*, a great amount of money and effort has been spent on developing the North American plague-resistant crayfish Pacifastacus lenisculus in large hatcheries. Extensive restocking programs in hundreds of lakes have been underway for a decade and have met with variable success (Brinck 1977). Furst (1977b) documented an unsuccessful restocking attempt due to depressed pH and Abrahamsson (1972a) reported that the cessation of liming by fishery biologists to an inflowing watercourse during a single month period resulted in a 96% mortality of the crayfish population in the lake. Consequently, Scandinavian crayfish fishermen and hatchery managers are deeply interested in the economic problems associated with acidification (S. Karlsson, Simtrop Hatcheries, pers. comm.).

Orconectes virilis is the most widely distributed and abundant crayfish species in Canada (Crocker and Barr 1968; Fig. 2). Its range extends from north-central Alberta (Aiken 1968) throughout the geologically acid-sensitive Precambrian Shield including the Haliburton-Muskoka region of south-central Ontario (Berrill 1977), one of the most heavily acidified areas of the globe (Dr. D.W. Schindler, FWI, pers. comm.), and to the northeastern United States. O. virilis is of commercial importance and supports an extensive fishery for bait and biological specimens (Crocker and Barr 1968). O. virilis has been harvested commercially in northern Wisconsin for decades (Threinen 1958) and recently the economic feasibility of opening a new fishery in Vermont has been investigated (Nolfi and Miltner 1978; Nolfi 1981). Furthermore, the importance of this crayfish species in maintaining an efficient energy flow, especially within those oligotrophic glacial lakes most vulnerable to acid precipitation (Momot et al. 1978), suggests that its disappearance could generate

Figure 2: Distribution of crayfish <u>Orconectes virilis</u> in North America from Crocker and Barr (1968). Star denotes Experimental Lakes Area field location of present study.


94°
90°
86°
82°
78°
74°
56°
54°
52°
50°
48°
46°
44°
42°
SCALE OF MILES
0
100
200
86°
82°
78°
I.S.

serious disturbances within lake ecosystem dynamics.

The purpose of this study was to investigate the life history and eco-physiological/toxicological response of Orconectes virilis (Hagen) to experimental acidification. The major direction of research has been towards developing a comprehensive ecotoxicological study combining both laboratory and field data in an attempt to accurately describe the reaction of this crayfish species to low pH.

The relationships between life histories and environmental disturbances are usually subtle and difficult to detect or interpret. Laboratory studies provide precise dose-effect information concerning the predicted effects of a single pollutant but can never successfully duplicate all the interacting variables characteristic of the natural environment. For these same reasons, field studies, although considered by many to reflect the final proof of ecological significance of perturbative pressures in the 'real' world, cannot provide the sensitivity necessary to detect adverse effects before they reach crisis proportions. The failure to combine both laboratory and field information in concert, produces studies that have little relevance to solving contaminant problems. Despite this, Sprague's (1976) review of current toxicology testing protocol, indicated that the lowest number of water quality criteria were based on field observations. Klaverkamp (Fresh- water Institute, pers. comm.) has proposed a mechanism for intergrating both laboratory and field toxicology data based on reciprocal objectives of increasing or decreasing relevance and identification of mechanisms, thereby producing the 'integrated toxicity test design' stressed by

Buikema and Benfield (1979) as being the single most valuable tool in toxicology research.

Life history is the study of the multifaceted parameters impinging upon an organism during its life; essentially the role or 'niche' of that animal in relation to the ecosystem. With the exception of Crocker and Barr's (1968) and Berrill's (1977) zoogeographic studies, there is little information on crayfish populations in Canada. The life history section of this thesis was designed to provide a comparative study of the natural variability of crayfish among four ELA basins, the data of which can be used, first, to compare with previous studies on O. virilis from other geographic areas in N.W. Ontario (Weagle and Ozburn 1972; Momot 1978) and northern Wisconsin (Momot 1967; Momot and Gowing 1977a); second, to aid in the interpretation of the effects of experimental acidification of a small oligotrophic lake upon the numerical abundance and population structure of O. virilis (Davies in prep); and third, to provide information useful in the design and interpretation of laboratory toxicity tests.

The purpose of the laboratory pH experiments was to determine, first, how the tolerance of O. virilis changes throughout its life history, and second, to measure the acid sensitivity of this species for comparison with other invertebrate and fish species. Accomplishment of these objectives would allow predictions to be made regarding the survival of crayfish populations in lakes subjected to acid precipitation and more particularly, the rapid and severe spring pulses of acidified snowmelt. Further experiments investigated questions raised by field observations of crayfish in the acidified lake. Abilities of acclimatization and recovery from acid stress were studied, as were several aspects of the sublethal behavioral reactions of O. virilis to acidified water. In all cases, attempts were made to assimilate field-gathered life history information in the interpretation of experimental results.

The present study in conjunction with laboratory investigations of physiological mechanisms (Malley 1980; unpublished data) and whole-lake population estimates (Davies in prep) is hoped to allow prediction of the response of this invertebrate to cultural acidification.

1.4 DESCRIPTION OF ELA STUDY LAKES

The location of the present study is the Experimental Lakes Area (ELA) situated at 93°30'-94°00' W and 49°30'-49°45' N, approximately 300 km east of Winnipeg, Manitoba. ELA is a headwater region containing small glacially-formed basins in the Canadian Precambrian Shield and typical of those lakes occupying half the land surface area of Canada. Lakes are commonly ice-covered for the 5-6 month interval from November to May and have dimictic thermal regimes. ELA waters have among the lowest ionic concentrations reported in the limnological literature. Significant portions of cations are supplied directly from precipitation, which averages about pH 5.0. Primary production is low, and lakes are characteristically defined as oligotrophic. Periphyton covers littoral rocky areas with a 0.5-2 cm thickness and is composed of 60-70% diatoms. Detailed physical, chemical and biological characteristics of the ELA region can be found in the three "ELA volumes": J. Fish Res. Bd. Canada 28:121-304, 1971; J. Fish Res. Bd. Canada 30:14091552, 1973; and Can. J. Fish. Aquat. Sci. 37:311-559, 1980. Orconectes virilis is the only crayfish species present in these lakes.

Four ELA lakes were selected for detailed field studies of crayfish populations and/or sampling for laboratory experimentation. All lakes are within 5 km of the ELA field station. Lake 224 (L224) flows into L223, both being part of a northerly draining watershed eventually supplying water to the English River System. Lakes 240 and 239 are adjacent to the ELA field station and flow south into Lake-of-the Woods. Crayfish were also obtained from several other lakes such as L302 and Roddy Lake (L468) near the ELA field station.

Fundamental physical, chemical and biological characteristics of the four basins are provided in Table 1. Generally lakes are of a similar size although L239 and L224 are deeper. The extent of shoreline formations (beaches, boulders, swamps, cliffs) are similar for all lakes. Lake 240 has the fastest water renewal time; L224 the slowest. Annual thermal cycles are nearly identical. Lake 239 has the highest calcium content and conductivity; L224 the lowest. Nutrient levels are similar but water transparency is greatest in L224 and lowest in L239 due to the presence of a darker humic colour in the latter. All waters are extremely soft/poorly-buffered and have similar hydrogen ion concentrations, if pre-acidification values in L223 are included. Lake 239 is the most productive lake, L223 and L240 are intermediate and similar, and L224 is the most oligotrophic. Invertebrate faunas are similar but differences exist in both abundance and presence or absence of major fish species.

Table 1. Characteristics of ELA study lakes. Data have been compiled from Beamish et al. (1976), Prokopowich (1979), Fee (1980) and "ELA volume": J. Fish. Res. Board Can. 28: 121-304, 1971.

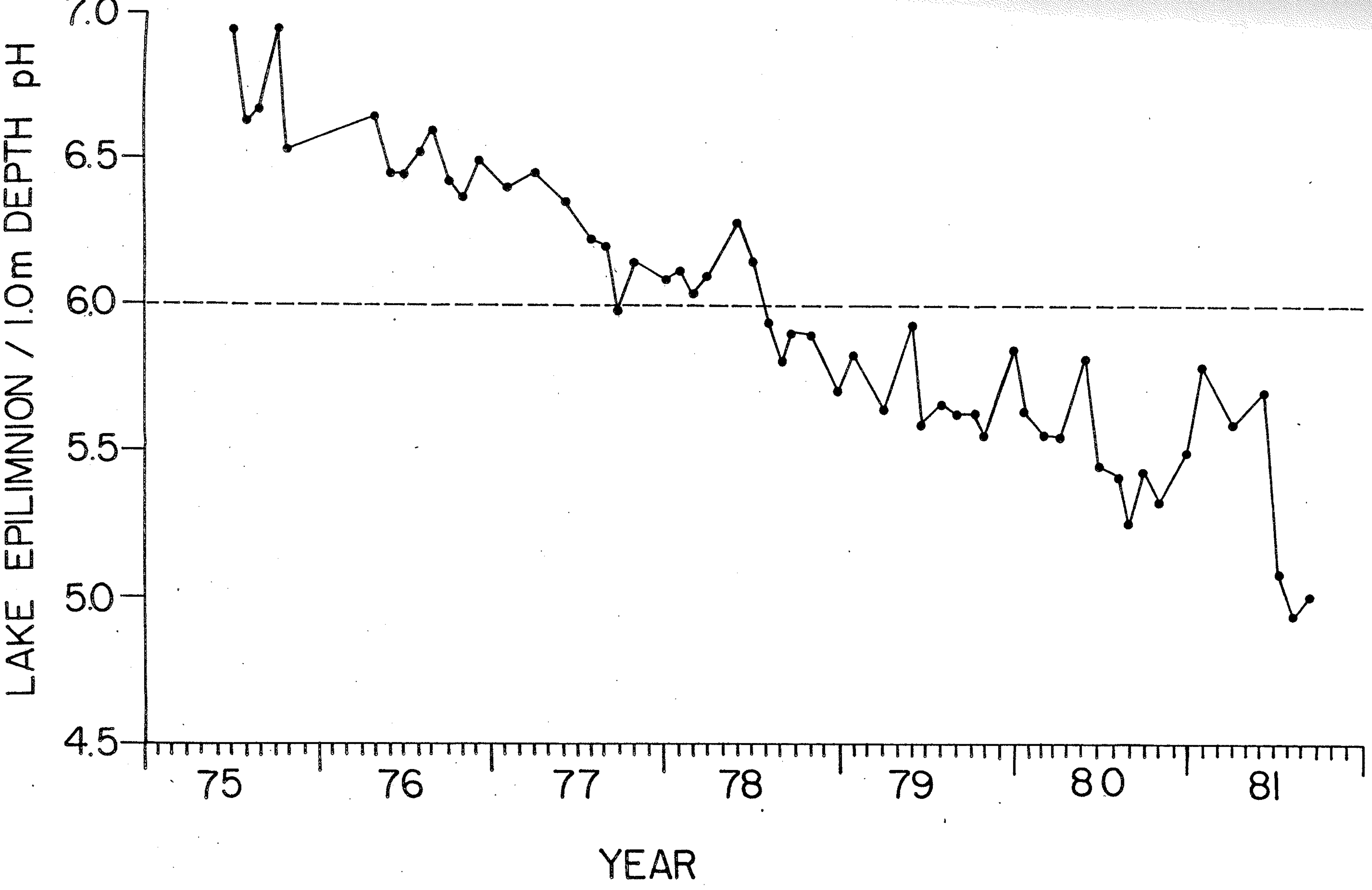
Characteristic	L223	L224	L239	L240
Lake surface area	27.3	25.9	56.1	44.1
Mean depth (m)	7.1	11.6	10.5	6.1
Maximum depth (m)	14.4	27.4	30.4	13.1
Mean epi-temperature (°C)	20.02	20.34	19.38	19.70
Ca ($mg \cdot L^{-1}$)	2.17	1.75	2.39	1.96
CDN (25°C)	20.7	18.0	25.8	22.5
CHL-a ($\mu g \cdot L^{-1}$)	1.6	1.3	2.2	1.9
Mean epi-primary production ($g\ C\ m^{-2} \cdot yr^{-1}$)	15.8	12.4	16.9	15.7
pH	-	6.71	7.03	6.99
Zoobenthos type	D	D	E	D
Fish (A = abundant R = rare)	lake trout A white sucker A slimy sculpin A	lake trout A white sucker A slimy sculpin R	lake trout R white sucker A slimy sculpin A northern pike R yellow perch A lake herring A	white sucker A slimy sculpin R northern pike A yellow perch A lake herring A

Lake 239 (Rawson Lake) and L240 are control basins for the ELA region. During 1976, a whole-lake radiotracer experiment was conducted in L224. The amounts of isotopes in the biota never exceeded 1% of the added amount and did not interfere with normal biological functions. Lake 224 has since been used as a control for the L223 experiment. Lake 223 has been undergoing experimental acidification with sul furic acid since 1976 to simulate the effects of acid precipitation. Epilimnion pH has decreased at a rate of approximately 0.25 pH units per annum (Table 2; Fig. 3). Methods of acid addition and detailed chemical and biological results are presented in Schindler et al. (1980), Schindler (1980), and Schindler and Turner (1982).

Table 2. Mean epilimnetic pH of Lake 223 in the ice-free season during the acidification experiment. Data from Schindler (1980) and Schindler (unpublished).

Year	pH
Pre-acidification	
1974	6.64
1975	6.61
During acidification	
1976	6.79
1977	6.08
1978	5.84
1979	5.60
1980	5.35
1981	5.05

Figure 3: Monthly pH measurements in Lake 223 following experimental acidification since 1976 (Schindler, unpublished data).


LAKE EPILIMNION / 1.0m DEPTH pH
7.0
6.5
6.0
5.5
5.0
4.5
75
76
77
78
79
80
81
YEAR

CHAPTER II

The Effects of Experimental Lake Acidification upon the Life History of the crayfish *Orconectes virilis* (Hagen)

2.1 INTRODUCTION

The effects of depressed pH on aquatic crustaceans are poorly understood and limited to only a few laboratory studies. It is not known if the results of these experiments can be extrapolated to natural populations (cf. 1.3). The aim of this study, in conjunction with whole-lake population estimates (Davies in prep.), was to describe the response of the crayfish Orconectes virilis to the experimental acidification of its lake habitat.

Of all the inorganic ions contributing to physiological maintenance in crustacean biology Ca^{++} is of leading importance (Robertson 1941). Because of this, the distribution of crayfish is to some degree controlled by the level of Ca^{++} in potentially habitable waters (Robertson 1941; Covegnas in Macan 1961; Rhoades 1962; Greenway 1974; Capelli 1975). The minimum threshold concentration of Ca^{++} necessary for survival of crayfish populations has been suggested by these studies to be 2-5 mg L^{-1}. The lakes at ELA average 1.6 mg L^{-1} Ca^{++}, among the lowest concentrations for this ion recorded anywhere in the world (Armstrong and Schindler 1971). The species O. virilis at ELA is therefore existing close to the lower environmental limits for Ca^{++} for crayfish and relatively minor disruptions of Ca^{++} metabolism can be expected to be critical. The close interrelationships between Ca^{++} and pH necessary for crustacean survival have been documented (Okland 1980).

Reproductive impairment is a good measure of environmental stress because the act of reproduction reflects the product of all the factors influencing metabolism (Buikima and Benfield 1979). This led Sprague (1976) to conclude that the most sensitive and productive toxicological experiments in setting water quality criteria are those concerned with the chronic effects on the reproductive cycle. Despite this, few toxicological studies involving crayfish have used reproductive impairment as an index of perturbation. Vey's (1977) physiopathological study of suspended clay particles upon A. leptodactylus is the only field investigation of pollution on crayfish reproduction. Although a plethora of data exist indicating the extreme sensitivity of fish reproduction to laboratory depressed pH (reviewed in Spry et al. 1982) and failure of reproduction is often cited as the prime cause for the disappearance of fish in many acidified lakes (Schofield 1976), no field studies have investigated the effects of low pH on invertebrate reproduction. An important understanding and recognization of the insidious sublethal effects of pollution upon reproduction is essential to detect stresses early before they culminate in irreparable population damage.

In recent years evidence has been put forward indicating an alarming increase in the concentration of many trace metals coincident with the acidification of watercourses in affected regions of North America and Scandinavia. Such increases have been attributed to widespread atmospheric fallout associated with the industrial contamination of precipitation (Jeffries and Synder 1981), to increased leaching within the watersheds by elevated hydrogen ion activity

(Troutmon and Peters 1980), or to a remobilization of heavy metals directly from the lake sediments (Schindler et al. 1980a, 1980b). Field investigations suggest that uptake of trace metals by fish is accelerated at low lake pH (Tomlinson et al. 1980). Crayfish are known to bioaccumulate certain heavy metals in relation to their water concentrations but the effect of acidification upon uptake is not known.

2.2 MATERIALS AND METHODS

2.2.1 General Sampling

Sampling of Orconectes virilis from both beaches and rocky areas in L239 showed that adult (age II, II and IV) individuals predominated in the sandy areas whereas a much more representative sample of all size/age classes was obtained from areas of rocky substrate (Fig. 4). The geomorphological structure of the collection sites in the present study (Figs. 5-8) corresponds to Capelli's (1975) "type-A substrate" (mostly rocks and gravel; high shelter potential) found to be most suitable for crayfish growth and abundance. Because traps were shown to exert a considerable bias with the capture of predominantly large adult males (Fig. 9), use was made of SCUBA diving. Crayfish were gathered at night over the depth range 0-6 m with the aid of a suction gun designed to collect a representative sample of the population and to prevent mechanical damage to the specimens (Davies and Ramsey, in prep.). Detailed descriptions of collection sites and sampling methodology are presented on Appendix A.

Figure 4: Comparison of length-frequency distributions of crayfish collected over areas of sand and rocky substratum. Sampling was during August 1980 in L239. Roman numerals represent age-groups (c.f. Appendix A).

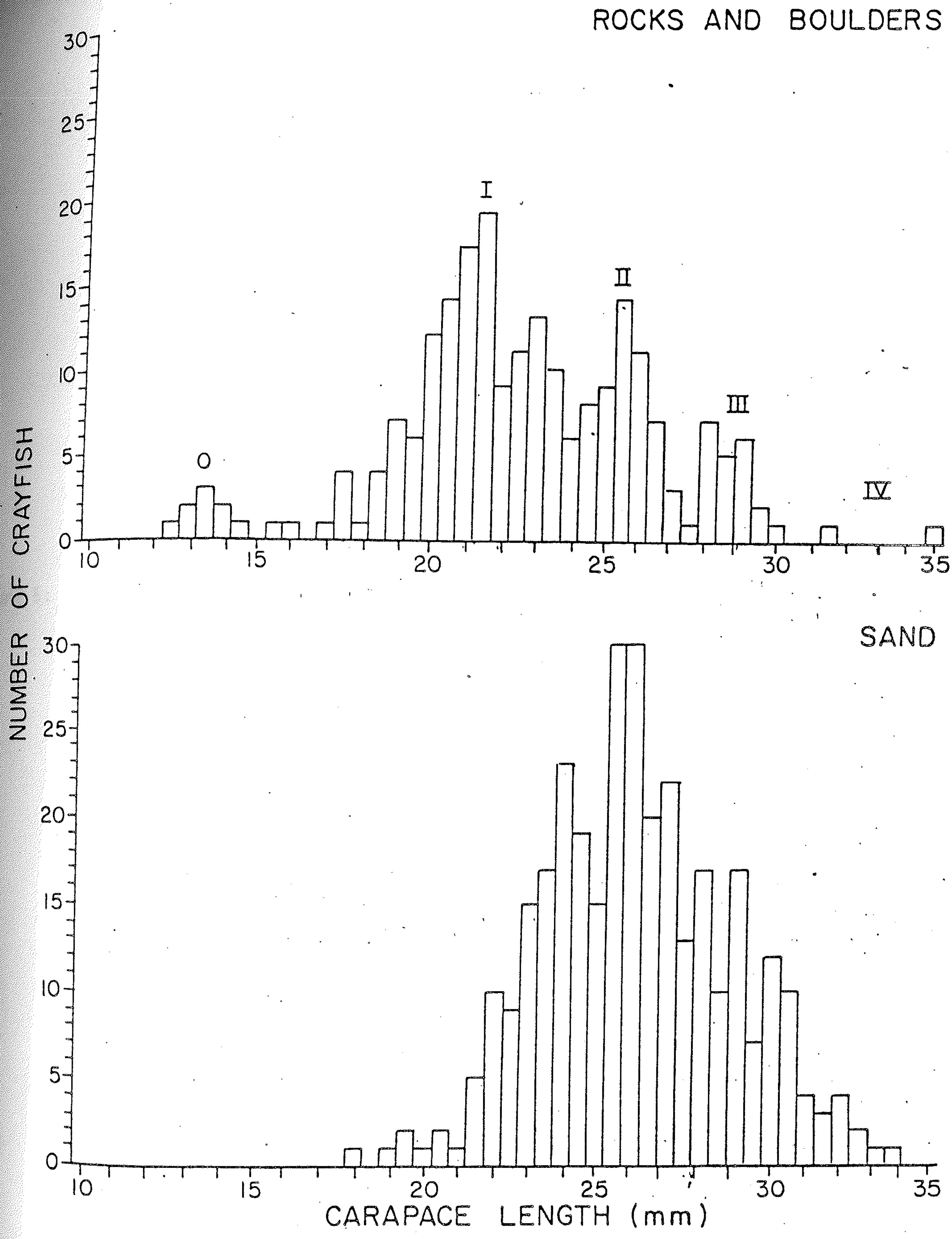

ROCKS AND BOULDERS
SAND
NUMBER OF CRAYFISH
CARAPACE LENGTH (mm)
O
I
II
III
IV
0
5
10
15
20
25
30
10
15
20
25
30
35

Figure 5: Bathymetric map of L239 showing type of littoral substratum. O. virilis collection sites designated by star symbols (After Stockner and Armstrong 1971).

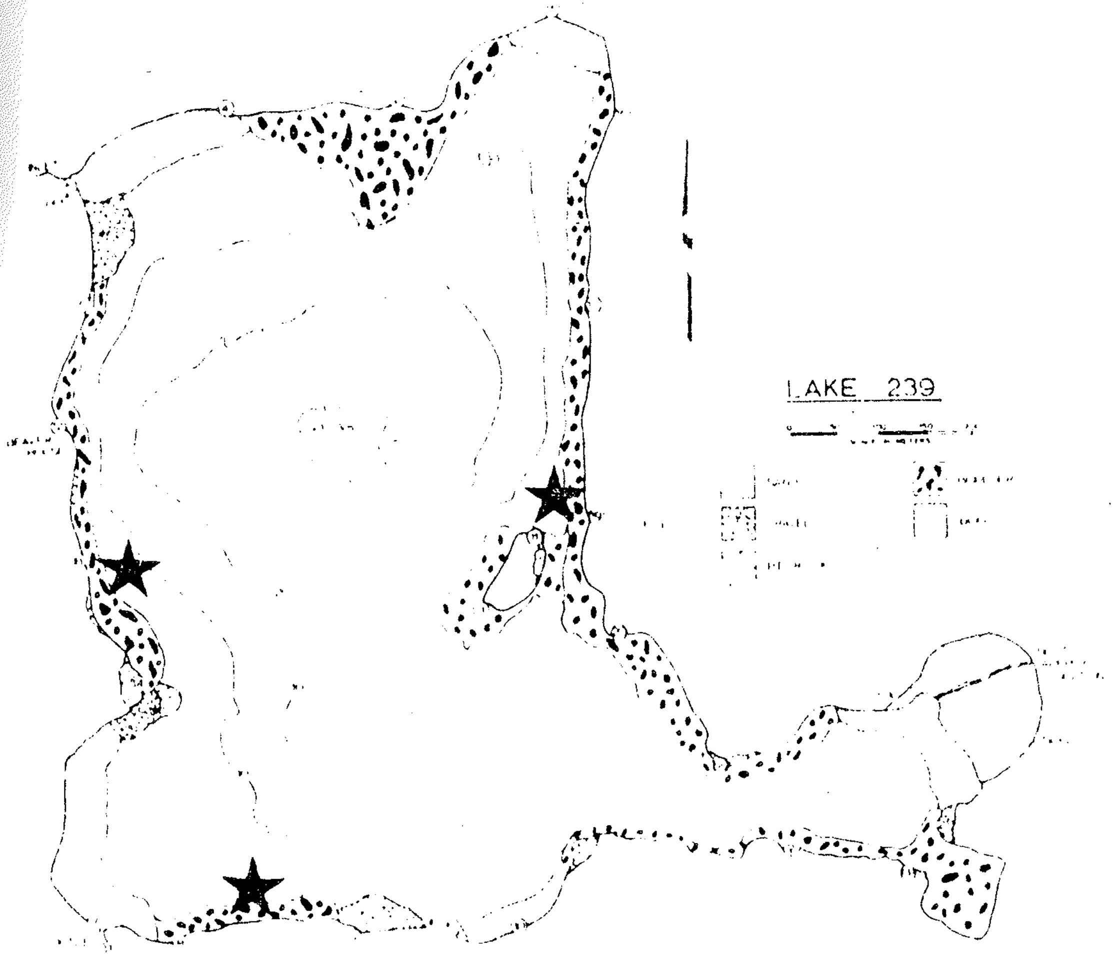

LAKE 239

Figure 6: Bathymetric map of L240 showing type of littoral substratum. *O. virilis* collection sites designated by star symbols (After Stockner and Armstrong 1971).

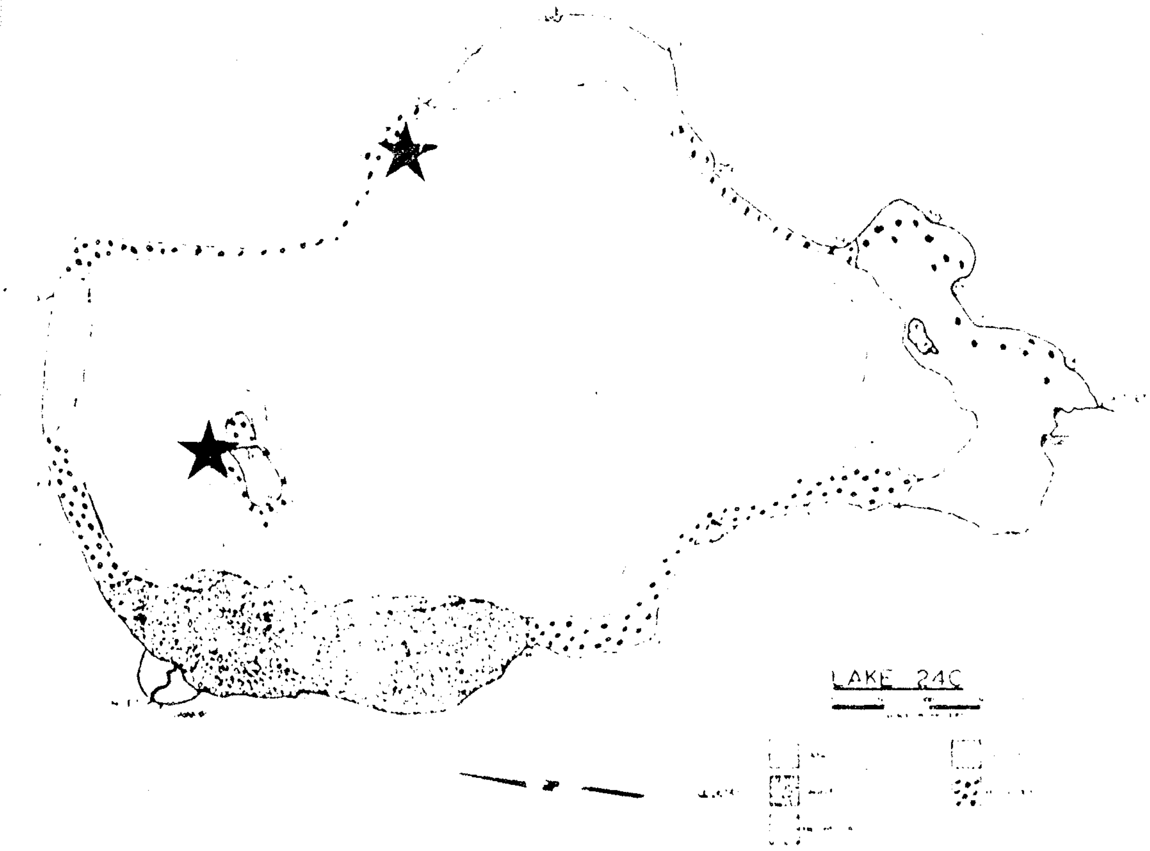
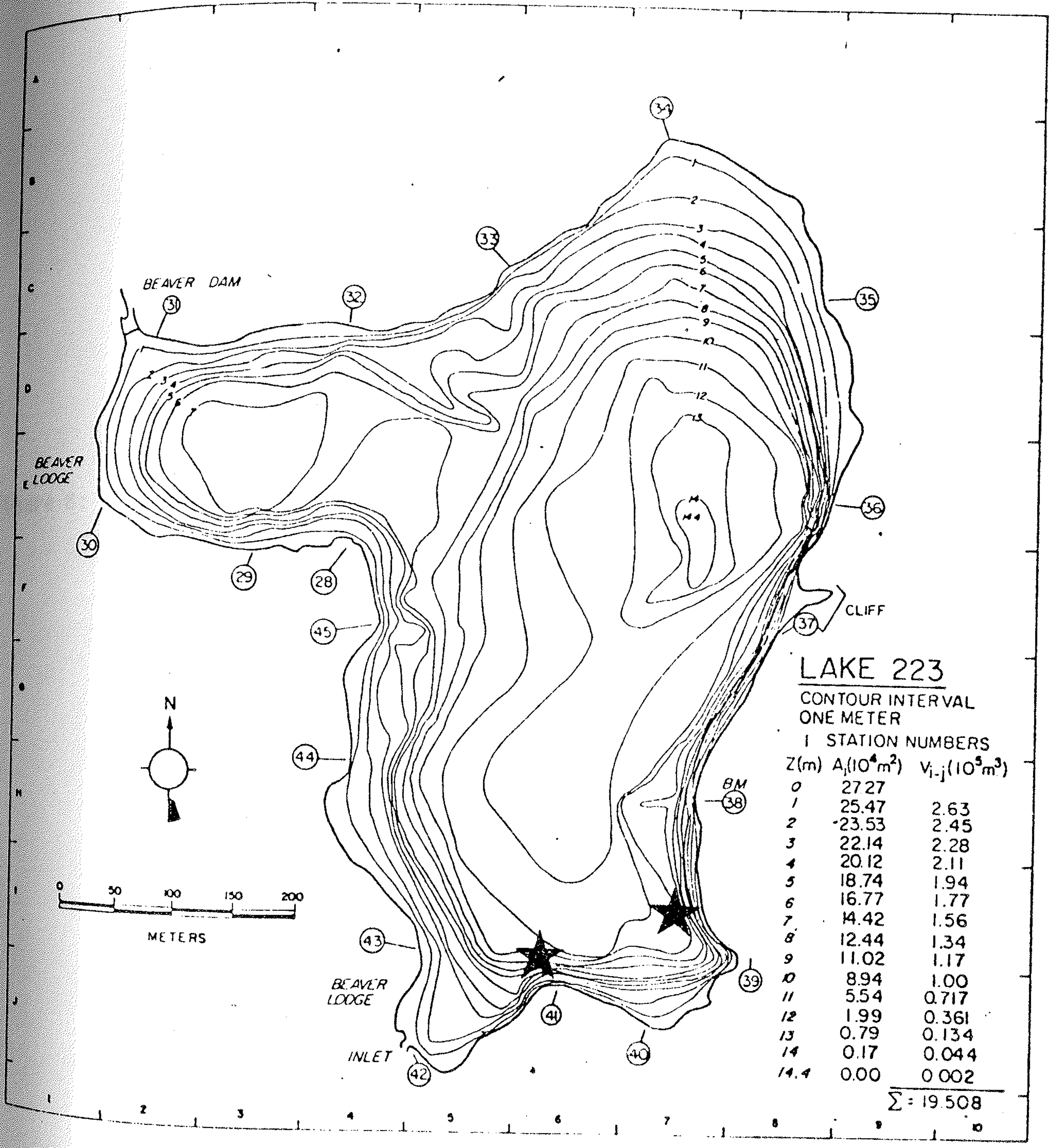

LAKE 24C

Figure 7: Bathymetric map of L223 with *O. virilis* collection sites designated by star symbols (After *Schindler* et al. 1980a).



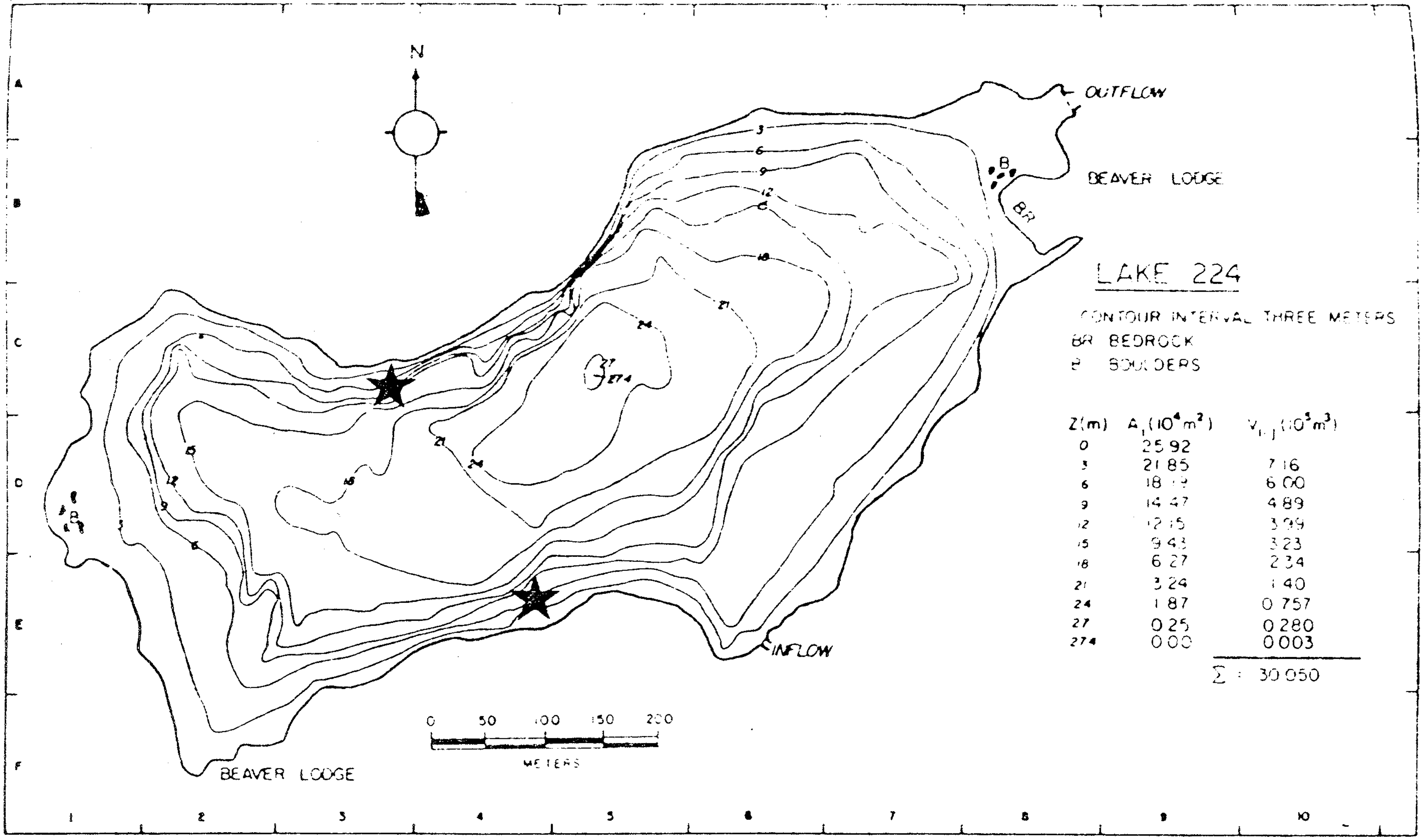
LAKE 223

CONTOUR INTERVAL
ONE METER

1 STATION NUMBERS

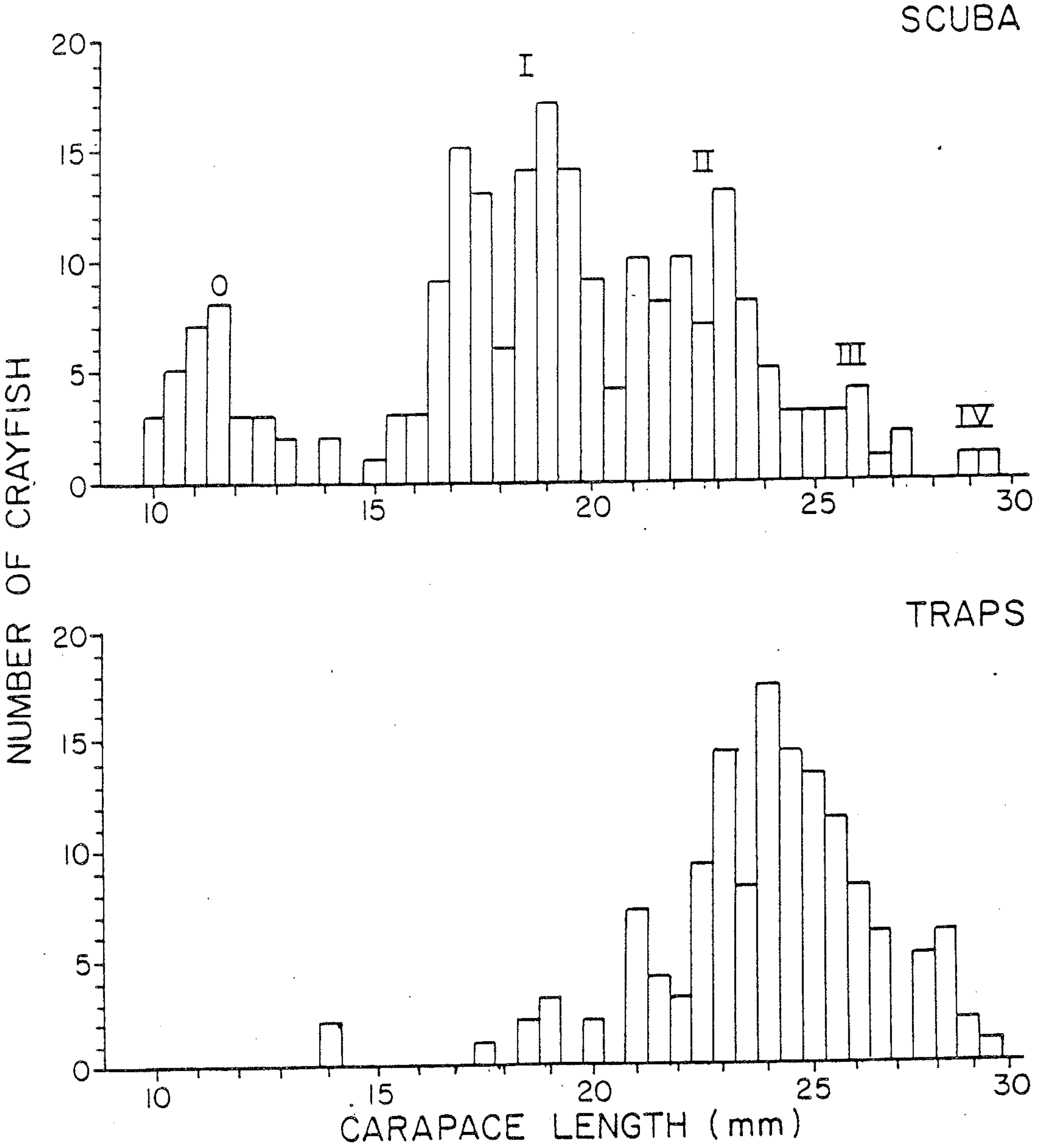
Z(m)	$A_i (10^4 m^2)$	$V_{i-j} (10^5 m^3)$
0	27.27	
1	25.47	2.63
2	23.53	2.45
3	22.14	2.28
4	20.12	2.11
5	18.74	1.94
6	16.77	1.77
7	14.42	1.56
8	12.44	1.34
9	11.02	1.17
10	8.94	1.00
11	5.54	0.717
12	1.99	0.361
13	0.79	0.134
14	0.17	0.044
14.4	0.00	0.002
		$\Sigma = 19.508$

Figure 8: Bathymetric map of L224 with O. virilis collection sites designated by star symbols (After Hesslein et al. 1980).



Z(m)	$A_i (10^4 m^2)$	$V_{i-j} (10^5 m^3)$
0	25.92	
3	21.85	7.16
6	18.19	6.00
9	14.47	4.89
12	12.15	3.99
15	9.43	3.23
18	6.27	2.34
21	3.24	1.40
24	1.87	0.757
27	0.25	0.280
27.4	0.00	0.003
		Σ = 30.050

Figure 9: Comparison of length-frequency distributions of crayfish sampled with SCUBA diving and trapping techniques. Collections were during August 1980 from L224. Roman numerals represent age-groups (c.f. Appendix A).


SCUBA
TRAPS
NUMBER OF CRAYFISH
CARAPACE LENGTH (mm)
0
5
10
15
20
25
30
O
I
II
III
IV

Crayfish were transported back to the ELA field station in plastic pails and stored overnight in aerated 5 L containers. Data recorded included (1) carapace length (C.L.) measured with vernier calipers to the nearest 0.1 mm from tip of the rostrum to posterior edge of the carapace; (2) occasionally total length measured to the nearest 0.1 mm, and weight in grams, of animals retaining all appendages; (3) sex characteristics, pleopod form I or II in males, presence of eggs or cement gland development in females; and (4) subjective measurement of exoskeleton carapace rigidity and/or molting stage assessment. Handling mortality did not occur and crayfish returned to the lake were in good condition.

2.2.2 Growth

Materials and methodology are presented in Section A.3 in Appendix A: Growth of the crayfish *Orconectes virilis* in small oligotrophic Canadian Shield lakes in the Experimental Lakes Area, northwestern Ontario.

2.2.3 Carapace Rigidity and Calcium Content

Due to the difficulty in measuring the rigidity of crayfish exoskeletons, use has been made of several subjective indices (e.g. Stevenson 1976). The carapace condition of postmolt and intermolt crayfish were determined by subjectively comparing the rigidity of the exoskeleton with the greatest degree of hardness observed within that particular population and scaling those other individuals below this. This led to the adoption of a six-level gradation system progressing from very soft (VS), soft (S), intermediate soft-hard (S/H) and hard-soft (H/S), hard (H), and very hard (VH) (cf. Davies, in prep.). Collections of crayfish and estimates of carapace rigidity were made from the four study lakes during autumn 1979 and 1980 following the last period of molting for the adult populations.

During early July 1980 late postmolt and intermolt crayfish were collected for comparative analysis of exoskeleton Ca^{++} content. Because the exoskeleton Ca^{++} concentration increases with crayfish size (McWhinnie et al. 1969; Greenway 1974; Mills et al. 1976) care was taken to include only those adults within a similar size range. For each lake attempts were made to incorporate individuals of all levels of carapace rigidity.

Exoskeleton Ca^{++} content was measured with procedures similar to those described by Malley (1980) and Adegboye (1977). Portions of the carapace branchiostegite were removed with a hole punch and both carapace and disc dried for 24 hr at 100 ± 5°C to obtain dry weights. Discs were then ashed at 500 ± 30°C for 24 hr, dissolved in 0.25 mL of 12N HCl and brought to 25 mL with deionized water. Calcium concentrations of the solutions were determined by atomic absorption spectrophotometry (Stainton et al. 1977).

2.2.4 Trace Metal Bioaccumulation

Crayfish were collected from the study lakes one week prior to ice formation in autumn 1980. Adult (age II-III; 20-30 mm C.L.) crayfish of both sexes were frozen within 1 hr following transport back to the ELA field station. The tail-sections of crayfish, including the abdominal muscle and exoskeleton covering, were dissected from the remaining body with stainless steel utensils. For each lake, a homogeneous sample was prepared by pooling together the abdomens of 20 to 30 individuals and, following drying at 100°C for 24 hrs, grinding them into a fine powder in a sample blender. Determination of tissue concentrations of Mn, Al, Zn, Cu, Cd, Pb, Hg and Se via flameless atomic absorption spectrometry were carried out by the Analytical Chemistry Unit at the Freshwater Institute, Winnipeg.

2.2.5 Reproduction

During the spring and early summer period (May 15-June 19) from 1979-81, collections were made of ovigerous females from the four study lakes. The distribution of eggs among the pleopods and the overall general condition of the female and attached eggs were closely examined under a dissecting microscope. Counts of eggs were made after gently scraping and removing the eggs from the pleopods with a blunt probe. Cursory examination of egg development stage was recorded and eggs were preserved in Davidson's fixative. Particular attention was directed to the occurrence and enumeration of abnormal conditions such as inviable eggs and/or empty egg capsules and stalks. After the removal of attached eggs, females were weighed, and estimates of mean relative fecundity (number of eggs per gram wt of female) were calculated. During 1979, a comparison was made of egg quality (weight and diameter) between the lakes. Occasionally females were sacrificed and ovaries removed and examined to investigate the occurrence and extent of egg reabsorption. In 1980 a simple experiment was conducted to ascertain the ease in which eggs could be removed from the pleopods, thereby providing information on the relative strength of egg attachment between crayfish from the lakes. Ovigerous crayfish from each lake were marked on the dorsal side of their carapaces in such a way that when performing the experiment the lake of origin could not be noted. The percentage of the total brood that could be easily removed with one uniform pass of the probe over the abdomen was determined. In autumn of 1979 and 1980 (September 25-October 28) adult females were caught, ovaries removed, and counts made of all visibly developing eggs. The general condition of the female at this time was also noted, i.e. occurrence of sperm plugs, cement gland development, and carapace rigidity.

2.2.6 Mortality Rates and Population Structure

Age composition was obtained from size-frequency graphs using Cassie's method of probit discrimination (cf. Appendix A). Annual survival (S) was estimated by combined catch curve analysis (Robson and Chapman 1961) based on the relative drop in frequency from one agegroup to the next, reflecting the combined effect of natural mortality and year-class strength. Estimates were obtained from autumn 1979 collections and refer to truncated distributions from age I-IV. To test assumptions of constant year-class strength and absence of sampling bias, Heincke's estimate was calculated and compared using the χ^2 test described in Robson and Chapman (1961). Instantaneous rates of mortality (Z) were computed from the data (Ricker 1975). Annual survival and mortality rates for individual ageclasses were analyzed by the logarithm catch curve procedure (Ricker 1975). By following the numerical progression of a series of stratified curves of catch per unit effort throughout the year for the L223 population (cf. Appendix A) the seasonal trends in survivorship for each agegroup were found. Sex ratio patterns were determined for the populations using procedures of Wenner and Frisaro (1979).

2.3 RESULTS

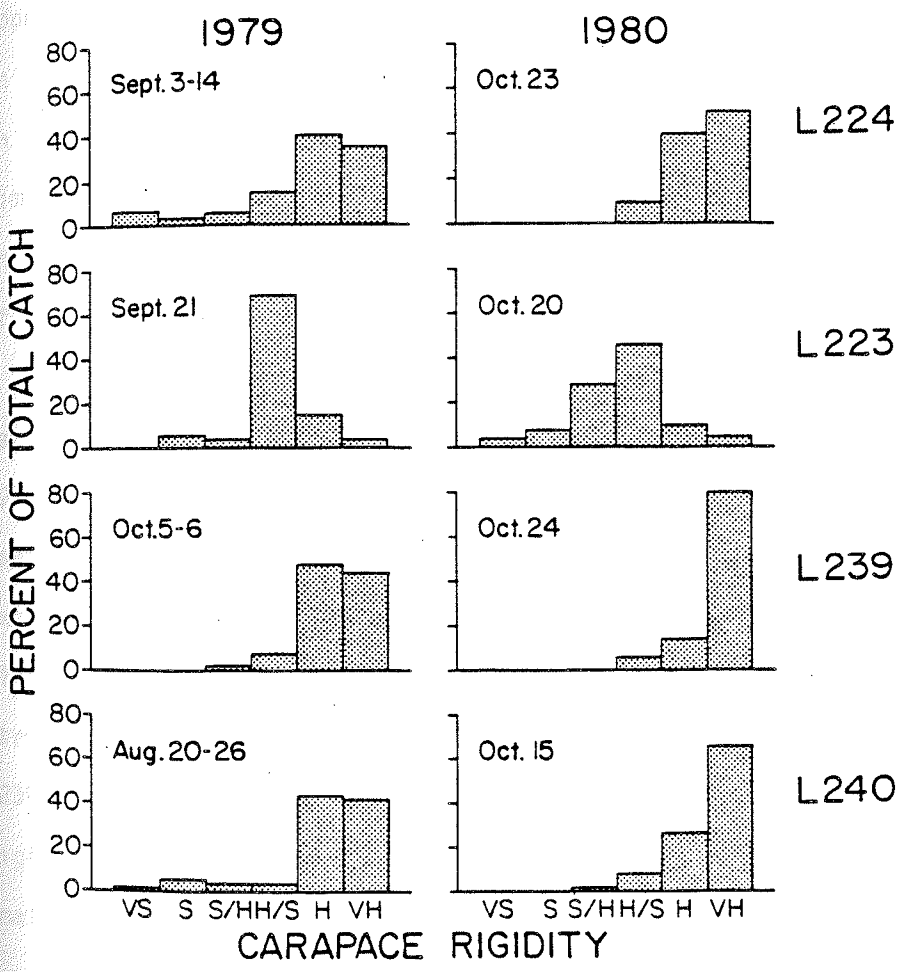
2.3.1 Growth

Size data and growth rates for the study lakes are presented in Appendix A (A.4).

2.3.2 Carapace Rigidity and Calcium Content

The medium carapace rigidity in L223 crayfish was lower compared to those from the three control lakes (Fig. 10). During autumn 1979 the majority of crayfish in the control populations were in the VH or H category in contrast to the modal H/S grouping prevalent in L223. The

Figure 10: Frequency distribution of subjective carapace rigidity for *O. virilus* from ELA lakes. Scale ranges from very soft (vs), soft (s), intermediate soft-hard (s/h) and hard-soft (h/s), hard (h), and very hard (vh).


1979
1980
PERCENT OF TOTAL CATCH
80
60
40
20
0
Sept. 3-14
Oct. 23
L224
Sept. 21
Oct. 20
L223
Oct.5-6
Oct. 24
L239
Aug. 20-26
Oct. 15
L240
VS
S
S/H
H/S
H
VH
CARAPACE RIGIDITY

occurrence of small numbers of softer individuals, even in the control lakes, suggests that, at the time of these collections, the crayfish had not reached their final overwintering state of exoskeleton hardness. To avoid this problem, collections in 1980 were delayed until a week prior to freezing and taken over a shorter time span to more accurately facilitate inter-lake comparisons. The bulk of crayfish in the control populations had attained the VH level at this time whereas individuals in L223 had formed a mode about the S/H-H/S divisions. The divergence in carapace rigidity between the L223 and control populations was not simply due to slower exoskeletal hardening as carapaces were still soft in the next year's spring collections, indicating an absolute limitation of the hardening process.

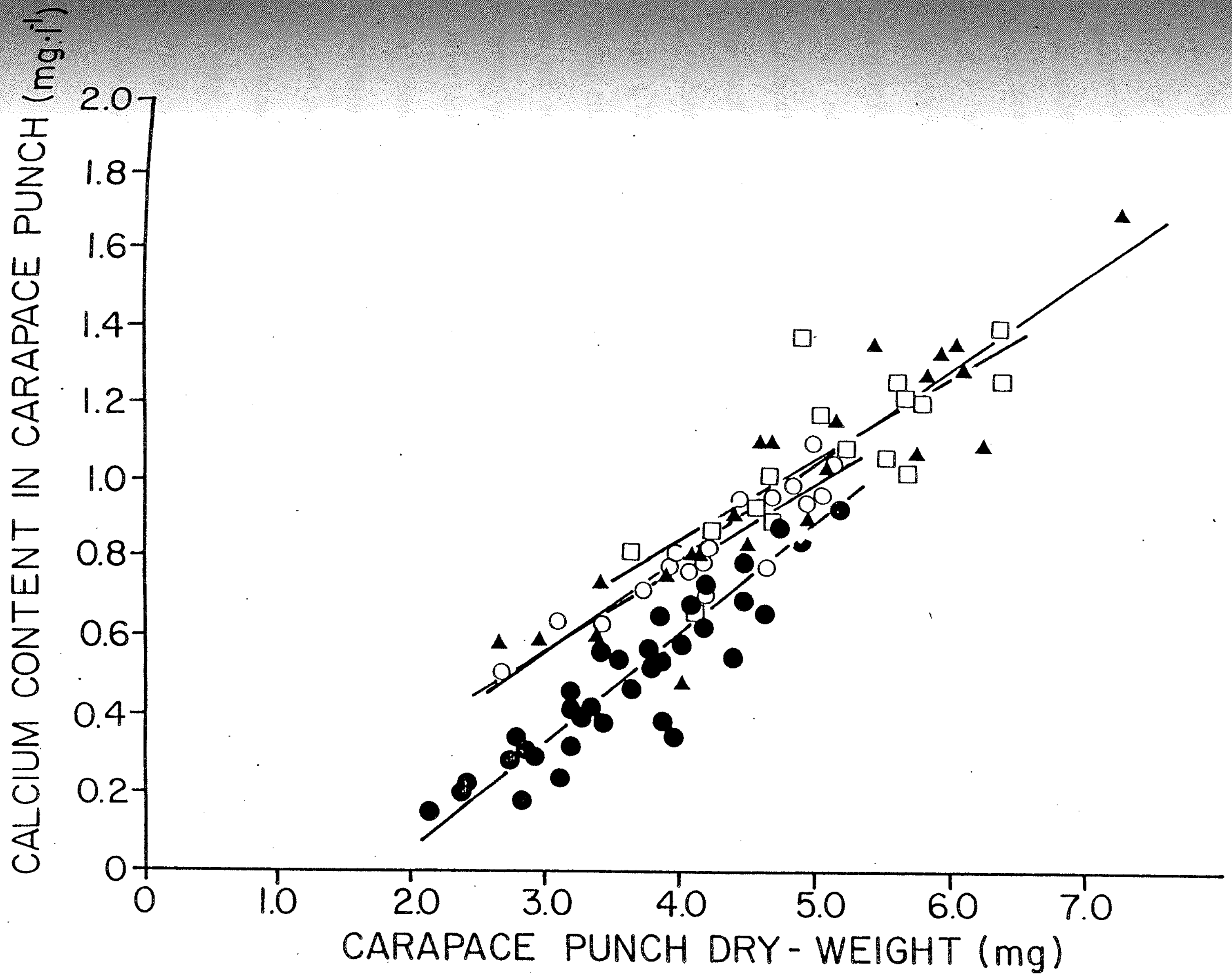
The ratio of carapace dry wt per unit length was calculated as a partial measure of organic material in the carapaces (Huner et al. 1978). For each lake, there was a distinct relationship between the mean carapace ratio or the dry wt of carapace disc to the index of exoskeleton rigidity (Table 3). This indicates that the subjective division of the population into the hardness classes is justified on a structural basis. A between-lake comparison shows that the dry wts of L239 and L240 carapaces were similar whereas L224 carapaces in the upper rigidity levels tend to weigh less, and those from L223 were the smallest in mass.

There was a strong relationship between the carapace disc dry wt and Ca^{++} content (Fig. 11). From these data the Ca^{++} content (x ± 95%

Table 3. Relationship of carapace rigidity index to carapace ratio (carapace dry wt./unit length) and carapace disc (area 33.2 mm^2) dry wt. in ELA <u>O. virilis</u>

	L239		L240		L224		L223	
	n = 23		n = 17		n = 18		n = 35	
Index	Carapace ratio	Disc dry wt.	Carapace ratio	Disc dry wt.	Carapace ratio	Disc dry wt.	Carapace ratio	Disc dry wt.
VH	4.04	5.71	4.89	5.84	3.35	4.79	3.32	4.44
H	3.20	5.01	3.44	5.70	3.00	4.32	3.15	3.58
H/S	2.50	4.34	2.15	4.74	2.89	4.29	2.69	3.72
S/H	1.72	4.14	1.65	4.19	2.65	3.99	2.12	3.22
S	1.24	2.67	1.32	3.14	1.17	3.02	1.08	2.97

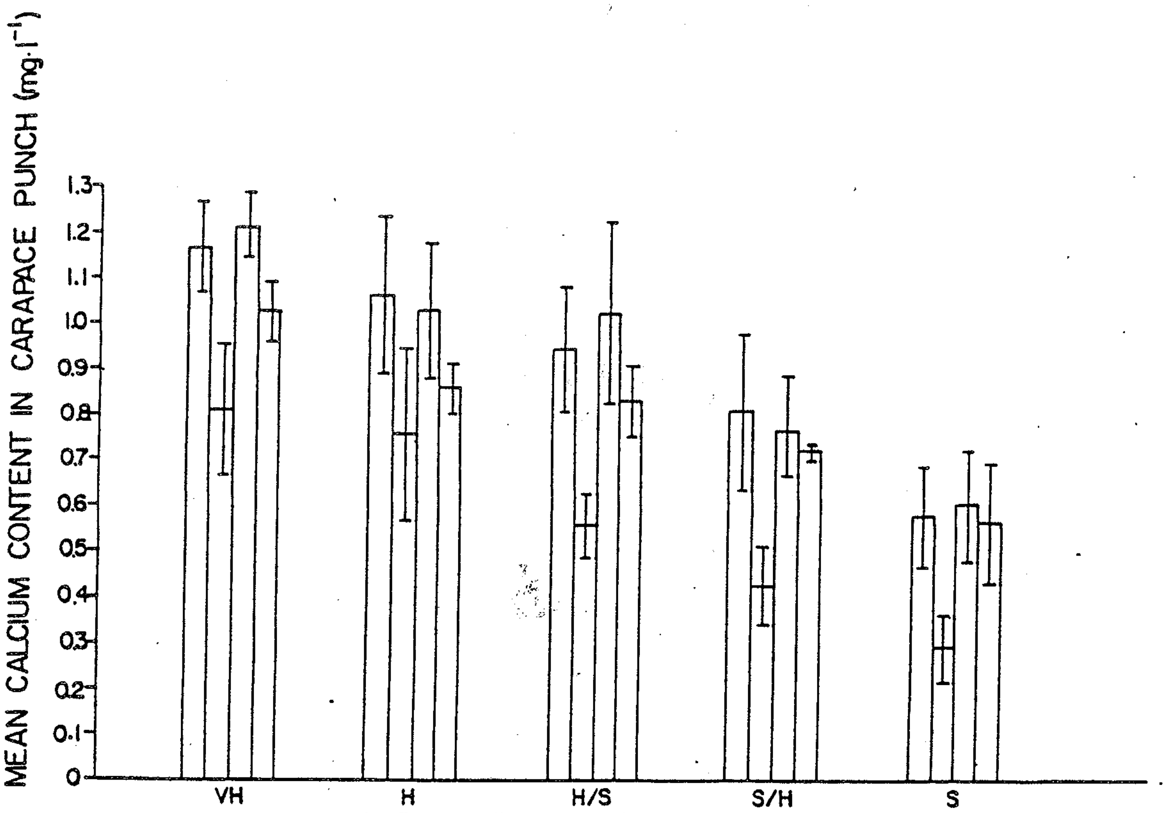
Figure 11: Linear relationships between carapace disc dry wt. and calcium content. L223 (closed circles): Y = -0.47 + 0.28X, r = 0.911; L224 (open circles): Y = -0.68 + 0.21X, r = 0.931 L240 (open squares): Y = -0.32 + 0.20X, r = 0.733; L239 (triangles): Y = -0.15 + 0.24X, r = 0.892.


CALCIUM CONTENT IN CARAPACE PUNCH (mg·l⁻¹)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
CARAPACE PUNCH DRY-WEIGHT (mg)

C.I.) for each index level of carapace rigidity was calculated (Fig. 12). In each lake there was a decrease in the Ca^{++} content with progression from VH to S hardness levels, again supporting the use of the subjective rigidity index as a predictive tool. Calcium content was significantly lower ($p < .05$) in VH L224 individuals than in VH L239 and L240 animals. Values of Ca^{++} content were also significantly lower ($p < .001$) in L223 crayfish than control organisms at every level of carapace rigidity.

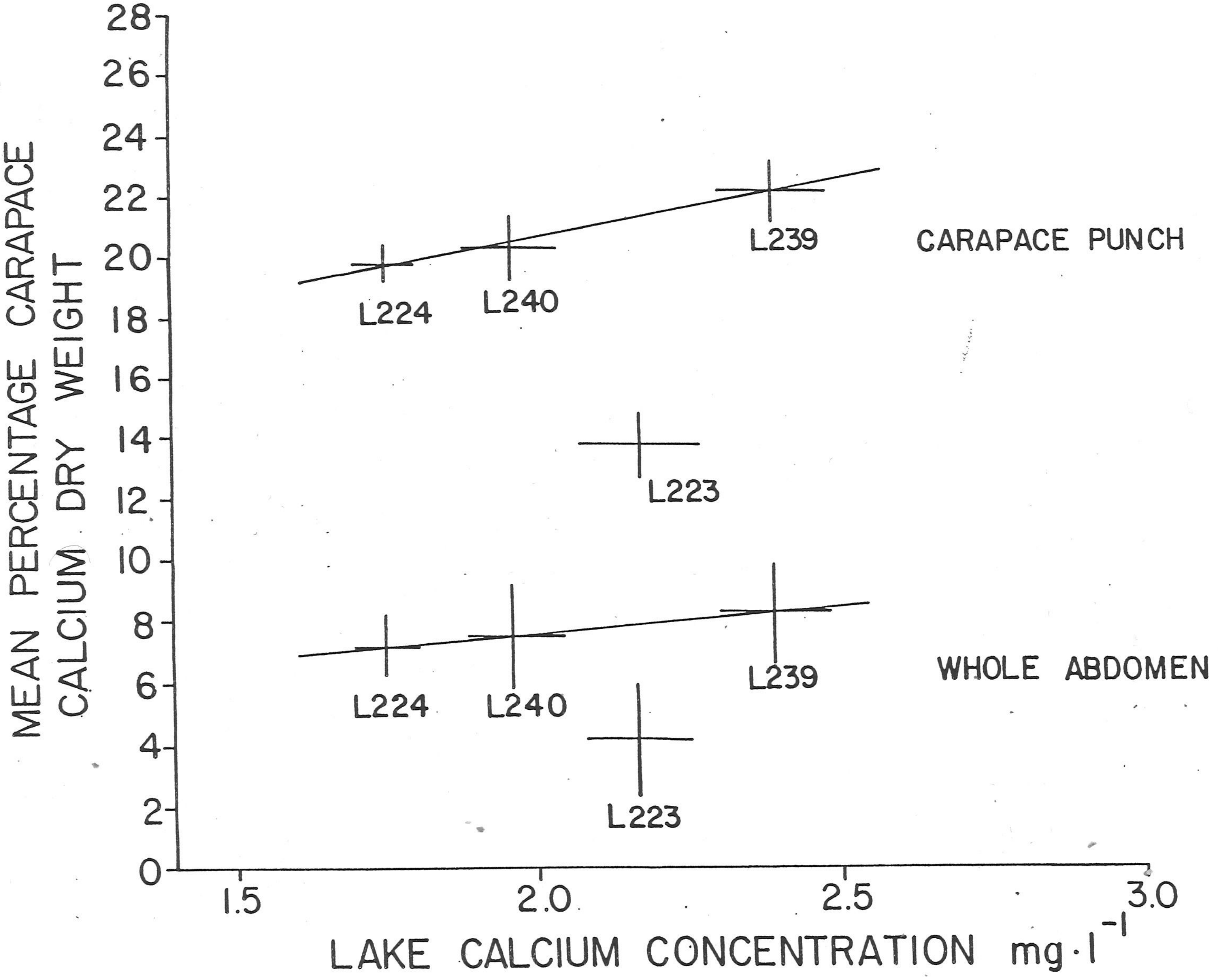
Individual measurements of carapace disc Ca^{++} content were standardized by removing inherent differences in dry wt and expressed for each lake on a mean percentage dry wt basis (Fig. 13). Carapace Ca^{++} content was significantly lower in L223 crayfish (x % dry wt ± 95% C.I. = 13.90 ± 0.89) than in animals from the control lakes (19.82 ± 0.55; 20.34 ± 1.04; 22.18 ± 0.84). It is known that O. virilis from ELA do not achieve the same degree of hardness the same as species from harder waters (Malley 1980). If the assumption is made that there is a relationship between the Ca^{++} content of crayfish exoskeletons and the Ca^{++} concentration of the lake water they inhabit, then an approximate estimate can be obtained of the percentage Ca^{++} reduction of L223 crayfish. From this it was calculated that L223 crayfish have suffered a 35% decrease in carapace Ca^{++}. The sample sizes used though for the present analysis were too small to reflect the natural distribution of carapace rigidities observed in the field and therefore might not accurately describe the mean population exoskeleton Ca^{++} content. Data

Figure 12: Comparison of mean calcium content (±95% C.I.) as related to carapace rigidity index between ELA study lakes. Groupings for each set arranged in order of decreasing lake calcium concentration (c.f. Fig. 15).



(Lakes for each set arranged in order of decreasing calcium content : L239-L223-L240-L224)

Figure 13: Plot of mean percentage calcium dry wt. of carapace disc and whole abdomen preparations against lake mean calcium concentration. Vertical and horizontal lines denote 95% confidence limits. Lake chemistry data from Prokopowich (1979).


MEAN PERCENTAGE CARAPACE CALCIUM DRY WEIGHT
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
1.5
2.0
2.5
3.0
LAKE CALCIUM CONCENTRATION mg·l⁻¹
L224
L240
L239
CARAPACE PUNCH
L223
L224
L240
L239
WHOLE ABDOMEN
L223

on the mean disc dry wt and Ca^{++} content were combined with the actual rigidity frequency distribution observed in late autumn 1980 to obtain a population weighted mean % Ca^{++} content: L224 - 20.57%; L239 - 20.59%; L240 - 20.07%; and with the L223 value - 14.77% about 25% lower than the control lake populations. Independent measurements of Ca^{++} concentration performed on whole abdomens used for heavy metal analysis provide further evidence that L223 crayfish have significantly less body Ca^{++} than animals from control populations.

2.3.3 Trace Metal Bioaccumulation

Metal concentrations in ELA waters are low, characteristic of an unpolluted region (Table 4; Beamish et al. 1976). As a result of the acidification of L223, several increases have been observed in the volume-weighted average concentrations of metal species (Schindler and Turner 1982). The most dramatic effect has been a steady increase in the Mn concentration from 12.5 μg/L in 1976 to 127.0 μg/L in 1980 (Table 4). Increases in Al and Zn from 15.4 to 27.0 μg/L and from 1.9 to 5.6 μg/L respectively, are also attributed to lake acidification, whereas levels of Cu, Cd, Cr and Pb have remained unaltered.

Cursory examination of the data shows that 5 of the 7 metals (excluding Se) have the highest concentration in L223 crayfish whereas 4 of the 7 are lowest in the L224 sample (Table 5). A Friedman test (Hollander and Wolfe 1973) was performed on ranked data and indicated that there was no overall significant difference ($p > .05$) in the

Table 4. Mean ice-free epilimnetic trace metal concentrations (ug L^{-1}) for ELA lakes (values for L239 are for lake outflow). All data from Schindler (unpublished) except for L240 from Beamish et al. (1976).

Lake	Year	Mn	Al	Zn	Cu	Cd	Pb
L224	1976-77	3.8	11.0	2.4	1.2	<1	<1
L239	1976-77	5.4	-	3.9	1.1	<1	<1
L240	1975	-	-	<2	<2	<1	<1
L223	1976	12.5	15.4	1.9	1.3	<1	<1
	1977	33.0	26.8	3.5	2.9	<1	<1
	1978	74.3	29.7	3.3	1.9	<1	<1
	1979	84.6	34.5	3.0	2.8	<1	<1
	1980	127.0	27.0	5.6	1.8	<1	<1

Table 5. Mean abdominal muscle trace metal concentrations in ug g^{-1} dry wt. for O. virilis from ELA study lakes.

Lake	Mn	Al	Zn	Cu	Hg	Cd	Pb	Se
L223	240	46	74	79	0.52	0.10	3.8	1.6
L224	33	37	66	77	0.20	0.22	1.1	2.1
L240	60	58	69	66	0.36	0.12	1.7	1.3
L239	41	52	48	64	0.24	0.15	3.1	0.86

ordering of tissue metal concentrations between lakes. Individually, however, L223 crayfish have responded with an increased bioaccumulation of Mn (L223 value of 240 $\mu g \cdot g^{-1}$ dry wt compared to a mean for the control lakes of 61 $\mu g \cdot g^{-1}$ dry wt) and Hg (L223 value of 0.52 $\mu g \cdot g^{-1}$ dry wt compared to control mean of 0.27 $\mu g \cdot g^{-1}$ dry wt).

2.3.4 Prevalence of Parasitism

Occasionally crayfish from ELA lakes were found to have a markedly white-opaque coloration of their abdominal musculature (Plate 1) and decreased defence reactions. Observation under a dissecting microscope showed the white coloration in affected animals was prominent throughout their entire body musculature. Further work by Dr. L. Graham at the University of Manitoba has shown that the whitening is a result of the presence of the microsporian parasite *Thelohania* sp..

Crayfish having *Thelohania* sp. infection were found only during the autumn collection period. In 1979 (Table 6), the prevalence of parasitism in the L223 population was 1.7% compared to an average occurrence of 0.3% for the 3 control lakes (absent in L239). In autumn 1980 the prevalence in L223 had increased to 6.5% (range 4.3-13.3% for individual collections) whereas the control lakes maintained a substantially lower level with only 0.6% of the sampled individuals being infected (absent in L240). Crayfish of age classes I, II, III and IV and of both sexes were equally susceptible to parasitism.

Plate 1: O. *virilis* infected with *Thelohania* sp. (left) and unparasitized (right) crayfish.

Table 6. Infection of *Orconectes virilis* at ELA with the microsporidian *Thelohania* sp.

Year	Lake	Date	Prevalence	Mean
1979	L223	Sept 21	3/192 = 1.5%	1.7%
		Oct 27-28	1/43 = 2.3%	
	L224	Sept 5-14	1/178 = 0.6%	0.3%
	L239	Oct 5-6	0/236 = 0.0%	
	L240	Aug 20-Sept 2	1/262 = 0.4%	
		Oct 23	0/49 = 0.0%	
1980	L223	Sept 18-25	10/196 = 5.1%	6.5%
		Oct 5	3/50 = 6.0%	
		Oct 15	3/47 = 6.4%	
		Oct 20	6/45 = 13.3%	
		Oct 26	2/47 = 4.3%	
		Oct 28	4/42 = 9.5%	
	L224	Oct 23	1/68 = 1.5%	0.6%
	L239	Oct 24	1/112 = 0.9%	
	L240	Sept 24-Oct 27	0/172 = 0.0%	

2.3.5 Reproduction

The distribution of eggs on ovigerous O. virilis (Fig. 14) is similar to that shown by other crayfish species (Mason 1978; Hopkins 1967). Most of the eggs (93.1%) were fastened to the pleopods, those remaining being attached to the medial sternal ridges between abdominal segments (Plate 2). The percentage loading of eggs among the pleopods reflects differences between the relative area of these appendages and length of their non-plumose setae.

During early spring 1979 it was noted that eggs of L223 females could be more easily removed for counting compared to animals from the three control lakes. In 1980 this was tested (Table 7) and the ANOVA/LSD analysis showed that the mean number of eggs detached with one uniform pass of a probe along the abdomen of L223 crayfish was significantly greater ($p < .025$) than that observed for either L240 or L239 crayfish.

Failure of secure attachment of pleopod eggs in L223 females has resulted in a much higher proportion of individuals with only a partial complement of eggs in that lake (Figs. 15-22). In all cases there was a significant relationship ($p < .05$) between female carapace length and number of attached eggs. Individuals with an incomplete number of eggs for a particular length are designated as open circles and not included in the regression equations. Covariant analysis showed that regressions did not differ significantly between lakes or for the same lake between different years. This is supported by calculations of relative fecundity (number of eggs per gram wt of female) which showed no

Figure 14: Percentage distribution of attached-eggs over the abdomen of ovigerous O. virilis. Sample size was 22 and vertical lines represent standard deviations about the mean.

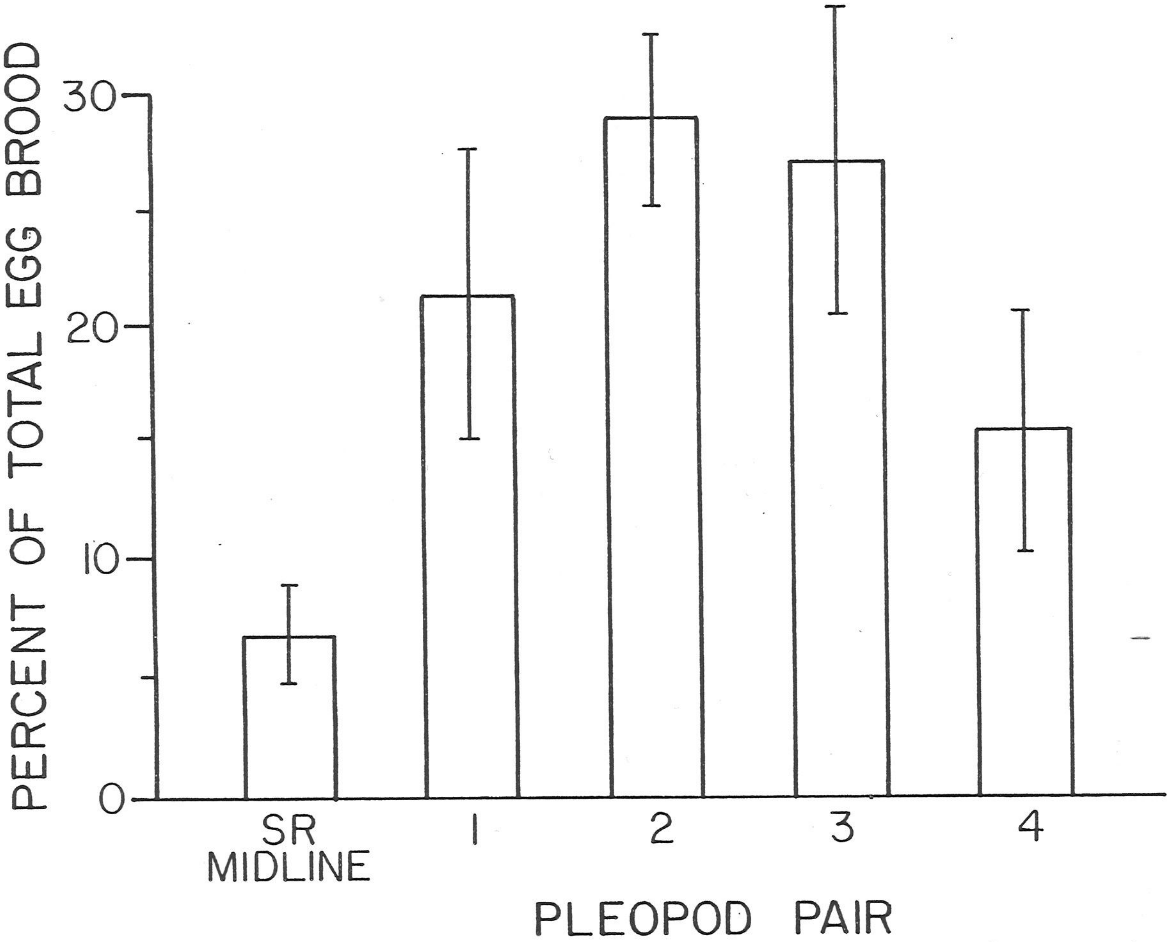

PERCENT OF TOTAL EGG BROOD
30
20
10
0
SR
MIDLINE
1
2
3
4
PLEOPOD PAIR

Plate 2: Uneffected crayfish from L223 with full compliment of eggs.

Table 7. Mean percentage of eggs detached (±S.E.) with one uniform pass of a probe over the abdomen of ovigerous crayfish.

Lake	No. of crayfish	Mean percentage of total brood removed ± S.E.	Range
L239	18	27.8 ± 6.4	20.4 - 37.2
L240	11	30.5 ± 7.0	19.1 - 53.6
L223	23	54.1 ± 4.9	27.7 - 83.3

Figure 15: Relationship of carapace length to number of pleopod eggs in L239 crayfish during 1979. Open circles represent females with an incomplete egg complement and were not included in the regression equation.

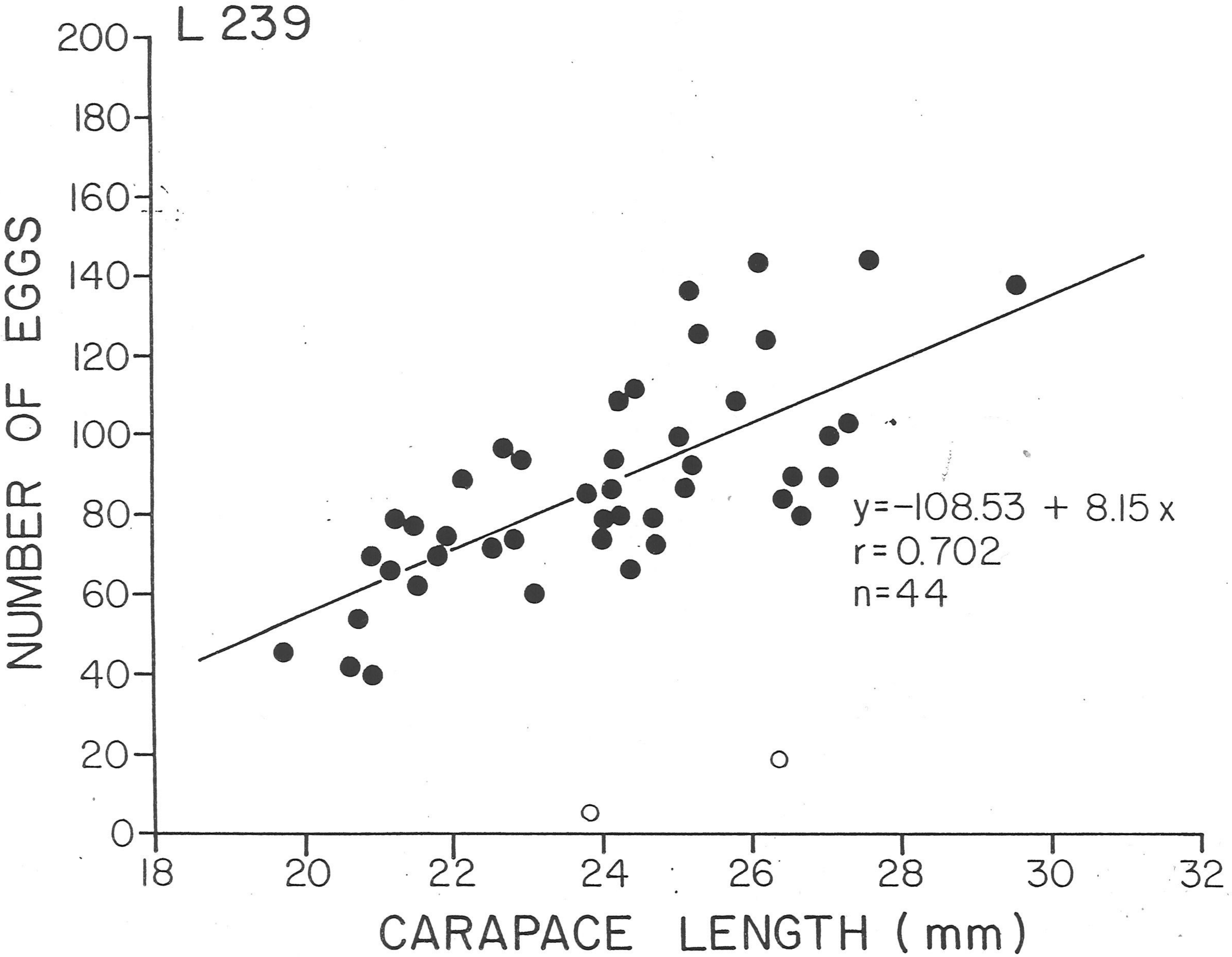

L 239
NUMBER OF EGGS
200
180
160
140
120
100
80
60
40
20
0
18
20
22
24
26
28
30
32
CARAPACE LENGTH (mm)
y=−108.53 + 8.15 x
r=0.702
n=44

Figure 16: Relationship of carapace length to number of pleopod eggs in L240 crayfish during 1979. Open circles represent females with an incomplete egg complement and were not included in the regression equation.

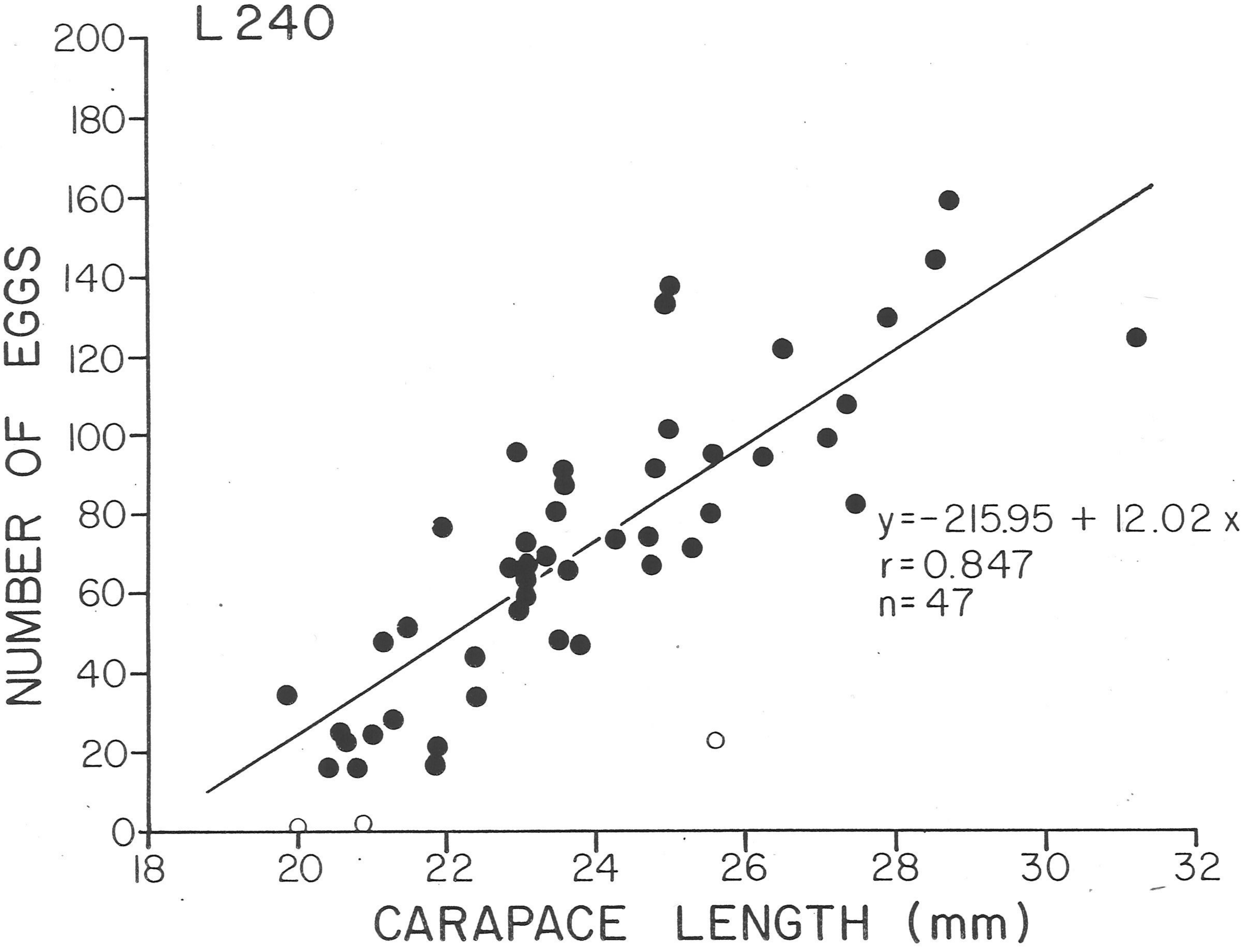

L 240
NUMBER OF EGGS
0
20
40
60
80
100
120
140
160
180
200
18
20
22
24
26
28
30
32
CARAPACE LENGTH (mm)
y=-215.95 + 12.02 x
r=0.847
n=47

Figure 17: Relationship of carapace length to number of pleopod eggs in L223 crayfish during 1979. Open circles represent females with an incomplete egg complement and were not included in the regression equation.

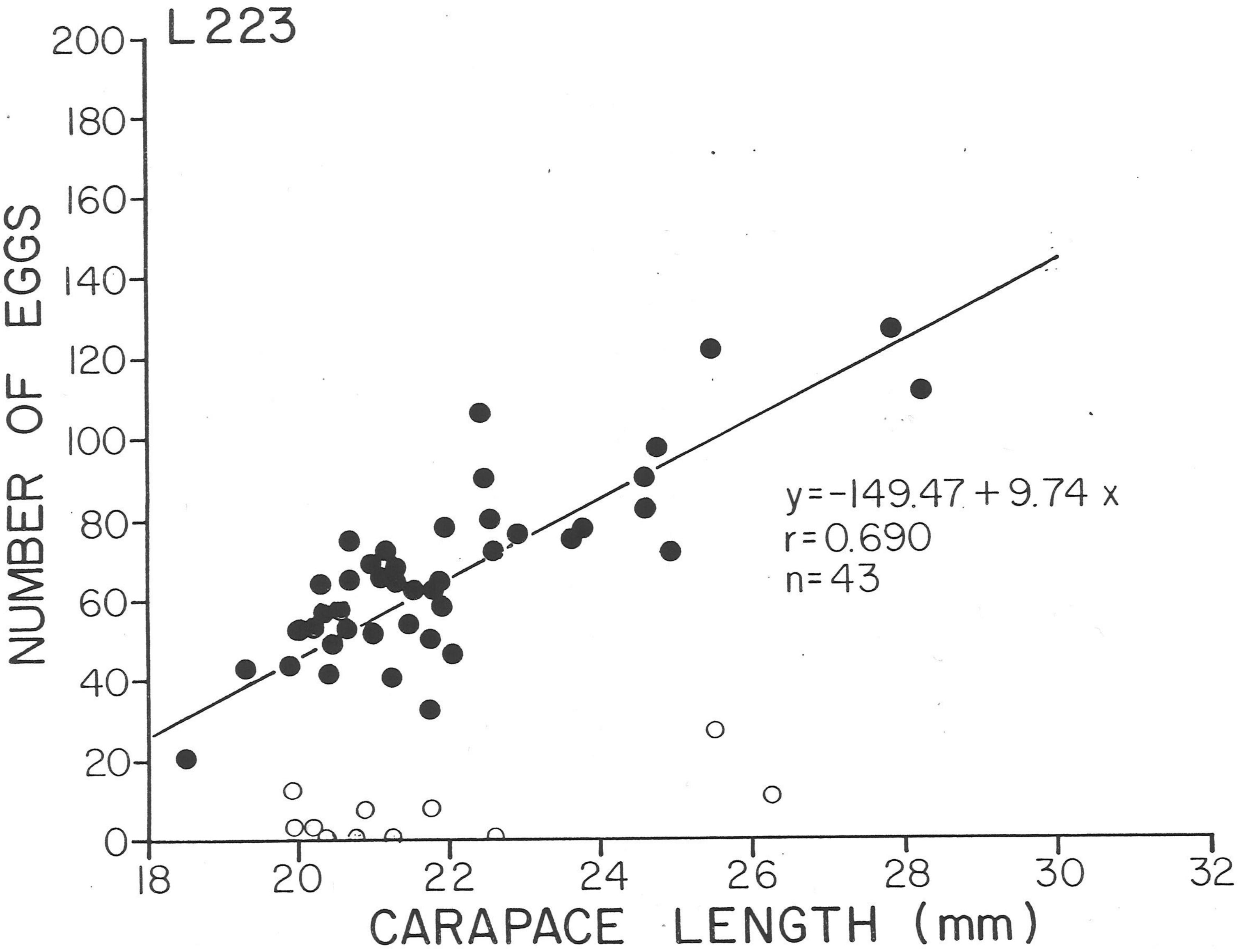

L223
NUMBER OF EGGS
0
20
40
60
80
100
120
140
160
180
200
CARAPACE LENGTH (mm)
18
20
22
24
26
28
30
32
y=-149.47+9.74 x
r=0.690
n=43

Figure 18: Relationship of carapace length to number of eggs in L239 crayfish. Open squares denote ovarian egg counts during fall 1979 and closed circles denote pleopod eggs in spring 1980. Open circles represent females with an incomplete pleopod egg complement and were not included in the regression equation.

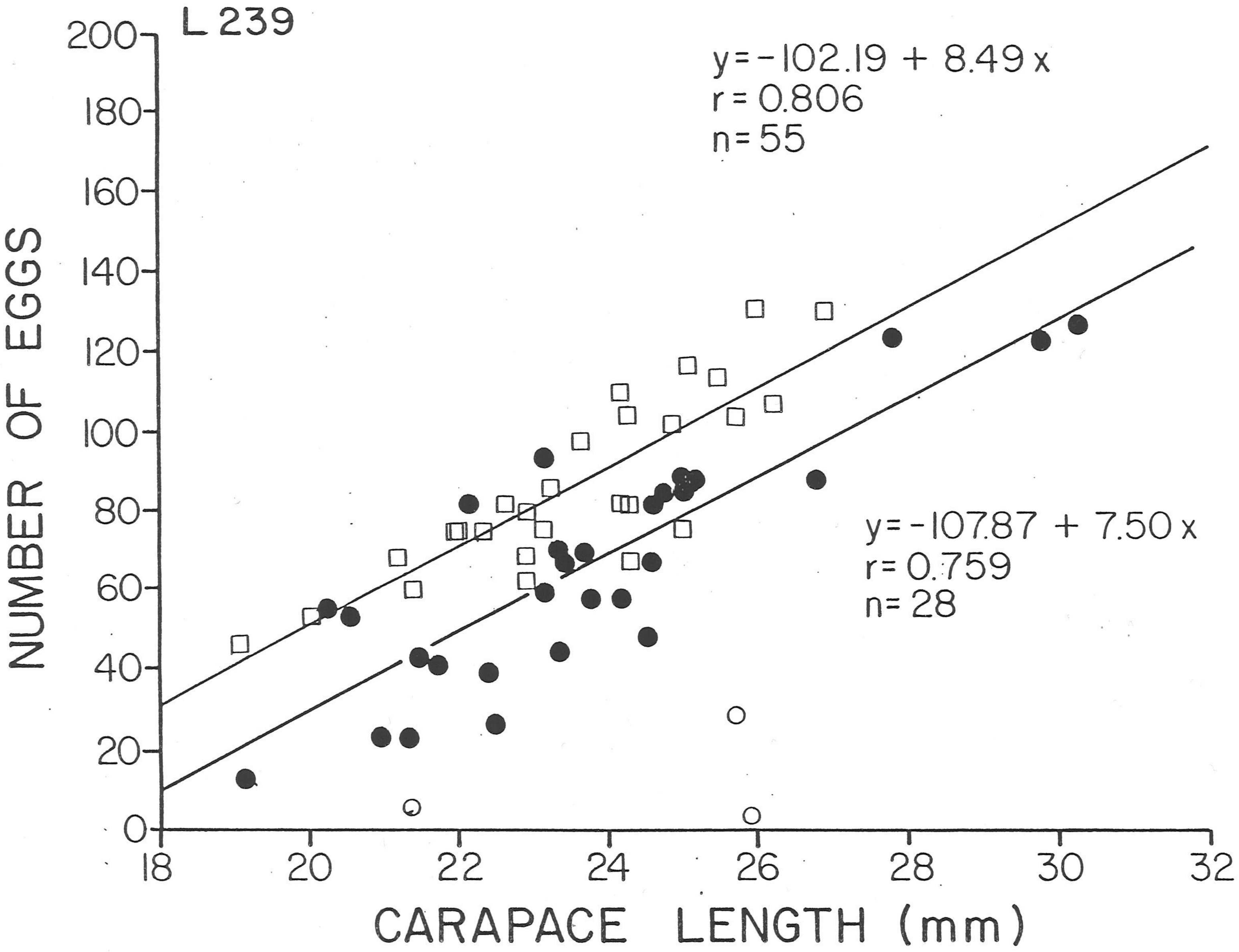

L 239
y = -102.19 + 8.49 x
r = 0.806
n = 55
y = -107.87 + 7.50 x
r = 0.759
n = 28
NUMBER OF EGGS
0
20
40
60
80
100
120
140
160
180
200
CARAPACE LENGTH (mm)
18
20
22
24
26
28
30
32

Figure 19: Relationship of carapace length to number of eggs in L240 crayfish. Open squares denote ovarian egg counts during fall 1979 and closed circles denote pleopod eggs in spring 1980. Open circle represents a female with an incomplete pleopod egg complement and was not included in the regression equation.

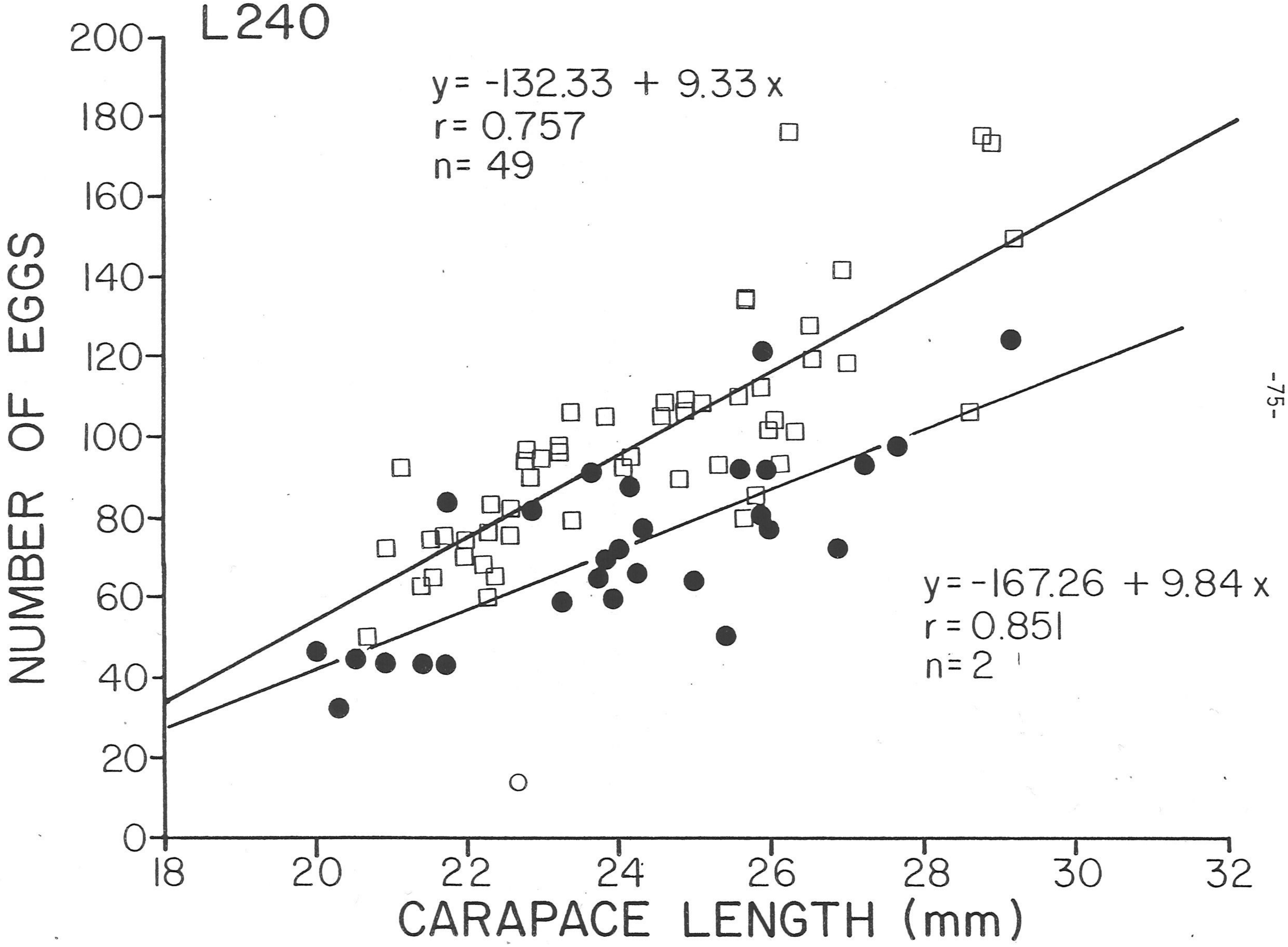

L240
y= -132.33 + 9.33 x
r= 0.757
n= 49
y= -167.26 + 9.84 x
r = 0.851
n= 21
NUMBER OF EGGS
0
20
40
60
80
100
120
140
160
180
200
CARAPACE LENGTH (mm)
18
20
22
24
26
28
30
32

Figure 20: Relationship of carapace length to number of pleopod eggs in L223 crayfish during 1980. Open circles represent females with an incomplete egg complement and were not included in the regression equation. Dashed lines and triangles represent counts of empty egg capsules. Regression of ovarian eggs to carapace length not shown: $Y = -80.53 + 7.50X$, $r = 0.710$, $n=43$.

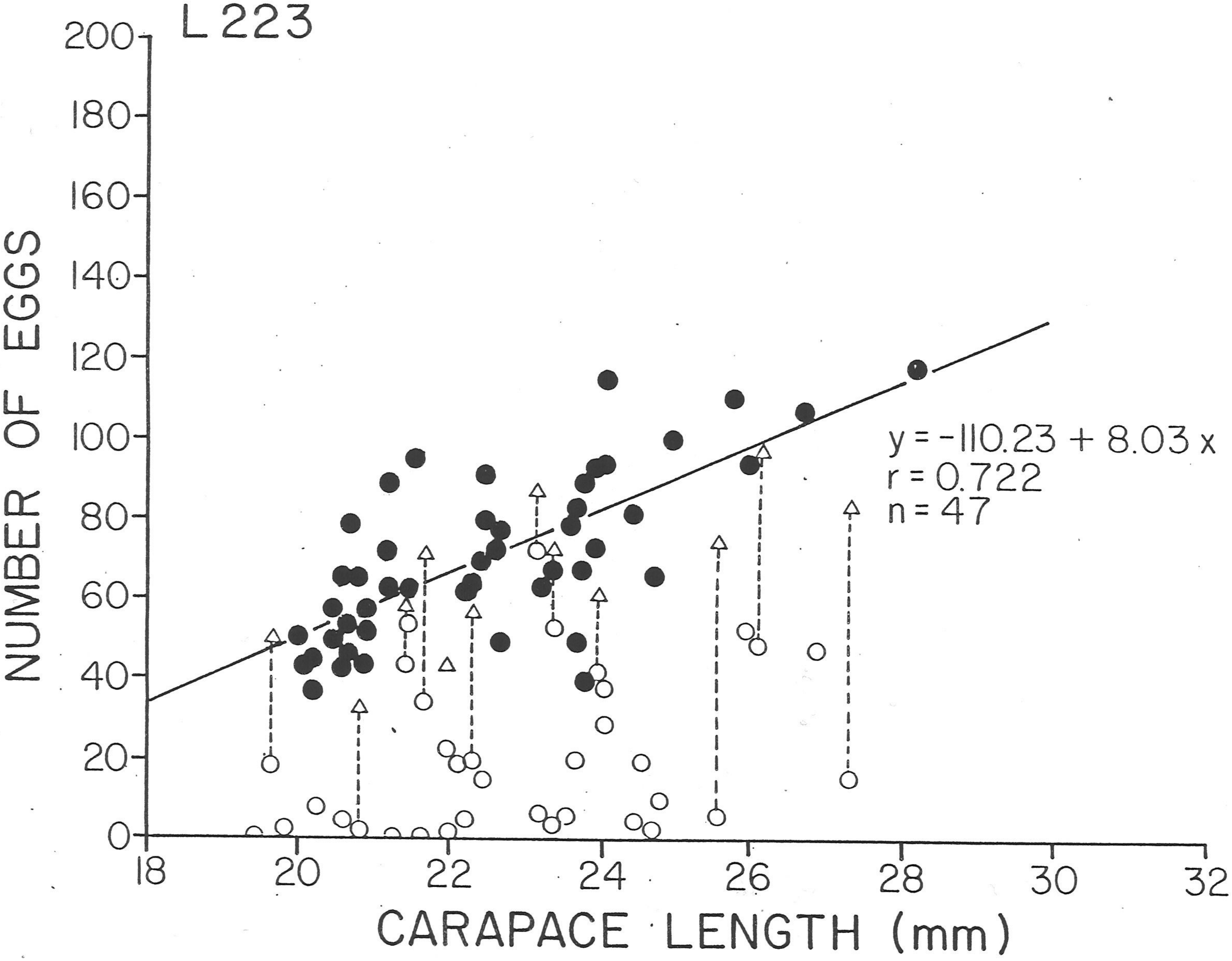

L 223
200
180
160
140
120
100
80
60
40
20
0
NUMBER OF EGGS
18
20
22
24
26
28
30
32
CARAPACE LENGTH (mm)
y = -110.23 + 8.03 x
r = 0.722
n = 47

Figure 21: Relationship of carapace length to number of eggs in L239 crayfish. Open squares denote ovarian egg counts during fall 1980 and closed circles denote pleopod eggs in spring 1981. Open circles represent a female with an incomplete pleopod egg complement and was not included in the regression equation. Dashed line and triangle represents count of empty egg capsules.

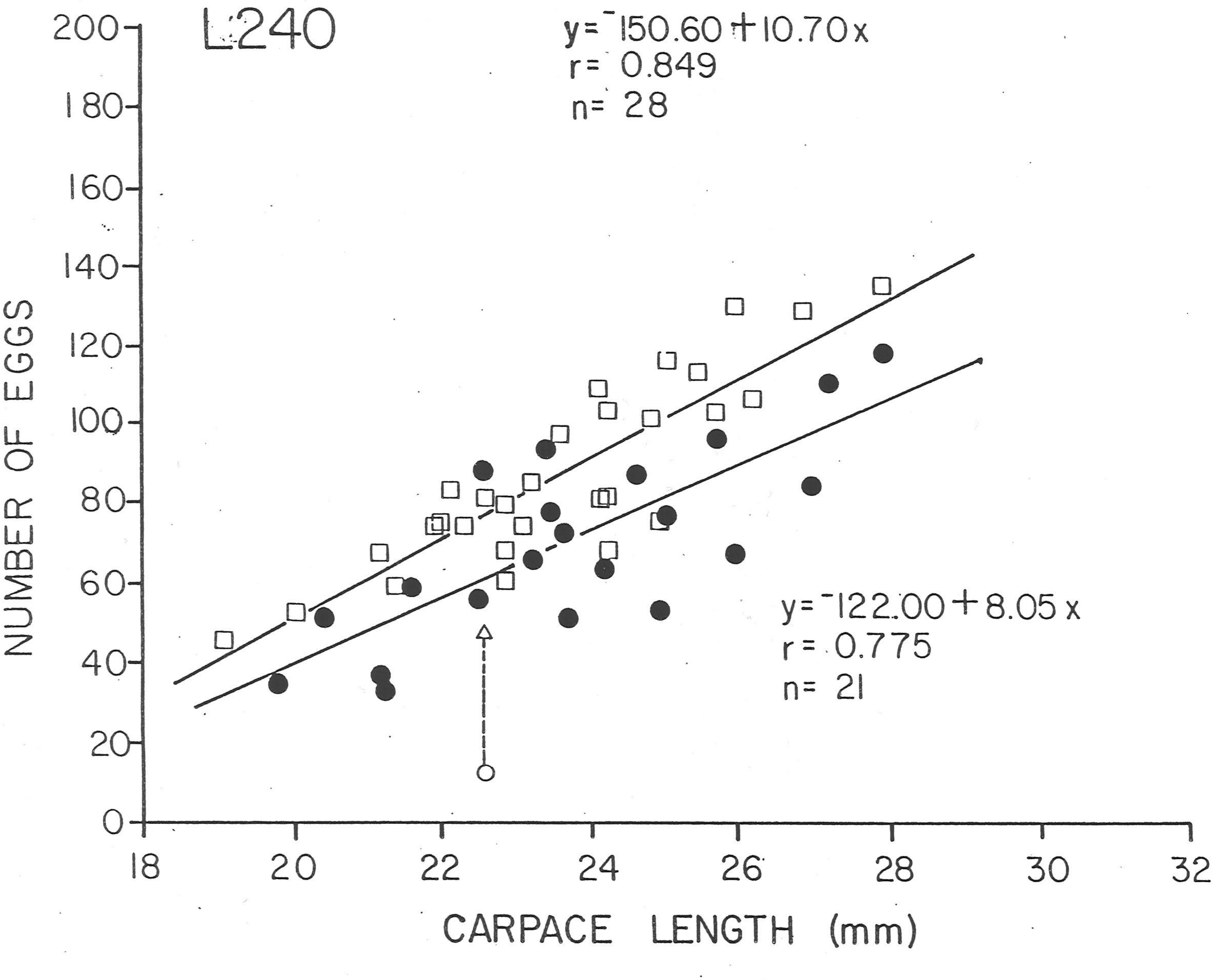
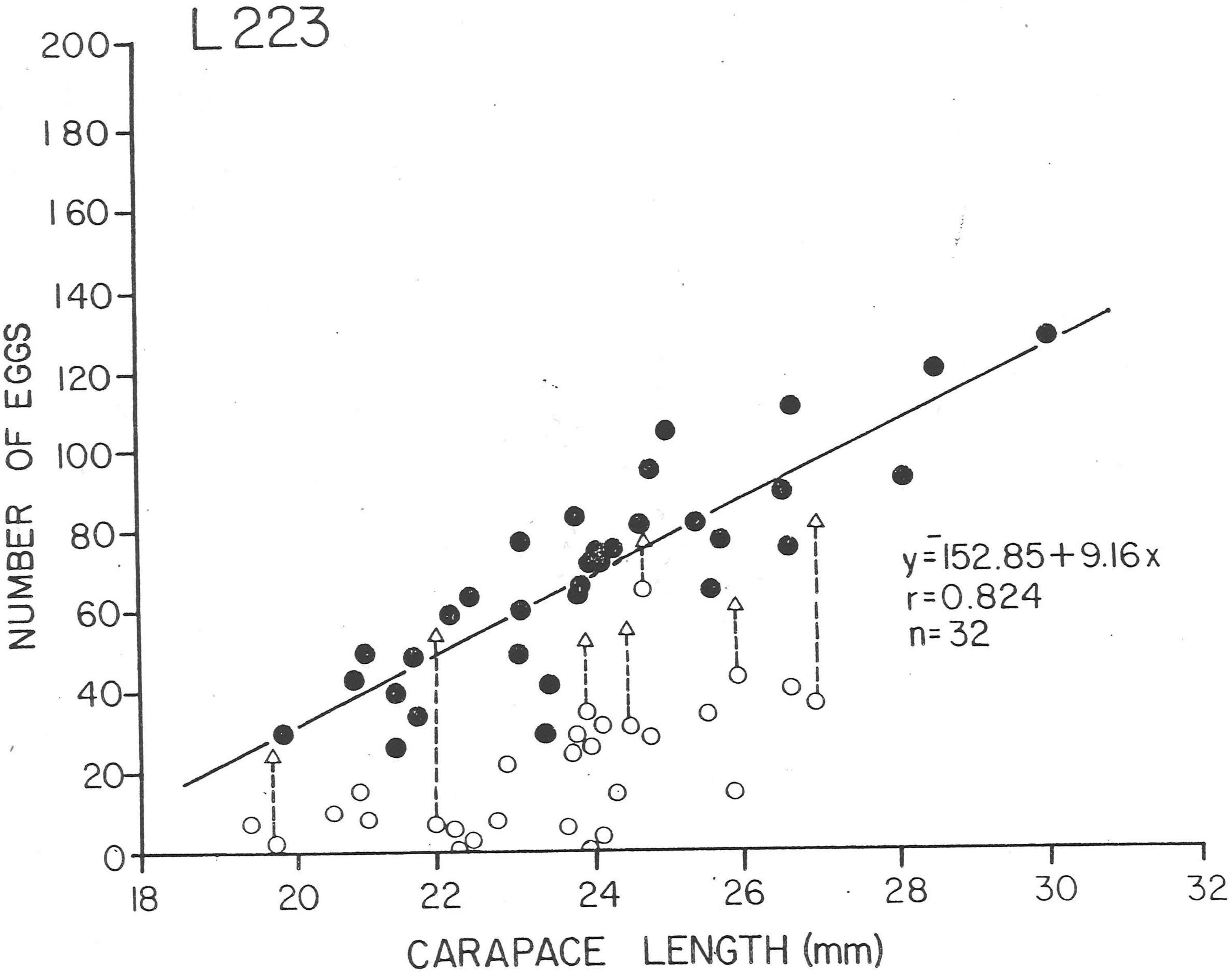

L240
y= ⁻150.60 + 10.70 x
r= 0.849
n= 28
y= ⁻122.00 + 8.05 x
r= 0.775
n= 21
NUMBER OF EGGS
0
20
40
60
80
100
120
140
160
180
200
CARPACE LENGTH (mm)
18
20
22
24
26
28
30
32

Figure 22: Relationship of carapace length to number of pleopod eggs in L223 crayfish during 1981. Open circles represent females with an incomplete egg complement and were not included in the regression equation. Dashed lines and triangles represent counts of empty egg capsules. Regression of ovarian eggs to carapace length not known: $Y = -105.13 + 8.50X$, $r = 0.849$, $n=44$.


L 223
NUMBER OF EGGS
0
20
40
60
80
100
120
140
160
180
200
CARAPACE LENGTH (mm)
18
20
22
24
26
28
30
32
y=152.85+9.16x
r=0.824
n=32

significant ($p > .05$) difference between lakes (Table 8). The inclusion of crayfish experiencing reproductive impairment indicated, not surprisingly, that the overall estimate of relative fecundity for the L223 population was significantly ($p < .05$) lower than those for the control lakes. The number of ovarian eggs produced by mature _O. virilis_ is linearly correlated with female carapace length and always greater than the number of successfully extruded and attached pleopod eggs the subsequent spring (Figs. 18, 19, 21; L223 regressions given but not graphed). The disparity or % drop in count between mean ovarian and pleopod eggs over a similar size range was over two-fold greater in L223 than in the control lakes (Table 9).

The percentage of L223 females with a partial complement of eggs was 19.0% in 1979, 42.4% in 1980, and 47.7% in 1981 in contrast to levels of approximately 5% over the three years in the control lakes (Table 10). The manner in which the eggs were scattered over the abdominal pleopods indicated that these few eggs were the remnants of a much larger brood, the majority of which had been lost (Plate 3; Fig. 23a). Often the pleopods of such individuals would be intertwined and bound together into a single mass by a gelatenous amber-coloured substance that covered the abdomen of the maternal female (Plate 4). The glue-like nature of this viscous compound actually fixed the pleopods together and occasionally completely enveloped the few remaining eggs. Closer examination under a dissecting microscope revealed this "glair-compound" to be composed of numerous egg-stalks and

Table 8. Comparison of relative fecundity (no. eggs/g wt of ♀) between study lakes. Data presented as $\bar{x}$ ± S.E. (n) for each fecundity estimate; * = significantly ($p < .05$) different from L223 value.

Year	Condition	L239	L240	L223	L224
1979	successfully reproducing ♀♀	22.2 ± 0.7 (44)	19.7 ± 0.8 (47)	19.5 ± 0.7 (43)	-
	all ovigerous ♀♀	21.1 ± 1.0 (46)*	18.6 ± 0.7 (50)	16.4 ± 1.0 (54)	-
1980	successfully reproducing ♀♀	20.8 ± 1.2 (28)	19.8 ± 1.1 (29)	20.9 ± 0.8 (47)	20.4 ± 1.8 (13)
	all ovigerous ♀♀	20.3 ± 1.2 (31)*	17.9 ± 1.1 (30)	14.7 ± 0.9 (82)	20.4 ± 1.8 (13)*
1981	successfully reproducing ♀♀	-	20.1 ± 1.5 (21)	19.8 ± 1.1 (32)	-
	all ovigerous ♀♀	-	19.9 ± 1.5 (22)*	14.3 ± 1.2 (60)	-

Table 9. Disparity between potential and realized reproductive capacity of *O. virilis* in ELA lakes. Data for each egg count presented as x ± S.E. (n).

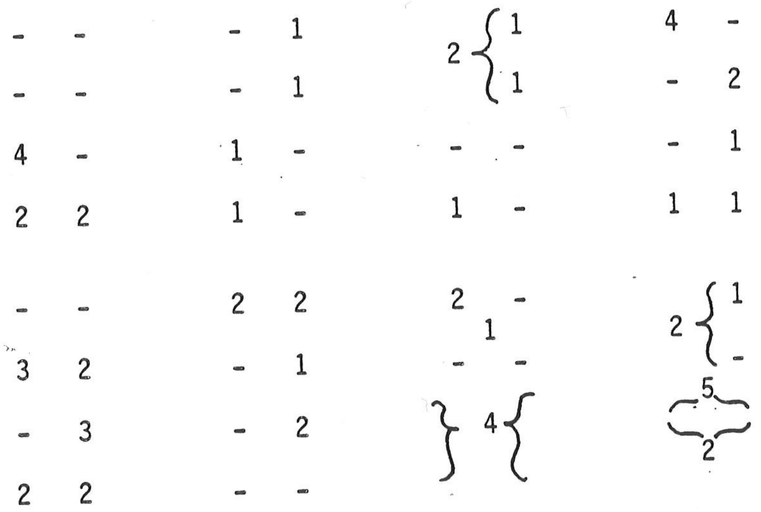
Year	Lake	Ovarian egg count	Pleopod egg count	Disparity (% drop in egg count)
1979-80	L239	98.1 ± 2.7 (55)	77.2 ± 4.6 (31)	21.3
	L240	88.3 ± 3.1 (49)	67.1 ± 6.4 (30)	24.0
	L223	89.7 ± 2.8 (43)	45.8 ± 5.8 (82)	48.9
	L224	77.7 ± 2.9 (31)	65.5 ± 6.3 (13)	15.7
1980-81	L240	86.2 ± 4.1 (28)	71.0 ± 5.1 (22)	17.6
	L223	91.8 ± 3.1 (44)	45.4 ± 6.4 (60)	50.6

Table 10. Percentage of crayfish from ELA populations experiencing reproductive impairment during 1979-81. Ovigerous females with partial egg complement (i.e. loss of eggs) or egg mortality are not mutually exclusive.

Lake	n	% females with partial egg complement	% females with dead eggs	n	% females with partial egg complement	% females with dead eggs	n	% females with partial egg complement	% females with dead eggs
L239	46	4.4	0	29	3.5	0	-	-	-
L240	55	5.5	0	54	5.6	0	21	4.8	0
L224	-	-	-	20	0	0	-	-	-
L223	58	19.0	6.9	83	42.2	16.9	56	47.5	13.8

Plate 3: Distribution of eggs over the pleopods of L223 crayfish that have lost the majority of their broods.

Figure 23: Schematic representation of the distribution of (A) eggs and (B) empty egg capsules over the four pleopod sets and medial sternal ridges of individual ovigerous crayfish from L223 showing an incomplete complement of eggs. (Blank lines represent no eggs present). Parenthesis denote pleopods bound together by unhardened glair-cement.



(A)

4	4	4	7	4	4	5	3
6	5	9	11	4	6	2	4
5	6	9	8	8	7	0	9
4	0	7	5	6	8	1	8
+		+		+		+	
18 eggs		22 eggs		16 eggs		8 eggs	

(B)

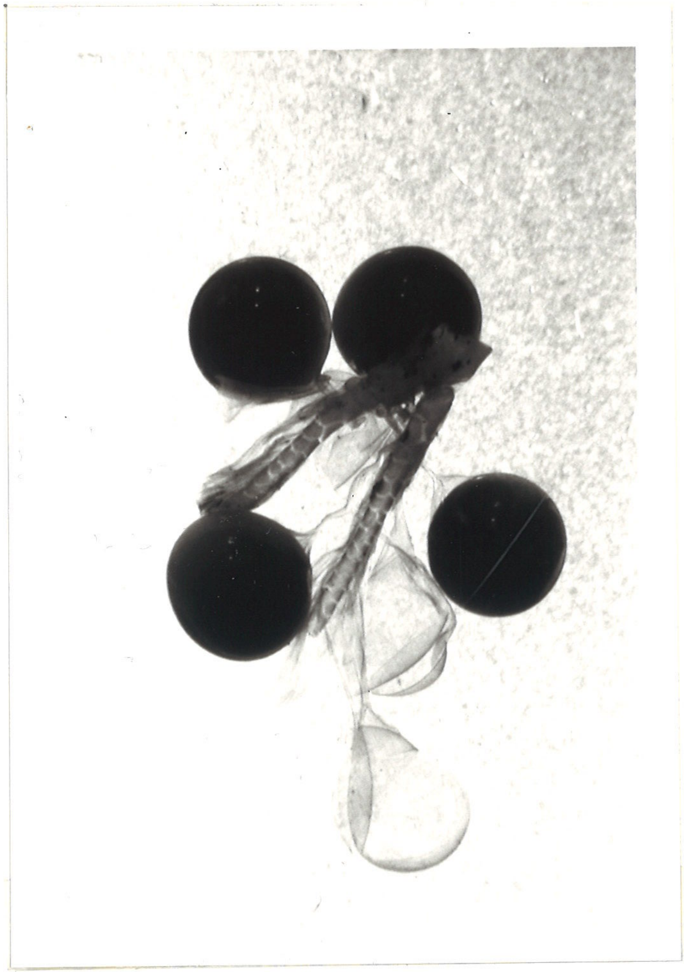
Plate 4: Pleopods of L223 crayfish bound together with unhardened glair-cement.

empty capsules that had ruptured at the distal end and lost their eggs (Plate 5). These empty egg cases displayed no location bias and were distributed evenly over all pleopod sets (Fig. 23b). Individual pleopods were also uniformly covered by egg capsules along their entire length and on both endo- and exopodite branches (Plate 6) indicating that egg loss was not restricted to the terminus. Occasionally pleopods would be removed and the number of empty capsules and stalks enumerated. The summation of both eggs and empty capsules projected the total potential number of eggs close to that which would be expected on the basis of the particular organism's carapace length (Figs. 20, 22; and L240 Fig. 21). This condition of reproductive impairment was not related to size of mature female and varied considerably among individual crayfish in its relative severity. The extent of egg loss was generally high with about 80% of those crayfish displaying this condition having lost more than half of their brood (Fig. 24a). Further, about one-quarter of the animals had lost greater than 87-94% of their clutch, some in fact retaining only the empty capsules. Egg loss, although rarely occurring in the control lakes, was always severe in such situations.

The percentage of females with dead eggs in L223 was 6.9% in 1979, 16.9% in 1980, and 13.8% in 1981 in contrast to an absence of this condition in control populations (Table 10). The extent of egg mortality varied greatly among affected crayfish. Dead eggs are easily distinguished from healthy, fertilized eggs by being bright orange or

Plate 5: Structure of empty egg capsules from L223 crayfish.

Plate 6: Distribution of empty egg capsules over pleopods from L223 crayfish.



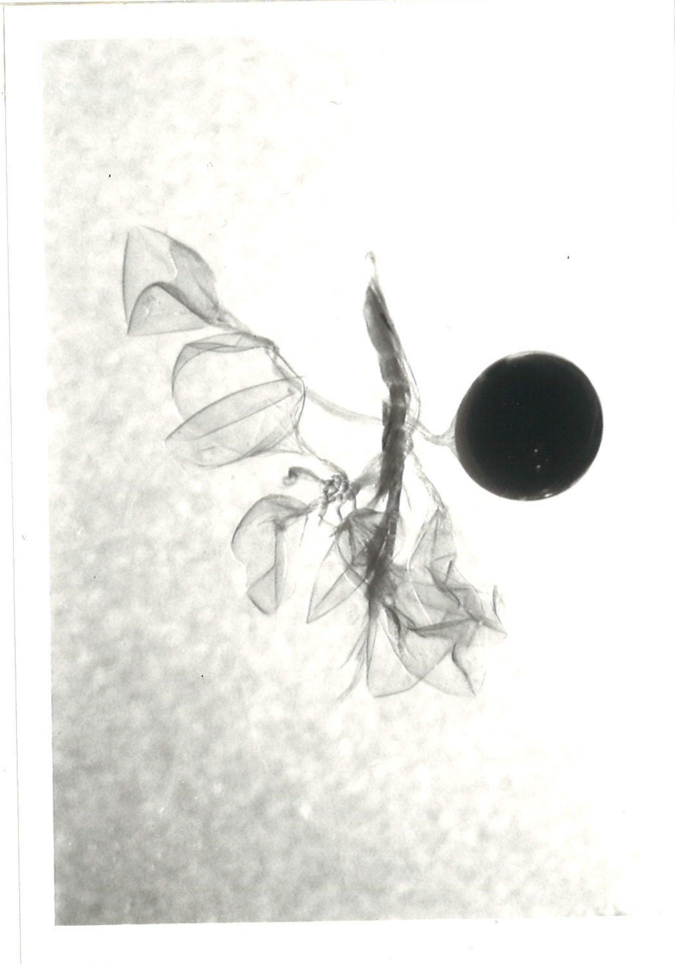
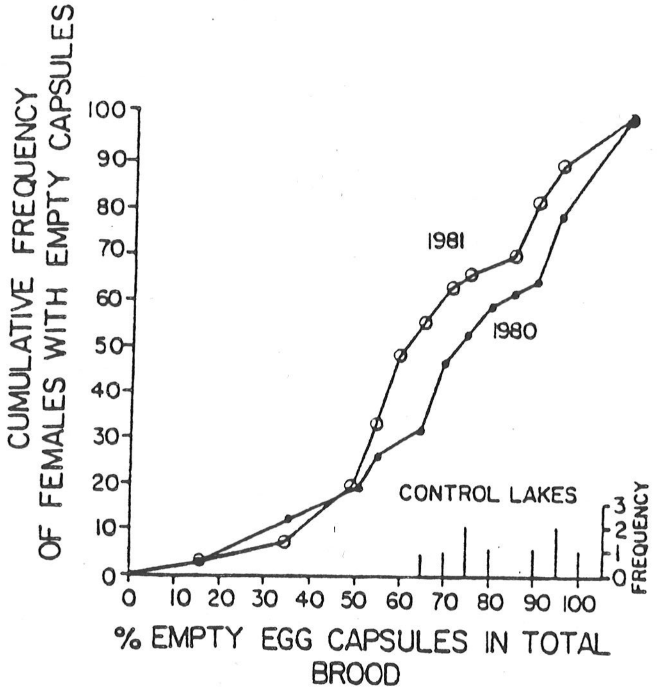
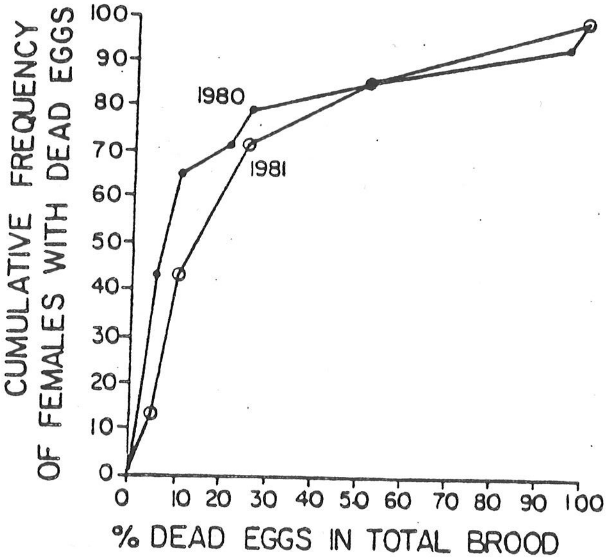


Figure 24: Cumulative frequency distribution of (A) eggs lost, represented as empty capsules, and (B) egg mortality from total brood in affected L223 crayfish during 1980 and 1981. Histogram depicts results of 10 control lake crayfish with this condition.


CUMULATIVE FREQUENCY
OF FEMALES WITH EMPTY CAPSULES
100
90
80
70
60
50
40
30
20
10
0
1981
1980
CONTROL LAKES
FREQUENCY
0
1
2
3
0
10
20
30
40
50
60
70
80
90
100
% EMPTY EGG CAPSULES IN TOTAL BROOD


CUMULATIVE FREQUENCY
OF FEMALES WITH DEAD EGGS
100
90
80
70
60
50
40
30
20
10
0
1980
1981
0
10
20
30
40
50
60
70
80
90
100
% DEAD EGGS IN TOTAL BROOD

yellow and often, depending upon when mortality occurred, a much smaller size relative to the successfully developing eggs around them. The most common state observed in L223 crayfish was the presence of only a few nonviable eggs randomly located over the pleopod egg mass. Thus the severity of egg mortality was generally low such that in 50% of those crayfish that carried dead eggs less than 7-14% of their total brood had expired (Fig. 24b). Only about 15% of the affected females had lost over half their brood. In such cases the pattern of egg mortality formed distinct groupings about individual pleopod clusters (Plate 7). The dead eggs were bound together into large discoloured masses surrounded by an amorphous, granular, opaque-white matrix. The quality of eggs within these dead clusters varied considerably including small undeveloped orange eggs, pure white or deep red eggs, and even an occasional large (3x normal size) green egg.

Due to the wide variability of the percent egg loss or egg mortality among individual crayfish, a more accurate assessment of reproductive impairment can be obtained on a population egg production basis (Table 11). The % population reproductive failure (defined as the decrease in the number of viable eggs available prior to hatching) in the three control populations averaged 3.2 ± 1.8 (±95 C.I.) while values of 18.7, 36.2, and 29.4% were obtained during 1979-81 in L223.

The degree of ovarian reabsorption of unlaid oocytes during the spring was categorized into 5 descriptively-based levels reflecting the general appearance of the ovary:

Plate 7: Crayfish from L223 with partial egg mortality.

Table 11. Reproductive impariment of <u>O. virilis</u> at ELA calculated on a total egg production basis per population.

Reproductive characteristic	Control lakes 1979-81 n = 6	Lake 223 1979	 1980	 1981
Total number of eggs examined	12132	2685	3963	2695
Estimated number of empty capsules	435	605	1760	993
% eggs lost	3.2 ± 1.8	18.3	30.8	26.9
Number of dead eggs	0	11	314	90
% dead eggs	0	0.4	7.9	3.3
Population % reproductive failure	3.2	18.7	36.2	29.4

Level 1: ovary is a small, homogeneous, white-opaque mass of condensed particles; indicative of no resorption.

Level 2: ovary is voluminous with translucent white particles more distinct and spherical than Level 1; also indicative of no resorption with the white flecs representing the formation of minute new eggs.

Level 3: progressively larger bi-lobed ovary with old eggs in the late stages of resorption and reduced to isolated irregular yellow masses scattered admix the white newly forming oocytes; transition level.

Level 4: large ovary filled with oocytes of various sizes and colours representing different stages of resorption of yolk substances. Characterized by distinctive presence of large orange or red semi-spherical oocytes of a similar size to the successfully extruded eggs.

Level 5: occurrence of large black oocytes rich in vitellus and identical in size and shape to successfully laid eggs; indicative of the earliest stages of degeneration prior to reabsorption of released yolk substances; rest of ovary similar to Level 4.

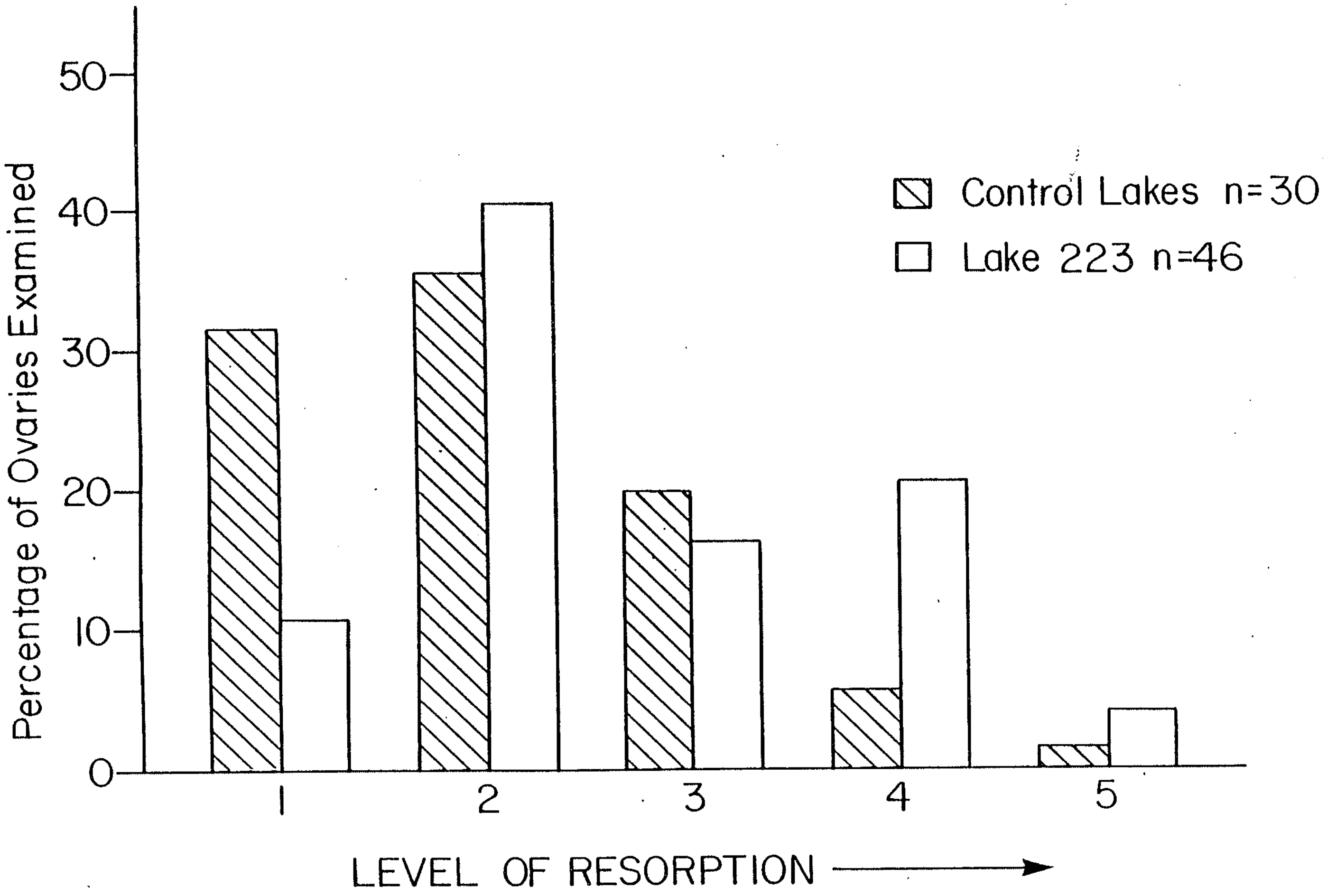
In summary, Levels 1 and 2 represent no resorption of ovarian eggs, Level 3 - a transition point, and Levels 4 and 5 distinctly showing resorption to be occurring.

Ovaries from L223 ovigerous females were almost always more voluminous than those from control lakes. In the control lakes (L239 and L240 combined data) 10.0% of the crayfish caught showed obvious signs of ovarian egg resorption at levels 4 and 5 compared to a value of 29.4% in L223 females (Fig. 25). The percentage of eggs in the process of being degenerated in Levels 4 and 5 ranged between 1.4-6.5% (x ± S.E. of 4.3 ± 0.6%) of the total number successfully extruded and attached. The degree of resorption in L223 crayfish was not correlated with the presence or absence of abnormal characteristics (i.e. dead eggs and/or empty egg capsules) of the extruded pleopod eggs. Sampling of mature yet unmated females (recognized by presence of cement glands and absence of sperm plugs) during the same time period showed ovaries that were completely filled with oocytes characteristic of the Level 4 or 5 stage of resorption, in contrast to the occurrence of only a few such oocytes in ovigerous females undergoing partial resorption.

In 1979 the comparison of egg quality parameters (Table 12) indicated that there was no significant difference between egg size and weight within the four study lakes. A close relationship exists between egg developmental stage and external coloration of crayfish eggs. In the present study the condition of pleopod egg maturation during 1980 was categorized into 7 descriptively-based levels reflecting easily observed alterations in external colour and/or morphological characteristics:

Level 1: newly-laid eggs characterized by a uniform black colour.
Level 2: eggs uniform black-grey.
Level 3: eggs grey with large light-brown spots developing.

Figure 25: Comparative percent distribution of the level of ovarian egg resorption in crayfish sampled from L223 and control lakes during spring 1980. For explanation of resorption index see text.



Percentage of Ovaries Examined
50
40
30
20
10
0
▨ Control Lakes n=30
☐ Lake 223 n=46
1
2
3
4
5
LEVEL OF RESORPTION →

Table 12. Crayfish egg quality parameters in ELA study lakes during 1980. Data presented as x ± S.E. (n). There was no significant (p > .05) difference between lakes.

Lake	Egg diameter (mm)	Egg dry weight (mg)
L223	2.31 ± 0.08 (55)	1.55 ± 0.05 (39)
L224	2.24 ± 0.10 (58)	1.48 ± 0.09 (15)
L239	2.36 ± 0.04 (25)	1.49 ± 0.03 (42)
L240	2.20 ± 0.06 (73)	1.54 ± 0.04 (40)

Level 4: muddy-grey appearance with small polar concentration of purple/lavender colour.

Level 5: polar developmental disc becomes light-pink in colour, thorasic appendages becoming evident.

Level 6: "eyed-stage" with obvious orange carapace and black eye-spots forming just prior to hatching.

Level 7: presence of eggs in the process of hatching and a few attached S-I young.

There is no difference in the rate of crayfish egg development in L223 at a mean pH of 5.35 compared to a control population (Fig. 26). Further, examination of egg colour and time to hatching during spring 1981 suggests that this conclusion also holds true for lake acidification to pH 5.05.

2.3.6 Mortality Rates and Population Structure

Annual survival rates (S) were 8% lower in L223 than in the control lakes (Table 13). The good agreement between Robson and Chapman's "best" estimate with Heincke's method indicates that errors in sampling or variable year-class strength are not important. Albiet the precision of estimation from a segment of the catch curve is substantially less than that obtained by using the entire curve, a general indication of the shift in age-specific survival can be made. For any lake there is a progressive increase in instantaneous mortality (Z) with age.

Figure 26: Comparison of the rate of egg development through 7 stages (described in text) between L223 and L240 crayfish during spring 1980.

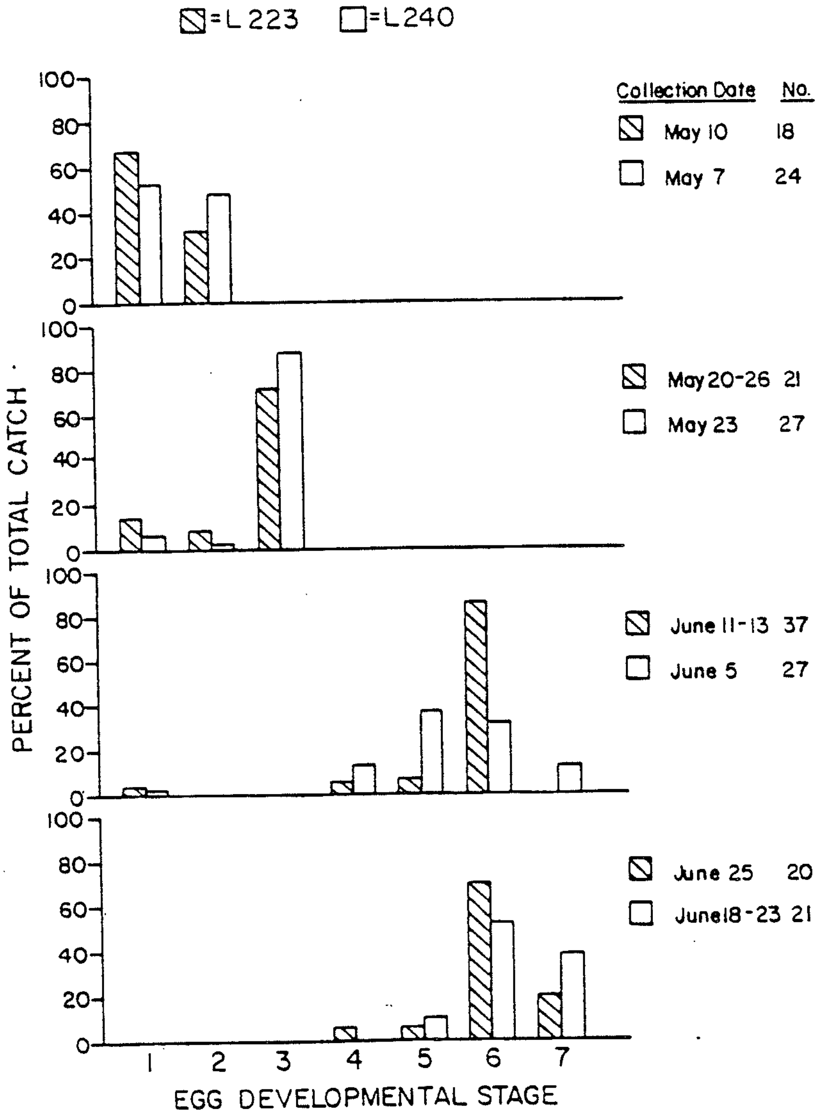

▨=L 223 ☐=L240
Collection Date No.
▨ May 10 18
☐ May 7 24
▨ May 20-26 21
☐ May 23 27
▨ June 11-13 37
☐ June 5 27
▨ June 25 20
☐ June 18-23 21
PERCENT OF TOTAL CATCH
0
20
40
60
80
100
1
2
3
4
5
6
7
EGG DEVELOPMENTAL STAGE

Table 13. Annual survival (S) and instantaneous mortality (Z) rates for ELA crayfish populations. Robson and Chapman's (±95% C.I.) and Heincke's estimates were obtained from combined catch curve analysis (Robson and Chapman 1961). Annual rates for individual age-classes were calculated by the logarithmic catch curve procedure (Ricker 1975).

	L223		L224		L239		L240	
Procedure	S	Z	S	Z	S	Z	S	Z
Robson and Chapman	0.28±0.05	1.27	0.36±0.06	1.02	0.34±0.05	1.08	0.38±0.06	0.97
Heincke	0.32	1.14	0.41	0.89	0.39	0.94	0.41	0.89
Catch curve								
I-II	0.38	0.97	0.46	0.78	0.54	0.62	0.48	0.73
II-III	0.25	1.39	0.36	1.02	0.29	1.24	0.33	1.11
III-IV	0.06	2.81	0.10	2.30	0.13	2.04	0.14	1.97

Calculations of S and Z are annual rates representing all forms of natural mortality including predation, disease, cannibalism, and physiological aging. Mortality though is not constant over a yearly period at ELA (e.g. Fig. 27). There is little overwinter mortality of age I or II crayfish relative to the dramatic disappearance of the age II group during the summer period. In the fourth summer (age III) no discernible mortality occurs until the end of the season. A constant die-off of age III-IV crayfish occurs with only a few individuals (all males) surviving the duration of their fifth summer before expiring.

A 1:1 sex ratio among mature animals is often the exception rather than the rule. Studies have shown that the sex ratio for Crustacea will vary with size in a pattern characteristic for individual species (Wenner and Fusaro 1979). The oscillating sex ratio pattern displayed for crayfish from L240 reflects differential growth rates between the sexes and greater longevity of males (Fig. 28).

Histograms of population structure reflect the relative catchability of the different age-groups over the year (Fig. 29). In all cases the difficulty in location and capture, and therefore underrepresentation, of the youngest age-group prevents a quantitative comparison with the remaining age components within the lake. It still allows, however, a relative comparison of this group to be made between lakes.

In spring 1979, the age composition of the two populations with slower growing crayfish, L224 and L223, were similar. In L224 the age I

Figure 27: Seasonal trends in survivorship of age I-IV *O. virilis* in L223 during 1980-81 (s=summer, w=winter). Line extrapolated from data of Momot (1967).

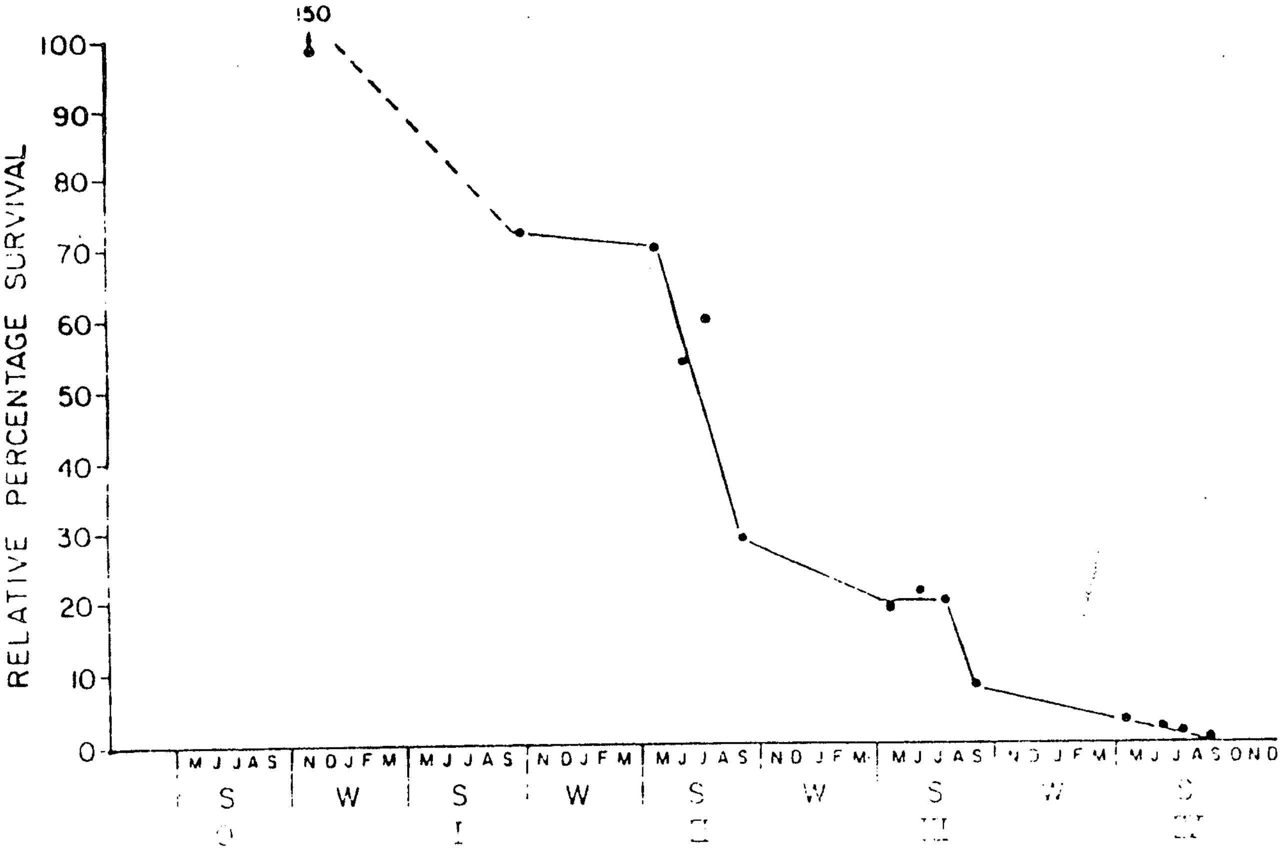

150
RELATIVE PERCENTAGE SURVIVAL
100
90
80
70
60
50
40
30
20
10
0
M J J A S
N D J F M
M J J A S
N D J F M
M J J A S
N D J F M
M J J A S
N D J F M
M J J A S O N D
S
W
S
W
S
W
S
W
S
0
I
II
III
IV

Figure 28: Sex ratio pattern of L224 crayfish in autumn 1980. Curves for other study lakes were similar.

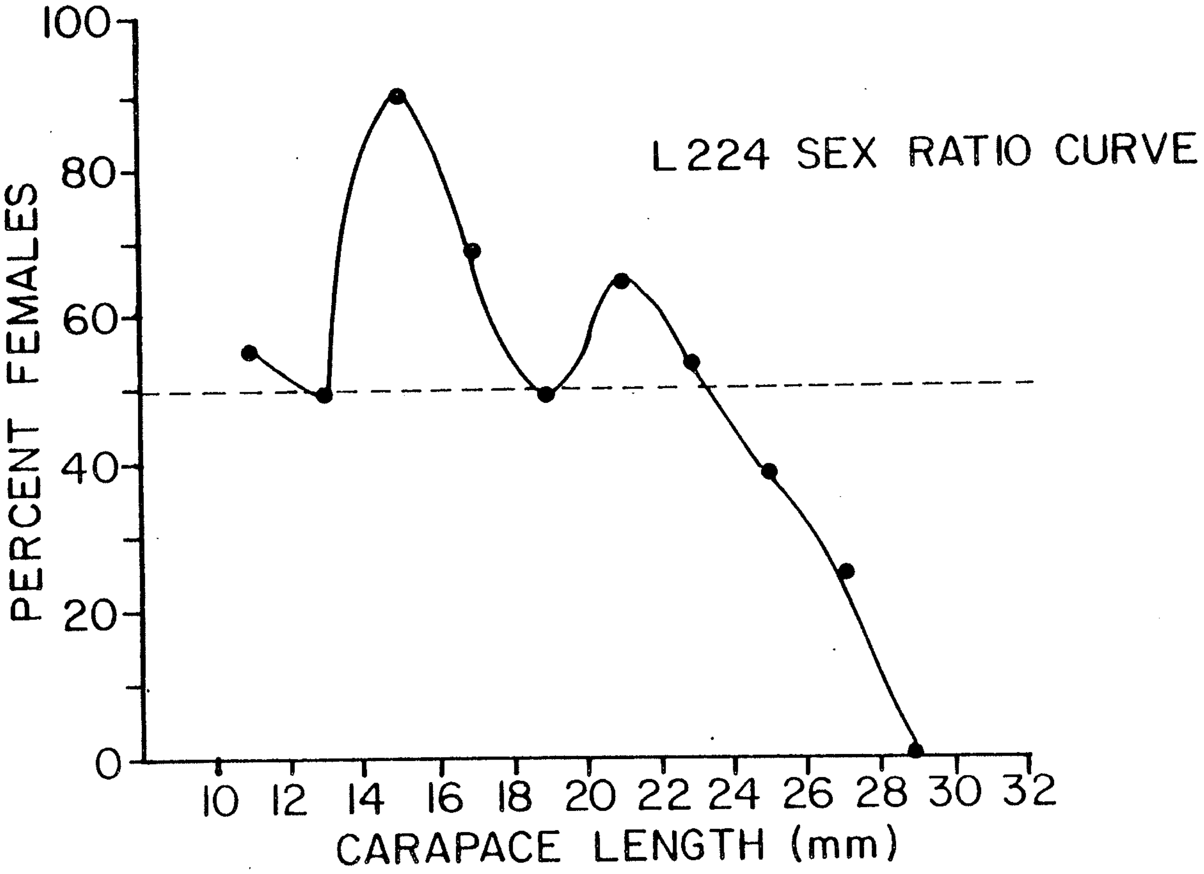
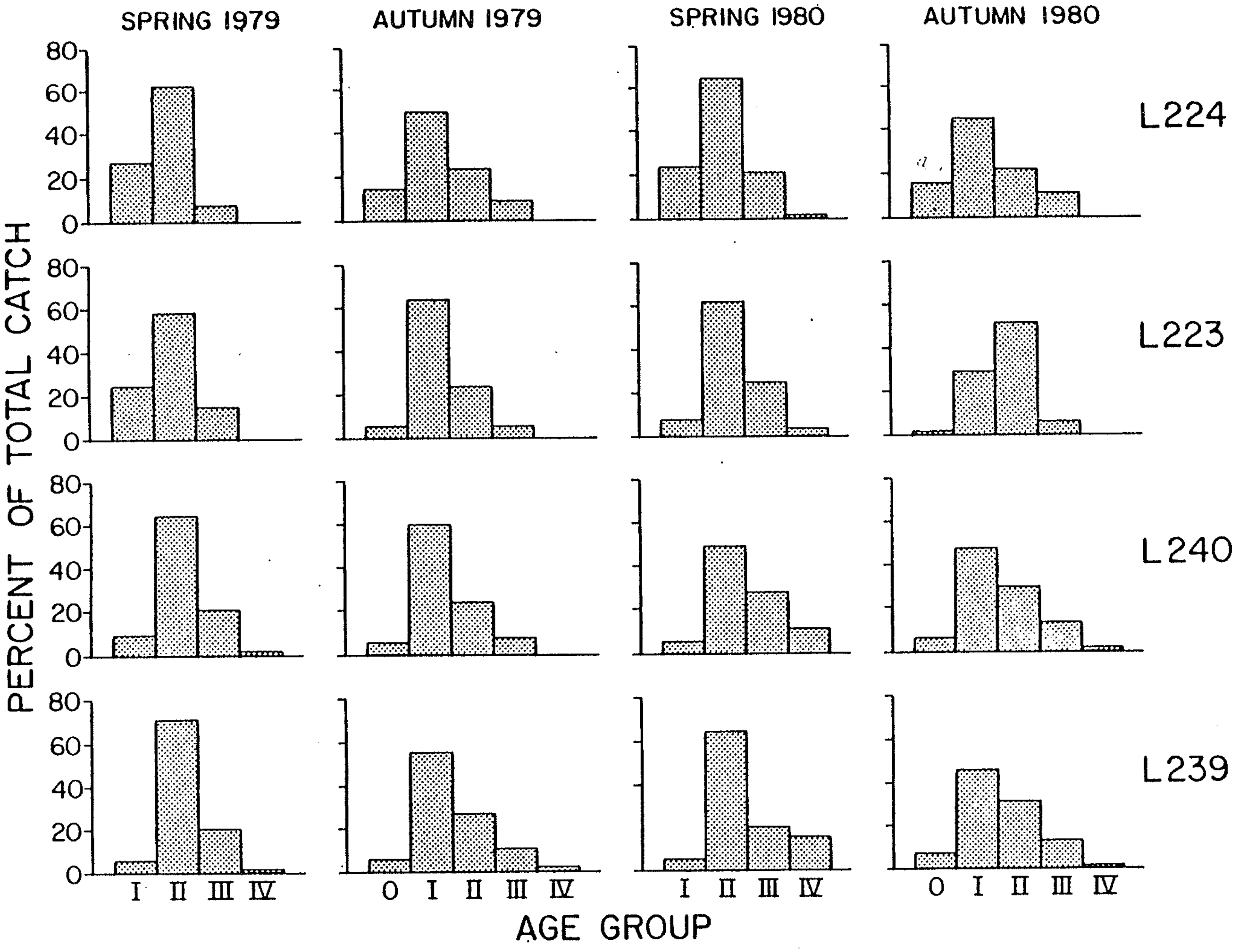

L224 SEX RATIO CURVE
PERCENT FEMALES
0
20
40
60
80
100
10
12
14
16
18
20
22
24
26
28
30
32
CARAPACE LENGTH (mm)

Figure 29: Age composition data of ELA *O. virilis* populations.


SPRING 1979
AUTUMN 1979
SPRING 1980
AUTUMN 1980
L224
L223
L240
L239
PERCENT OF TOTAL CATCH
0
20
40
60
80
I
II
III
IV
0
AGE GROUP

group, representing those individuals born the previous summer, comprised 28.9% of the total catch, while the same age group in L223 subsumed 25.7% of that lake's catch. The catch in L239 and L240, because of the faster growth rate and predominance of larger individuals, was more biased toward the age II and III group at the expense of the age I individuals. With growth and maturation during the summer the age I group assumed a numerical dominance in all four lakes during the autumn 1979 collections. The age class 0, representing young-of-the-year born two months previously, represented 16.0% of the total catch in L224 but only 7.0% of the L223 catch, similar to those levels recorded in the two populations with faster growth.

In spring 1980, the age compositions of crayfish from the three control lakes resembled those of the previous spring. The age 0 group, now represented as age I, in L224 subsumed 21.5% of the total catch while the same age-group comprised only 8.0% of the L223 catch, a large decrease in relative prevalence from the previous spring collection. Age I representation in L239 and L240, like L224, was similar to spring 1979. Growth during the summer produced fall 1980 collections in the control lakes that closely resembled those of the previous autumn. The disparate population structure in L223 indicates that there was a partial recruitment failure during summer 1979. This coincides with a 18.7% decrease in the production of L223 eggs at that time. During autumn 1980 in L223 only 2.0% of the total catch was now represented by this age group (values of 8.5-18.7% for control lakes). This dramatic decrease in 1980 recruitment must partially represent the 36.2% reproductive failure estimated by the spring egg counts.

In spring 1981 (data not shown) the numerical abundance of age I crayfish in L223 was 1.9% of the total catch, significantly lower than levels of 8.0% in spring 1980 or 25.7% in spring 1979. Fecundity studies in 1981 again suggested a 29.4% reduction in L223 reproduction, a result in agreement with autumn 1981 collections in the lake that showed almost complete absence of the 0 and I groups (Davies in prep.).

2.4 DISCUSSION

2.4.1 Trace Metal Bioaccumulation

Although numerous investigations involving fish-heavy metal interactions have been performed, little work has been directed toward the study of trace metal accumulation in freshwater invertebrate populations. Crayfish, because of their large size, unique polytrophic life habits, and ubiquitous distribution, may serve an important indicator function in acidified heavy metal stressed environments.

The Hg tissue concentrations in this study (0.20-0.52 $\mu g \cdot g^{-1}$) are similar to values previously recorded for O. virilis at ELA (0.17 $\mu g \cdot g^{-1}$, Hamilton 1972a), and intermediate between levels for O. virilis from northern Manitoba (0.04 $\mu g \cdot g^{-1}$) and for Clay Lake (0.95-10.12 $\mu g \cdot g^{-1}$), a mercury polluted system near ELA (Vermer 1976; Hamilton 1972b). Gale et al. (1973) record whole-body concentrations of Pb, Mn and Cd from an unidentified crayfish species sampled in a heavily polluted mining area in Missouri that are 84-95% greater than those for ELA O. virilis. Anderson and Brown (1978) found that O. virilis populations accumulate trace metals in relation to their environmental concentration. Body concentrations of Cd, Pb, and Zn even for their "control" populations were substantially higher than those at ELA. Alternatively, Procambarus sp. from a South Carolina stream receiving coal ash inputs (Cherry et al. 1979) had lower body concentrations of Cu and Zn than ELA crayfish despite much higher water concentrations of metals. Clearly factor(s) other than the aqueous metal concentration can influence the uptake, accumulation, and regulation of heavy metals by crayfish, making comparisons between different studies difficult, especially if background water chemistry data are not provided.

Calcium can modify the uptake of heavy metals. Franzin and McFarlane (1980) and Franzin (1980) found that discrepencies in bioaccumulation for fish and macrophytes could be explained on the basis of the lake Ca^{++} concentration. Laboratory studies have shown that aqueous Ca^{++} provides an ameliorative protection against the uptake and toxicity of heavy metals in fish (Kinkade and Erdman 1975) and invertebrates (Bryn 1967; Wright 1980). Hypothetical mechanisms include competition for active cellular binding sites between the heavy metals and Ca^{++}, complexing of Ca ions to metals thereby inhibiting their uptake, or interference in the "accidental" metal uptake via a Ca^{++} regulatory mechanism. The range of Ca^{++} concentrations in ELA lakes studied (1.7-2.4 mg$\cdot$L^{-1}) is too narrow to explain differences in metal uptake, indicating that elevated tissue concentrations of Mn and Hg in L223 crayfish were probably caused by acidification.

Average metal concentrations in _O. virilis_ were similar to those of _Mysis relicta_ in many of the same ELA lakes (Nero 1981), suggesting that similar uptake, regulation and sequestation mechanisms may be present in these freshwater crustaceans. Bioaccumulation of Hg, Se, Cd and Cu by _O. virilis_ occurs directly from the water (Hamilton 1972a; Gillespie et al. 1977; Evans 1980). Zinc uptake from soluton by crayfish occurs largely by surface adsorption on the carapace and gills and, if ingested with food, can be regulated via loss in the urine or feces (Bryn 1967; Giesy et al. 1980).

Anderson and Brower (1978) emphasize that the effectiveness of crayfish as biomonitors of heavy metal pollution is dependent upon the physiological role of the metal. It is important to distinguish between trace elements such as Cu or Zn which are biologically essential to invertebrates and thus expected to be homeostatically regulated, and those like Cd and Pb which serve no physiological function and can be expected to vary in tissues in relation to environmental inputs. _O. virilis_ in ELA lakes bioconcentrate essential/regulated elements such as Cu and Zn more than nonessential ones like Cd, Pb, Al, and Mn.

The L223 acidification experiment was designed to separate the confounding effects of low pH and elevated heavy metals found to occur within polluted regions. The increased metal concentrations observed in

L223 are from sediment remobilization, and are small compared to lakes receiving both atmospheric fallout and increased drainage basin leaching in addition to such autochthonous sources. Nonetheless, that the crayfish O. virilis could still respond to increases of Mn and possibly Hg, strongly promotes the utilization of this organism in the biomonitoring of more seriously polluted regions.

2.4.2 Carapace Rigidity and Calcium Content

The crayfish exoskeleton is composed of four major layers: (1) the outer non-calcified epicuticle which is hardened by tanning, (2) the exocuticle hardened by both tanning and calcification, (3) the thick endocuticle hardened only by calcification, and (4) the thin non-mineralized membranous layer. Calcium carbonate is the chief inorganic component, and Ca^{++} by weight, the commonest ion present in the crayfish exoskeleton.

The close relationship between carapace disc dry wt and Ca^{++} content found in this study agrees with previous relationships found between carapace length or weight and Ca^{++} content in other crayfish species (Appelberg 1980; Adegboye et al. 1977; Greenaway 1974). The percentage dry wt of Ca^{++} in intermolt O. virilis carapaces in the control lakes was found to be 20-23% comparable to values of 20-25% previously recorded for O. virilis (Travis 1963; McWhinnie et al. 1969). Mills et al. (1976) and Bretonne et al. (1969) found that the concentration of exoskeletal Ca^{++} was correlated with the amount of available Ca^{++} in the water inhabited by crayfish in support of the relationship between O. virilis Ca^{++} content and Ca^{++} concentrations in lakes in this study.

It has long been known in aquaculture practise that crayfish in acid waters tend to have thinner shells (LeCaze 1970). Appelberg (1980) held molting A. astacus in 10 mg Ca^{++} L^{-1} water for 79 days and found a significant decrease in carapace dry wt and Ca^{++} content with decreasing pH. The calcification of exoskeletons was inhibited such that individuals molting in pH 4.55 test water had paper-thin carapaces. Similarly, O. virilis held at pH 5.0 for 10 d had slower rates of exoskeleton calcification and lower Ca^{++} contents than animals at pH 6.0 or higher (Malley 1980). The significantly lower carapace rigidity and Ca^{++} content observed in L223 crayfish (estimated 25-35% reduction in Ca^{++}) compared to animals from the 3 control lakes indicates that calcification inhibitory mechanisms operating in the field are similar to those previously observed in the laboratory.

Postmolt crayfish depend largely upon the uptake of Ca^{++} from the aquatic environment to calcify their new exoskeletons, as the amount stored in the gastroliths or taken up through feeding is of insufficient quantity (Adegboye et al. 1977). Borgstrom and Hendrey (1976) suggested that the effect of low pH on crustaceans may be to interfere with the uptake of Ca^{++} at molting. Greenaway (1974) found a 60% reduction in postmolt Ca^{++} uptake in the absence of HCO_3^- which was thought to accompany Ca^{++} for electrical neutrality. The remaining 40% of Ca^{++}

uptake occurred through ion exchange for internal H^+ or by HCO_3^- production via metabolic CO_2 formation. The HCO_3^- is deposited together with Ca^{++} as $CaCO_3$ during calcification. Malley (1980) found that inhibition of postmolt Ca^{++} uptake by O. virilis began below pH 5.75 and was complete at about pH 4.0 due to either (1) the scarcity of HCO_3^- to accompany Ca^{++} for electrical neutrality, (2) interference with the exchange of internal H^+ for external Ca^{++} due to the elevated concentration of H^+ in the ambient environment, or (3) the direct effect of H+ on the active transport system by altering molecular configuration.

Calcium influx for L223 crayfish in 1978 was lower than that measured in 1977 for comparable molt states, suggesting the possibility that the L223 population was making physiological adjustments associated with the decreasing HCO_3^- in the lake (Malley 1980). From Fig. 2 in Malley (1980) it can be predicted that during 1979 the L223 population experienced a 13% inhibition in Ca^{++} uptake at a mean lake epilimnion pH of 5.60; with further acidification in 1980 to pH 5.35 causing a 35-40% reduction in calcification. The carapace Ca^{++} content as % dry wt ±95% C.I. for L223 intermolt crayfish was 16.8 ± 0.6 in 1977(Malley 1980) significantly ($p < .05$) higher than the value 13.90 ± 0.89 obtained in 1980 in this study. Huner et al. (1978) found that P. clarkii have adequate body Ca^{++} reserves to maintain normal metabolic functions and to calcify their exoskeletons to stage C3 of the molt cycle (13.70% carapace Ca^{++} content - a reduction of almost half the amount present in intermolt animals) in the complete absence of dissolved environmental Ca^{++}. It is not known if the carapace Ca^{++} content of L223 crayfish will continue to decrease as a result of further acidification. Long-term trends in L223 crayfish exoskeleton Ca^{++} from 1975-82 are currently being analysed (Davies in prep.).

Chaisemartin (in Greenaway 1972) determined that 10% of the total crayfish body Ca^{++} was easily mobilized. During premolt, large amounts of Ca^{++} were removed from the old exoskeleton, reabsorbed into the blood and then stored or eliminated. Greenaway (1972) suggested that the reservoir of Ca^{++} in the exoskeleton is probably sufficient to enable healthy crayfish to tolerate the net Ca^{++} loss during the overwinter intermolt period without a loss of skeletal function. McMahon and Morgan (1981) held adult *O. rusticus* at pH 3.5 for 96 hr and found a gradual increase in hemolymph Ca^{++} suggesting a dissolution of $CaCO_3$ from the exoskeleton to provide the hemolymph with more HCO_3^- buffer. This decalcification may also limit exoskeleton rigidity in acid waters.

Although calcification is the primary source of exoskeleton hardening in Crustacea, it is not the sole cause and only the integrated operation of a large complex of physiological processes results in the final rigidity. The principal chemical change that occurs following the molt in those layers secreted before the molt, is phenolic tanning or sclerotization (Stevenson 1977). An enzyme incorporated into the outer cuticular layers as they are laid down by the epidermis, catalyses the oxidation of phenols to quinones, which react with and link together the proteins of the cuticle, producing greater strength, rigidity and hardness prior to calcification. This initial hardening via tanning will also afford an effective control of permeability while leaving the cuticle elastic enough to allow for its extension with the subsequent onset of calcification (Dennell 1955). Yonge (1936) investigated the hardening mechanism of decapod cuticle and found it to be sensitive to corrosion by acids producing a loss of rigidity. The cuticle was also found to reach its lowest permeability at pH 5.2, its iso-electric point. The hormone crustecdysone is thought to play an important role in the deposition of Ca^{++} while another hormone in O. virilis eyestalks promotes Ca^{++} reabsorption from the exoskeleton (McWhinnie et al. 1972). The pH must be sufficiently high to facilitate the precipitation of $CaCO_3$ which is influenced by the enzymes alkaline phosphatase and carbonic anhydrase.

Delay or limitation of calcification or other hardening mechanisms, thereby producing prolonged periods of soft exoskeletons, would make populations more vulnerable to abrasion-mechanical damage and cannibalism, both of which can limit crayfish distribution and production in softwater environments (Abrahamsson and Goldman 1970; Bretonne et al. 1969). Although small crayfish may be preyed upon by fish at any molt cycle stage, larger individuals are predominately taken only following ecdysis while exoskeletons are still soft (Scott and Duncan 1967; Stein 1977), suggesting that an indirect effect of acidification on crayfish populations will be to increase predation pressure. Digestion rates of fishes are known to depend upon the cuticular composition and therefore hardness of their crayfish prey. Because crayfish lose 75% of their mineral salts and chitin through a molt, such individuals have nearly two times the digestible organic material of intermolts and are thus considerably more palatable (Stein and Murphy 1976). The behavioral interaction between predator and prey that causes the highly susceptible recent molts to seek shelter until their exoskeleton hardens (Mobberly 1973; Stein 1977) is absent in intermolt crayfish with reduced carapace rigidity caused by acidification. In such cases predation will be of great severity to the population. Chaisemartin (in Mills et al. 1976) speculated that crayfish inhabiting low Ca^{++} environments may need to conserve Ca^{++} by restricting its distribution to areas where it is most beneficial in terms of rigidity. If this occurs in the Ca^{++} stressed L223 population it may additionally promote the incomplete hardening of the male form I copulatory appendages, interfering with mating by leaving males impotent. Imbalances in Ca^{++} uptake may eventually delay, or interrupt the molting cycle itself (Borgstrom and Hendrey 1976; Malley 1980; Appelberg 1980) to a degree that individual growth will be retarded and onset of sexual maturity stayed.

Preliminary examination suggests that the size and weight of gastroliths from premolt L223 animals are less than those from crayfish inhabiting the control lakes. Due to the length of time (4-7 days) needed to assimilate Ca^{++} from solution at low concentrations, the presence of large gastroliths may be of enhanced importance in softwater lakes, providing a small store of readily available Ca^{++} which could be used for initial strengthening of the exoskeleton following ecdysis, thereby allowing a minimum level of activity and defence during this critical period (Scott and Duncan 1967).

The percentage of the total catch that had lost or were regenerating one or both chelae was obtained from the autumn 1980 collections and was higher in L223 (6.7%) than in the control lakes (L224 - 1.5%, L240 - 2.1%, L239 - 2.7%). While this may indicate increased cannibalism in the softer L223 crayfish, it may also reflect an inhibition of limb regeneration by low pH. Needham (1952) found that the rate of limb regeneration in the isopod Asellus was sensitive to small pH variations, with a 17.2% difference in mean rate between pH 7.5 to 6.0. Chelae serve an important behavioral and ecological role in the dominance ranking among crayfish (Bovberg 1953) as well as deterring predation (Stein 1977).

In addition to serving a protective function, exoskeletons provide a relatively efficient barrier to the free diffusion of ions and to changes in osmotic pressure (Robertson 1941). Incomplete exoskeleton hardening would therefore not only render crayfish unprotected but would also decrease the effective control over their salt balance and resistance to adverse osmotic pressure, both of which can be expected to be important in maintaining internal homeostasis in acidified environments.

2.4.3 Growth

Crayfish abundance in oligotrophic ELA lakes is thought to be regulated by the density-independent control of growth, operating through reproduction (cf. Appendix A). The effects of acidification upon growth can therefore be expected to be of profound importance. A reduction in the growth rate of the L239 population by only 10% would result in a 17% decrease in population size; further growth decreases to 30% of the initial value would produce a population of only half the original size. Alterations in growth by acidification can result from three processes, (1) a change in the physiological and/or metabolic mechanisms, (2) modification in feeding behavior, and (3) variation in the quality or quantity of food available per animal. The growth of invertebrates in acidified lakes has never been investigated.

The most dramatic effect in potential crayfish food in L223 has been the development of a dense mat of the alga Mougeotia throughout the littoral zone (Schindler 1980). Muller (1980) suggested that the ecological consequences of a simplification of the periphyton community due to acidification would be a decrease in the variety of food available to benthic grazers. Crayfish, however, are extremely opportunistic feeders, so that the shifts in species diversity as a result of lake acidification are unlikely to cause food shortages. This is supported by a comparison of the length-weight relationships among crayfish from the study lakes which showed no significant difference in the relative fitness of L223 crayfish (A.4). Laboratory experiments (3.A.3.1) have shown that both adult and young-of-the-year *O. virilis* do not cease feeding, even at chronically lethal pH levels. Crayfish growth may also be affected by acidification through physiological interference with the molting cycle producing either a slower (Malley 1980; Borgstrom and Hendrey 1976) or faster (Appelberg 1980) progression through molt stages or length of the intermolt period. Contrary to these laboratory studies, the timing of the adult *O. virilis* molting events in L223 compared to those in the control populations has not been altered with acidification to pH 5.05 during 1981. During 1979 the growth rate of the L223 population was what would have been expected on the basis of its relationship to lake phytoplankton productivity (Appendix A). Continued acidification from the mean epilimnion pH of 5.60 in 1979 to a value of 5.35 in 1980 did not change the ordering of crayfish growth rates among the lakes. Further, the better climatic conditions during 1980 actually increased crayfish growth in L223 corresponding to similar increases observed within the other study lakes. Acidification-related decreases in crayfish abundance will therefore be caused by other processes long before any alteration in growth will pose a significant threat.

2.4.4 Parasitism by Microsporidia

Microsporidia parasitize most major animal groups including a wide variety of crustaceans (Johnson 1977; Sprague and Cough 1971).

Thelohania contejeani is well-distributed throughout northern Europe, being reported from the following crayfish hosts: *A. astacus*, *A. fluviatalis*, *A. leptodactylus*, *Austrapotamobius pallipes* and *C. affinis* in Finland, U.S.S.R, Lithuanla, Britian, Ireland, France, Poland and Germany (Sumari and Westman 1969; Mazylis 1978; Cossins 1972, Vey and Vago 1972; O'Keefe and Reynolds 1981; Unestram 1972). *Thelohania* (possibly *contejeani*) has been found to infect the New Zealand crayfishes *Paranephraps zealandicus* and *P. planiforms* (Quilter 1976; Jones 1980) and the Australian crayfish *Cherax destructor* (Carstairs 1978). In North America, Sprague (1950) described a species *T. cambari* in *C. baratoni* from Georgia, Sogandares-Bernal (1962) reported an undescribed species in *Cambarellus schufeldti* from Louisiana and D. McGriff (pers. comm.) found infected *P. leniusculus* in California. This is the first report of *Thelohania* from Canada and the first mention of its occurrence in the crayfish *O. virilis*. Further work is presently underway to determine if this is a new species or one previously described (L. Graham, pers. comm.). The occurrence of this protozoan disease can be of considerable ecological and economic importance (Cossins 1972; Carstairs 1978; Unestam 1972). Vey and Vago (1972) emphasize that even if the proportion of animals parasitized in natural populations is relatively low, these parasites are nevertheless of extreme importance in the fishery rearing of crayfish.

Thelohania is an intracellular parasite that is tissue-specific for striated muscles, eventually invading all skeletal muscles causing increasing deterioration and finally death. Histological and ultrastructural studies by Cossins (1972) and Vey and Vago (1972) showed that as the parasite invades the muscle fibres a rapid asexual schizogony occurs, resulting in an enormous increase in protozoan spores which dissolve all the myofibrils, leaving only the sarcolemma. This in turn then ruptures, releasing the spores into adjacent fibres. As the disease progresses, the ventral surface of the abdominal muscle becomes progressively whiter, in contrast to the translucent coloration of normal uninfected muscle, thereby producing the common name "porcelain disease" in Europe. Information on the life cycle of Thelohania is largely conjective (Cossins 1972; Johnson 1977). Many believe that there is only one host and that disease transmission occurs as a result of cannibalism of infected muscle from dead crayfish (Cossins 1972; Mazyles 1978). Preliminary work with O. virilis however, (L. Graham, U. of Man.) supports previous observations with A. astacus (Kai Westman, Finnish Game and Fisheries, pers. comm.) that strongly questions the generally accepted assumption of cannibalism etiology. Thelohania parasitism is an irreversible chronic disease, gradually developing over a time period of 5-6 months. Infected O. virilis died within 3 months of being held in the laboratory.

Due to the destruction of myofibrils, the contractile function of affected muscles is greatly reduced and muscular activity must emanate from adjacent unparasitized fibres (Cossins 1972). Carstairs (1978), Quilter (1976) and Cossins (1972) have all noted that individuals with advanced Thelohonia infection have a noticeably weaker tail flick than uninfected specimens. Comparative measurements of abdominal muscle contraction of uninfected O. virilis from L240 and L223 with parasitized L223 crayfish (T. Wiens, U. of Man., unpubl. data) have shown that the strength of muscular contraction was reduced ten-fold in crayfish infected with Thelohania. This total reduction of muscular power available to parasitized crayfish produces an ineffectual escape response, making such individuals more susceptible to fish predation.

Sumari and Westman (1969) found the maintenance of low prevalences of Thelohania parasitism of <1.0% in four Finnish watercourses over a four year period. Mazyles (1978) recorded that between 0.7-3.7% of the crayfish caught from separate Lithuanian lakes were infected, being dependent upon population density. Jones (1980) reported a 1.8% infection level in Lake Tupo, New Zealand. Reports of higher prevalences (6.5-30%) have been recorded in small streams in more temperate climates and probably reflect the much higher densities of crayfish in these habitats (Vey and Vago 1973; Carstairs 1978). Quilter (1976), however, has cautioned that because the musculature of infected individuals becomes white only in the late stages when the parasite is mature and dispersed, crayfish with a light attack would have been classed as healthy. As a result, all infection rates, including those in the present study, are probably underestimates. For example, O'Keefe and Reynolds (1981) found that 0.7-1.2% of Irish A. pallipes lake populations displayed white abdomens characteristic of Thelohania parasitism. Further microscopic examination revealed that 40% of diseased crayfish went undiscovered by macroscopic screening.

The etiology of the disease is not known. Cossins (1973) has suggested that different water conditions may enhance or disrupt the spread of the parasite depending on such factors as water flow, crayfish density, and the extent of cannibalism. Present ELA data are comparable to the climatically and limnologically similar areas of Finland and Lithuania where prevalence of Thelohania is generally very low, possibly as a result of low crayfish densities in such lakes.

The higher prevalence of parasitism by Thelohania sp. in the L223 population compared with the control lakes suggests a number of hypotheses. First and most simply, if as was previously believed, the disease is spread via cannibalism, then it is possible that the general reduction in exoskeleton rigidity observed in L223 has allowed for an abnormally high degree of cannibalism to take place. Bretonne et al. (1969) have found a higher mortality rate in softwater pools than in those with higher water hardness, suggesting an elevated rate of cannibalism among the softer shelled crayfish.

Second, it is possible that the increased acidity in L223 has in some way been beneficial to the life cycle of Thelohania. The life cycles of certain fish parasites are known to be affected by pH in that they require acid environments for successful reproduction or attachment to the host (EIFAC 1969). There is some confusion as to how long the pansporoblast membrane remains around the Thelohania spores following their release wlth the death of the host (Quilter 1976). It is possible that under conditions of lowered pH the spores, either singly or still within the pansporoblast, are able to remain viable for much longer periods of time before they are picked up by another receptive host.

Third, little is known about how different environmental changes may enhance this infection in the host. Organisms that are weakened by sublethal exposure to environmental perturbations are considered to be under physiological stress which will increase their susceptibility to pathogenic infections. Such individuals have to exert more energy to maintain normal body homeostasis at the expense of resources needed to combat disease. Knittel (1980) found that experimental exposure of fish to sublethal concentrations of heavy metals will decrease their inflammatory response to bacterial infection. The aggravating effect of stress from various types of pollution such as eutrophication, raw sewage wastes, industrial chemicals, and pesticides causes higher levels of infectious diseases in fish compared to those living in unpolluted habitats (Snuesyko 1974). It has long been known in fish farming practise that low pH increases the susceptibility of fish to disease, for example, below a pH of 5.5 carp develop a hypersensitivity to bacteria (Ness _in_ EIFAC 1969). The discharge of heavy metals into a lake raised the percentage of _P. lenisculus_ infected with the crayfish fungal plague (_Aphanomyces_) from 5-11% to over 60% (Magnus Furst, pers. comm.). Vey and Vago (1972) have observed a cellular resistance reaction of host crayfish to _Thelohania_ consisting of the formation of protective capsules around the parasite spores. Sogandares-Bernal (1962) has suggested that certain crayfish may be permanent carriers of Thelohania, occasionally relapsing under adverse conditions of environmental or nutritional stress. Preliminary data also implies higher incidences of Thelohania parasitism in California (D. McGriff, pers. comm.) and Britian (C. O'Keefe, pers. comm.) possibly coincident with industrial contamination.

2.4.5 Mortality Rates

There are few data on the annual instantaneous mortality rates (Z) of decapod crustaceans. The annual mortality rate of the long-lived (12 yr) P. leniusculus population in Lake Tahoe was estimated to be 0.118-0.324 (Flint 1975b). Morrissy (1975) calculated a Z value of 1.095 for a Cherax tenuimanus population with a similar life span (3-4 yr) to O. virilis. Estimates of mortality rates for several O. virilis populations in Michigan (Momot 1967; Momot and Gowing 1977a) and N.W. Ontario (Momot 1978) have been made. The rate of mortality was always highest in the first summer (mean Z of 2.56-3.03), remained quite high during the age I year, declined in the third year (age II) and abruptly increased during the age III year. These rates showed considerable variation in response to changes in crayfish density from one year to the next. Without exception, the Z values for ELA O. virilis populations are substantially lower than those calculated for the more productive and faster-growing southern populations. This inverse relationship between growth and survivorship is well-known and has been demonstrated to occur in other crayfish species (Hopkins 1966).

The majority of the mortality of age I-IV *O. virilis* at ELA occurs almost exclusively during the summer period. High mortalities during molting periods and the subsequent sudden decreases in abundance of certain age groups has been reported in other species (Van Deventer 1937). These deaths occurred with the attainment of sexual maturity or at the close of the reproductive season among adults near maximum size, in agreement with the pattern of summer mortalities shown in the L223 population. The periodic mortality patterns caused by molting probably reflect internal physiological or mechanical origins rather than external causes such as predation, disease or starvation. *Orconectes* less than 1.5 yr old are consumed by trout and perch (Reid 1962; Gowing and Momot 1979). The present study calculated survival rates based on truncated distributions older than age I, so differences in species and quantity of fish between lakes would not greatly influence these estimates. It is possible that the lower survival rates in L223, especially among the older age III and IV crayfish, reflects increased *Thelohania* parasitism or enhanced predation and cannibalism pressures due to softer exoskeletons.

2.4.6 Reproduction

The reproductive efficiency of crayfish is generally considered to be low (Mason 1977). The number of ovarian eggs in *O. virilis* was always higher than the number of embryos successfully extruded and attached to the pleopods the following spring. The ovarian egg count represents a measure of the potential reproductive capacity of crayfish whereas counts of pleopod eggs provide an estimate of the realization of this potential, i.e. the net or ecological fecundity. The mean disparity between the number of ovary and pleopod eggs for *O. virilis* in ELA control lakes was about 20%, similar to values of 20-22% recorded for *O. limosus* (Kossakowski 1975) and *P. leniscu1us* (Flint 1975b) but less than levels of 20-50% documented for dense populations of *P. leniscu1us* (Mason 1974) and *O. virilis* (Momot and Gowing 1977a) depending on the relative severity of density-dependent feedback mechanisms of control. The larger correlation coefficients of ovarian, rather than pleopod eggs to carapace length signifies that, following ovarian maturation and prior to hatching, the eggs are subjected to a variety of stresses that increase the variability of carried eggs.

The magnitude of discrepancy between the potential and realized fecundities reflects the general physiological state of crayfish with reference to the intensity of adverse environmental factors. The two-fold increase in disparity in L223 crayfish egg counts is the result of a much higher proportion of ovigerous females with a reduced number of attached eggs, the severity of which is correlated with increasing lake acidification. Despite the vast number of crayfish life history studies that have monitored fecundity, less than 5 percent of these have made reference to the collection of ovigerous females with abnormally low numbers of pleopod eggs.

Discrepancies between ovarian and pleopod egg counts can be attributed to 1) an incomplete extrusion due to some disturbance during spawning, 2) a lack of all eggs to be fertilized at oviposition, 3) a failure of the extruded eggs to become securely attached to the maternal pleopods, 4) egg mortality, or 5) other causes such as dislocation of eggs due to physical contact with environmental obstacles or cannibalism by the brooding female. The occurrence of one of these mechanisms does not preclude the synergistic operation of any of the remaining factors.

2.4.6.1 Egg Resorption

Beamish et al. (1975) proposed that a failure of normal Ca^{++} metabolism causing mature fish to resorb unlaid oocytes may be the primary cause for the observed extinction of fish populations in several Ontario lakes. Internal degeneration-reabsorption of ovarian eggs and subsequent mobilization of yolk substances into the blood can occur for crayfish held under unfavorable laboratory conditions (Andrews 1906; Penn 1943; Mason 1970a) including *O. virilis* (Stephens 1952). Atresia has also been shown to occur in natural populations of crayfish subjected to physiologically stressful conditions (Vey 1977; Morrissy 1975).

Although more crayfish in L223 resorbed ovarian eggs than the number of females in the control lakes (29.4% compared to 10.0%), consideration must be given to the magnitude of this resorption with respect to the number of successfully extruded eggs. Atresia is therefore of little ecological importance influencing crayfish recruitment in acid environments, increasing the number of unlaid eggs from 0.4% in control situations to 1.3% in L223. The lack of correlation between extent of egg resorption and condition of attached eggs indicates that pleopod egg loss and mortality occur independently of atresia.

Lake 223 crayfish experiencing difficulties in normal Ca^{++} metabolism have not reached internal Ca^{++} levels low enough to jeopardize the formation of eggs nor to sacrifice their eggs and resorb the Ca^{++} into the hemolymph as a buffering agent to maintain body homeostasis. Instead it appears that the opposite has occurred. Collection of crayfish from L223 during autumn 1981 at a lake mean pH of 5.05 showed that females with developing ovaries had lower carapace rigidities than males, despite the fact that the females had undergone their molt three months before whereas the males had only molted less than a month and a half prior to sampling. This suggests that crayfish in acid environments will attempt to form and develop their full complement of eggs with Ca^{++} obtained at the expense of exoskeleton hardness via processes of internal carapace breakdown.

Contrary to previous field life history investigations (Penn 1943; Weagle and Ozburn 1972) this study supports the physiological laboratory evidence of Stephens (1952) that egg-laying and oocyte-yolk resorption in crayfish are not mutually exclusive processes but are closely associated simultaneous events.

2.4.6.2 Fertilization

Inhibition of mechanisms involved with fertilization, gametogenesis, and mobility and viability of sperm have been suggested as possible factors contributing to decreased fish reproduction in acid waters. Aiken and Waddy (in Ennis 1980) have demonstrated that mature female lobsters sometimes lay unfertilized eggs which are lost soon afterwards. Mating pairs of O. virilis in L223 were observed in fall 1980 at a lake mean pH of 5.35, during spring 1981 (contrary to Weagle and Ozburn's (1972) assumption of no spring copulation for this species in N.W. Ontario) and again in fall 1981 at a lake pH of 5.05. Formation of sperm plugs throughout the fall and winter and their subsequent complete absence in ovigerous females collected the following spring implies successful copulation. Furthermore, the percentage of mature females that were fertilized in L223 is what would be expected considering the growth rate of crayfish in that lake (A.5.5). The relative fecundity of successfully reproducing L223 crayfish and knowledge that summation of eggs and empty capsules projected the total potential fecundity of unsuccessful females to a value expected in relation to their carapace length indicates that pH values as low as 5.0 do not elicit effects on egg fertilization that could detrimentally influence crayfish reproduction.

2.4.6.3 Egg Attachment

Oviposition in crayfish has been closely examined. All studies report little, if any, loss of eggs during this period (Andrews 1904, 1906; Cheung 1966; Mason 1970a). Laboratory studies have shown that only if the female is physically disturbed will there be a significant loss of eggs before the binding-cement has completely hardened (Ebrenbarum *in* Herrick 1896; Andrews 1904; Scott *in* Yonge 1937; Woodland 1967). The most dramatic affect of lake acidification upon *O. virilis* reproduction has been the failure of secure attachment of pleopod eggs in L223 females. This has resulted in a much higher proportion of individuals with only a partial complement of eggs compared to crayfish from control lakes.

Lereboullet (1860) and Andrews (1904) found that for up to 12 hr following oviposition, the eggs may be easily removed from the pleopods as they are held solely by a viscous, mucoid secretion referred to as glair. Later, after hardening, considerable force is needed to sever the egg-stalks from the setal hairs. The funiculus or egg-stalk begins as a flat, exceedingly delicate band of material and with subsequent hardening, becomes a narrow, twisted stalk of great contractile strength, forming a thick elastic outer case around the developing egg. The funiculus has great elasticity, so that after it is stretched out to four or five times its normal length, it contracts back to its original length like a rubber band when released. Great effort is normally required to separate it when pulled (Andrews 1906, 1907). Yonge (1946) states: "This elasticity (of the outer membrane)...in the case of the eggs has biological significance. The outer membrane attaches the eggs to the pleopods in the decapod Crustacea, but it also protects them and has to withstand the pressure of adjacent eggs and the effort of the constant beating of the pleopods needed to produce the respiratory current around the developing mass of eggs. In this connection its elasticity as well as its firm consistency will be of real value. Moreover, during development the egg increases in size, becoming oval with the long axis 50% greater than the original diameter...Certainly the outer membrane could not remain around the developing egg securing it to the pleopods and protecting it were it not capable of stretching greatly". The demonstrated ease in which L223 eggs can be removed with one uniform pass of a blunt probe over the abdomen (54.1 ± 5.9% of total brood, significantly higher than values of 27.8 ± 6.4 and 30.5 ± 7.0 for control crayfish) is the result of a loss of this crutial property of elasticity. When viewed closely under a microscope the clear, hardened egg-stalk ligaments of crayfish from control lakes with normal attachment and number of eggs, can stretch greatly and display a great resilience when released. The amber-coloured and soft, amorphous L223 egg-stalk material, in contrast, spreads apart effortlessly when pulled, producing large gaps and severing easily. Because of its lack of firm consistency, no resilience is demonstrated upon release.

Lereboullet (1860) was the first to link the coagulation and hardening of the glair-cement and subsequent adherence of the eggs to the pleopod setae to some chemical interaction with the water. Later, Cano (in Herrick 1896) stated that: "It would seem that the water might explain the chemical change which the cement undergoes, a change analogous to that observed in the exoskeleton after the moult...". Yonge (1937) and Lang and Yonge (1935) found that the outer egg membrane and binding cement have properties indistinguishable from those of the cuticle. The success of egg attachment is dependent on the favorable physical properties of the cement which resemble those of the cuticle: low surface tension, slow solidification in water, final hardness, and limited permeability (Yonge 1936). The processes involved with the formation of decapod egg membranes are merely modifications of those present in the carapace following ecydsis (Yonge 1932; Lloyd and Yonge 1940).

The lack of structural resilience, subsequent failure of secure attachment, and loss of pleopod eggs from crayfish in L223 reflect an incomplete cuticular hardening of the glair-cement forming the egg capsule membrane and funiculus. In addition to the obvious physical differences displayed between egg attachment in L223 and control lakes, further evidence is supplied by the coloration of the substances involved. Previous life history investigations in the laboratory have revealed that the egg-stalk material becomes a clear highly-refractive sheet in its final hardened state (Andrews 1906, 1907; Yonge 1932, 1946; Burkenroad 1947; Cheung 1966; Hopkins 1967), a condition consistent with that observed in solidified cuticle (Yonge 1932; Dennell 1947) and in crayfish from ELA control lakes. Incompletely hardened, newly-formed cuticle, however, is amber-coloured, similar to the empty egg capsules observed on L223 crayfish. This is a result of the presence of orthoquinone - the coloured oxidation product of a polyphenol closely associated with cuticular phenolic tanning (Yonge 1932; Dennell 1947).

Mature L223 and L240 females with obvious cement glands and sperm plugs were collected in fall 1980 and held in control L239 water over the winter until oviposition occurred in April 1981. In all cases, there was no difference in the structural characteristics of the glair-cement in L223 and L240 crayfish. Eggs were healthy and firmly attached to the pleopods with no indication of egg loss, mortality or resorption. Clearly then, unhardened glair-cement is not a normal characteristic of L223 crayfish and must therefore be attributed to some factor(s) associated with the lake acidification experiment.

Yonge (1932, 1946) investigated the hardening of decapod cuticle and found it to be sensitive to acids, which caused a substantial loss of rigidity. The cuticle and egg glair-cement reach their lowest permeability at pH 5.2, their iso-electric point (Lang and Yonge 1935). Malley (1980) showed that the uptake of Ca^{++} by postmolt ELA O. virilis was inhibited below pH 5.75 resulting in incomplete exoskeleton hardening, a result verified by field investigation of the L223 population. It is probable that a somewhat similar process involving cuticular phenolic hardening and calcification has prevented a secure egg attachment in L223 crayfish. Ovigerous females of A. astacus from acidified Swedish waters have been noted to show a similar condition (Magnus Appelberg, pers. comm.) indicating that the phenomenon is a general response of crayfish to acidification. Similar processes may affect other crustacean species that carry external egg masses, if exposed to anthropogenic acidification. There is some evidence to suggest a progressive reduction in the number of eggs carried by diaptomids in increasing acidic lakes (Roen 1955).

The loss of eggs from their capsules represents a serious reproductive impairment because such eggs would never develop due to their dependence upon maternal pleopod activity to ensure constant aeration and protection from fouling (Andrews 1906). There are several lines of evidence which indicate that the presence of empty capsules during brooding is an abnormal condition and does not merely represent a successful production and release of independent young. Immediately following oviposition any shreds of glair that remain are soon removed by the grooming actions of the maternal female (Andrews 1904, 1906; Cheung 1966; Mason 1970b; Ingle and Thomas 1974). The rate of egg development has not been altered in L223 and the condition of the remaining eggs indicated that the time to hatching was always distant. Although a small loss of ova may occur during spawning at the stage of fertilization and oviposition, under normal circumstances there is little loss thereafter during the brooding period such that there is no detectable difference in the number of eggs per female between the egg and attached young developmental stages (Morrissy 1970, 1975; Rhoades and Holdrich 1982). Considerable evidence indicates that following hatching the dependent young immediately begin to consume their empty egg capsules and stalks as the first source of nourishment and that under normal circumstances the maternal pleopods show only small traces of egg capsule stems after the successful departure of the last dependent young (Andrews 1904, 1907; Mason 1970b). Alternatively, if the female has lost her young due to unknown causes or had them artificially removed and separated from her, only then will the ruptured egg capsules be retained (Mason 1970b, 1977). Woodland (1967) recorded a mean percentage loss of eggs as measured by empty capsules of 11.48 ± 2.12 (S.E.) in a very dense population of C. destructor due to intraspecific-related mechanical damage to the broods.

2.4.6.4 Egg Mortality

The eggs of decapods held within the laboratory are susceptible to fungal infection and eventual mortality (Herrick 1896; Andrews 1904, 1907; Mason 1977; Vey 1977). In contrast, there are few reports of egg mortality in unstressed natural populations of crayfish (Payne 1978; Rhoades and Holdich 1982). Enhanced egg mortality in decapod populations has been attributed to stressful environmental conditions such as great quantities of suspended sediment (Vey 1977), dense overcrowding (Woodland 1967), or nutrient-enriched polluted water (Fisher and Wickham 1976, 1977). The percentage of L223 females with dead eggs ranged from 6.9% to 16.9% during the study period compared to an absence of this condition in the control populations.

Several observations suggest that fungal attack occurs only as a result of mechanical damage to the developing crayfish eggs (Payne 1978; Woodland 1967). Work on the dungeness crab, however, implicates the fouling of eggs by epibiotic microorganisms as the cause the mortality, possibly by the restriction of gas exchange across the outer egg membrane (Fisher 1975). Increased prevalence of parasitic infection has been demonstrated for adult _O. virilis_ in L223, and it is possible that acidification has also promoted fungal egg infections. The discrete groupings of heavily affected egg clusters amid the total egg brood is in accordance with Woodland's (1967) observation that fungus does not spread to adjacent viable eggs. However, the much more common occurrence of low incidences of dead eggs without obvious signs of abnormal coverings, suggests that fungal infection is probably the consequence, rather than the direct cause, of the elevated egg mortalities in L223. Appelberg (1981) recorded a sublethal decrease in O_2 consumption in _A. astacus_ eggs exposed to water below pH 5.0. The hypothesis that egg mortality could be the result of a pH-induced behavioral modification of the maternal pleopod beating needed to oxygenate the developing eggs was examined in the laboratory and dismissed (3.A.3.3). The cause(s) and mechanism(s) of _O. virilis_ egg mortality as related to lake acidification are not known at this time.

Several decapod studies have indicated that infertile eggs are easily lost (Payne 1972), leaving only the ruptured empty egg cases and stalks remaining attached to the pleopods, perhaps for weeks (Woodland 1967; Fisher and Wickham 1976). It could then be argued that the elevated number of empty egg capsules present in L223 crayfish are simply the remnants of infertile or moribund eggs that had been lost previously. There are several observations however that indicate the mechanisms of egg mortality and egg loss in L223 crayfish are distinct. Although the occurrence of either of these conditions are not mutually exclusive, there is no evidence to suggest they are collaborative. Crayfish with a full complement of securely fastened eggs and no empty capsules still carry dead eggs whereas, alternatively, the remaining eggs of crayfish with many empty capsules are often all healthy. The fact that moribund eggs, whose small size indicates an early death, were successfully carried by females throughout the normal course of development of the remaining healthy eggs,indicates that they are not preferentially lost. Observations of the strength of egg attachment under a microscope showed no qualitative difference in the affixation of either live or dead eggs from the same female. This is supportive of Vey (1977) who also found that abortive eggs in an environmentally-stressed crayfish population still remained firmly attached to the abdomen of the female. This strengthens the argument that loss of eggs in L223 crayfish occurs as a result of incomplete hardening of the membrane and not merely a jettison of dead eggs.

2.4.6.5 Rate of Development

Borgstrom and Hendrey (1976) found that pH levels below 5.0 delayed the hatching of Lepidurus arcticus. The influence of low pH upon rate of egg development for crustaceans has not previously been monitored in the field. The present study indicates that the progression of O. virilis embryos through 7 developmental stages has not been altered with lake acidification to pH values between 5.05-5.35. Consequently, the basic parameters of crayfish egg quality (i.e. size and weight) have not been affected by L223 acidification. This is important because the survival ability or fitness of benthic invertebrates is often correlated with egg size (Thorson 1950). Balasunadarum and Pandian (1982) have recently proposed that egg loss in crustaceans could be the result of volumetric expansion of the eggs due to inhibition at the initial period of incubation. The resulting "struggle for space" among the developing eggs severs those less securely attached during the fanning movements of the pleopods. The fact that the size and weight of the remaining eggs in L223 crayfish are the same as those in the control lakes implies that a differential inhibition of water by L223 eggs cannot be suggested as the mechanism of increased recruitment failure in that lake.

2.4.7 Implication of L223 Results for the Survival of Crayfish Populations Exposed to Acidification

The present study suggests that the loss of crayfish populations to lake acidification will be brought about by reproductive impairment and possibly by increased susceptibility to parasitic infection long before those acid levels are reached that result in direct toxic damage to crayfish stocks.

The experimental acidification of L223 during 1979-81 has increased the population reproductive impairment 5-10 fold over background levels for the ELA region. The failure of secure attachment and subsequent loss of pleopod eggs due to incomplete cuticular hardening of the glair-cement accounted for 85-98% of this impairment. The extreme sensitivity of crayfish reproduction long before hatching is reached in late June, poses a serious threat to the continued sustenance of populations. Oviposition in Orconectes species occurs in mid-April, when the lake temperatures approximate 7°C (Berrill 1977), a time coincident with the spring melting of snow and severe pH depression in receiving waters (Jeffries et al. 1979). Several acid-sensitive fish species on the other hand spawn much later in the spring, after the occurrence of this pH shock, suggesting that crayfish may be more vulnerable to acidification than these fish. The present work verifies the tentative hypothesis of Furst (1977b) that it will be the sensitivity of reproduction to pH, particularly during the egg-laying and brooding stages, that will determine the long-term survival of crayfish populations exposed to lake acidification.

Indirect evidence from Swedish survey data supports this conclusion. Not only does the number of lakes with crayfish decrease below pH 6.0, but the fishing yield within these lakes is only 1/5 that for the circumneutral basins (Svardson 1974). Only one-half as many acid lakes have *A. astacus* yields of at least 60 crayfish per hectare compared to those levels obtained within basic lakes. This lead Svardson to conclude that the crayfish yields have decreased in response to acidification.

Population reproductive failure as measured in this study reflects only the decrease in viable eggs prior to hatching; the detailed effects of low pH upon *O. virilis* hatching are not known. Work by Furst (1977b) suggests that the successful hatching of *A. astacus* eggs is impaired below pH 5.6. Following hatching, the S-I young normally remain firmly connected to the maternal female by an elastic telson thread originating from the egg membrane that previously enclosed the embryo (Andrews 1907; Mason 1970b). Preliminary experiments with *O. virilis* hatching in low pH water indicated a lack of secure attachment, so that the newly-hatched young fell to the bottom of the aquaria (D. Malley, unpubl. data). Andrews (1907) stated that, "as it is some time before the respiratory movements become perfect, as the limbs only gradually acquire the ability to move, and as the body is globose and the creature cannot stand on its legs, the larvae would probably perish but for this temporary fastening of the egg stalk". If these additional stresses are experienced by the L223 population, the reproductive failure would, of course, be much higher.

Assuming a good knowledge and general confidence in the sampling methodology utilized, the resultant age composition data reflect the sum of all the dynamic forces acting upon the population and may therefore serve as the final measure of insight into the consequences of perturbative pressures. Analyses of population structure and whole-lake population estimates (Davies, in prep.) indicate that these reproductive stresses have in fact contributed to a recruitment failure and led to imbalances and decreases in the size of the L223 O. virilis population. The similarity of the age composition structure between L224 and L223 populations at the spring 1979 collection in this study, suggests that the recruitment of the L223 population was not reduced by acidification prior to that date. It is therefore recommended that to ensure the long-term survival of O. virilis populations, a "safe" limit be set of no lower than about pH 5.8; continued acidification to pH 5.05-5.60 can cause substantial failures in recruitment which may ultimately result in the local decline of the crayfish population. This conclusion is in agreement with European data that indicates the species A. astacus is seldom found in Swedish waters with a pH below 5.5-6.0 (Svardson 1974; Appelberg 1980; Almer et al. 1978) or below pH 6.0 in Denmark and Norway (Larsen; Munthe-Kaas in Abrahamsson 1972a) and that the failure of reproduction and disappearance of this species occurs with lake acidification to about pH 5.6 (Abrahamsson 1972a).

CHAPTER III

EXPERIMENTAL RESPONSE OF THE CRAYFISH *ORCONECTES VIRILIS* (HAGEN) TO LOW PH

III-A

LIFE CYCLE

3-A.1 INTRODUCTION

Despite the wealth of toxicological studies conducted on crayfish with regard to a wide variety of perturbations, most have utilized short-term static bioassays involving only adult organisms. The toxicological responses of fish life cycle stages to the effects of low pH have been extensively studied (as reviewed by Spry et al. 1981) but no comparative work has been undertaken with invertebrates. The purpose of the survival experiments was: 1) to determine how the low pH tolerance of O. virilis changes throughout its life history, 2) to measure the acid sensitivity of this species for comparison with invertebrate and fish species, and 3) to investigate the ability of O. virilis to recover from acid stress.

In any study of toxicant stress upon crayfish recruitment it is essential that the maternal and embryonic responses to the experimental perturbation be separated. The abdominal appendages to which the developing eggs are attached in ovigerous females are involved in a rhythmic vibration pattern which ensures a current of water is circulated about the eggs, helping to keep them well-aerated and free of disease (Andrews 1904). When pleopod movement slows or stops, egg development may be delayed or halted (Suko 1956). Furst (1977b) found that when ovigerous A. astacus were placed in holding containers in a river of varying acidity, eggs failed to hatch at pH values below 5.2. Appelberg (1981) recorded an inhibition of O_2 uptake in A. astacus eggs with acidification below pH 5.0 in the laboratory. In acidified L223, 6.9-16.9% of the egg-bearing females experienced partial mortality of their broods at pH 5.1-5.4 (Section 2.3.4). The purpose of this experiment was to determine if this mortality could be the result of low pH-induced behavioral modification of the maternal female rather than a direct acid toxicity to the developing eggs.

3-A.2 MATERIALS AND METHODS

3-A.2.1 Tolerance of Life Cycle Stages

Intermolt adult (age II-III; 20-30 mm carapace length) and juvenile (age 2 1/2 mo; 10-14 mm C.L.) crayfish from control Lake 240 were collected during September 1980 with the aid of a diver-operated suction gun designed to prevent mechanical damage to the specimens (Davies and Ramsey in prep.) and held for 48 hr prior to testing in aerated aquaria containing L239 water. Young (age 2 wk; 5 mm C.L.) were raised from L240 ovigerous females which were held in the laboratory until they produced their hatchlings in July 1980. The hatchlings had left their maternal pleopods and were in independent stage III during the experiment. Only healthy individuals were used.

Young-hatchling and juvenile crayfish were tested in 3 L glass aquaria contained within a water bath to regulate temperature. Treatment sample size was n = 20 for the young and n = 15 (approximate equal numbers of each sex) for the juveniles. Tests for adults were conducted in 50 L Plexiglass® aquaria (65 x 85 cm) subdivided into 20 compartments with perforated PVC plastic sheeting which separated crayfish to prevent cannibalism yet ensured free mixing of water and allowed animals to crawl about on the vertical sides, increasing their area of movement. A translucent heavy plastic cover was used to prevent crayfish from escaping and to provide a diffuse light source. Ten (5 male, 5 female) adult L240 crayfish were exposed in each aquarium in addition to 10 animals from L223 to compare for development of acclimatization (cf. 3B.2.1).

Lake 239 water was acidified to the appropriate test pH with H_2SO_4 and vigorously aerated for 1 hr to ensure removal of all excess CO_2 (Table 14). The following pH values were used: for the young hatchling experiment; pH 6.8 (control), 5.5, 5.0, 4.5 and 4.0; for the juveniles, pH 6.8, 5.0, 4.5, 4.0, 3.5 and 3.0; and for the adults pH 3.5, 3.0, 2.6, 2.3 and 2.0. Control adults were held in a 10 L glass aquarium. An additional test of young-hatchlings at pH 3.0 was performed in a 2 L glass beaker.

A 12 L:12 D photoperiod and continuous aeration for the adults and periodic aeration (30 min each day) for the juveniles and young were provided to keep oxygen at saturation throughout the experiments. Temperature approximated field conditions at the time of each test and was between 19-23°C for the young, 17-22°C for the juveniles, and 14-18°C for the adults. To maintain a high metabolic rate comparable to that found in natural situations the following feeding regimes were applied: young crayfish were provided throughout the experiment with a thin layer of flocculent organic detritus covering most of the aquarium

Table 14. Ionic composition of L239 test water following experimental exposure of young crayfish for 4 days. Control water was pH 6.8. Values for TDN in ug L^{-1} and for other ions in mg L^{-1}.

Treatment	TDN	Cl	SO_4	NA	K	Mg	Ca	Mn
control	993	1.4	5.8	1.55	1.44	0.90	3.47	<0.01
5.5	967	2.2	11.4	1.94	2.05	0.90	4.36	<0.01
5.0	913	1.8	18.6	1.93	1.62	0.90	3.69	<0.01
4.5	949	2.2	19.0	1.78	1.76	0.90	3.51	<0.01
4.0	876	1.4	21.0	1.72	1.30	0.92	3.24	<0.01

bottom and furnishing substrate as well as a food supply; juvenile crayfish were fed macerated Gainesburger® at 6 day intervals; and adults were fed to excess for 48 hr prior to testing but not during the test period itself. To remove metabolic waste products, seventy-five percent of the water was renewed with preacidified, aerated water at 4 day intervals for the young, 6 day intervals for the juveniles (following feeding), and at day 10 and 20 for the adults. Mortality and pH were checked at least six times daily during the first week and four times daily thereafter. Experiments were conducted 16, 24 and 28 day exposure periods for the young, juvenile, and adult life cycle stages, respectively. The ecologically more meaningful asympotic or incipient lethal levels, recommended by Sprague (1969) as being the single most useful criterion of toxicity, were emphasized. Periodic additions of acid were required to maintain each treatment pH. Values of pH never fluctuated more than ±0.1 units from the nominal levels. Behavior and carapace rigidity were observed and dead animals were removed, weighed, and measured.

The pH-mortality data were statistically analyzed by the log-probit transformation procedure of Litchfield (1949). Sprague (1969), in a comprehensive literature review, stressed the need for standardized statistical interpretation of toxicological information and strongly recommended this method. The dose-effect curve can be defined by its median lethal concentration (LC50) with its 95% confidence interval and slope function (S). This procedure is mathematically more conservative than other iterative computer techniques because it produces relatively larger confidence limits (Sprague 1969). To determine if two LC50's are statistically different, Litchfield's (1949) test for parallelism and potency ratios was performed. The incipient LC50 is defined as that level of environmental perturbation beyond which 50% of the population cannot live for an extended or indefinite period of time. i.e. the ultimate level at which the LC50 becomes effectively constant. Calculation and statistical testing of differences between median survival times (MST) for each pH treatment followed the similar graphic procedure presented in Litchfield and Wilconox (1949).

3-A.2.2 Recovery From Acid Stress

Intermolt juvenile crayfish (age 2 1/2 mo; 10-14 mm C.L.) were selected for this experiment because previous work had shown that the first symptoms of acid toxicity (ET50) for this life stage developed within 76-93% (mean 86%) of the time if takes them to expire (MST). Crayfish were collected with the suction gun from L240 in September 1980 and tested in 3 L glass aquaria with treated L239 water as described above.

Temperature was between 15-19°C throughout the test. Sample size was n = 10 for each treatment. Treatment pH levels 3.0 and 3.5 were chosen to provide both acute and chronic lethal acidities with MST's previously shown to be 3.15 and 9.20 days, respectively. Crayfish were exposed to each of these pH treatments until all individuals had died.

A set of replicate treatments was run in which, at the time of 50% mortality (MST), the remaining 5 juveniles were removed from the acid water and immediately transferred to control pH 6.8 conditions where recovery behaviour and/or mortality response was closely monitored. The experiment was run for 13 days.

3-A.2.3 Behavior of Ovigerous Females

Adult (age II-III; 20-30 mm C.L.) ovigerous females were collected from L240 during June 1980 with the suction gun. Organisms were held for 48 hr in holding aquaria and tests performed with L239 water treated as described above. Five crayfish displaying rhythmic pleopod beating of egg clusters were selected for experimental observation. Tests were performed in a 2 L glass beaker shielded on all sides except for an observation window in front of which was mounted a desk magnifying lens. Experiments were conducted in a secluded area to minimize external influences on crayfish behavior and extreme care taken at all times to not startle the individual being tested. Following placement inside the testing container the animal was allowed 30 min to adjust to the experimental conditions. Visual observations, timed with a stop watch, were then made of vibration frequency and the duration of vibration and interval (between beating) periods. Crayfish behavior was monitored for 15 cycles, which in most cases, amounted to 20-30 min. Following each test the organism was placed back into the holding aquarium and allowed to recover for about 4 hr before further testing at a different pH value.

3-A.3 RESULTS

3-A.3.1 Tolerance of Life Cycle Stages

Little mortality is suffered by the young-hatchling crayfish at pH values above 5.5 (Fig. 30). Acidification below this concentration caused progressively increasing mortality. A biphasic mortality response was evident for the pH 4.5 and 4.0 treatments with rapid mortality during the first 4 days of exposure followed by a plateau with little change in survival from day 4-7 and then a final period of rapid death. Acidity-induced mortality became apparent below pH 5.0 for juvenile *O. virilis* (Fig. 31). Again pH 4.5 and 4.0 treatments displayed a delayed or chronic effect with the highest mortality rates occurring during day 16-20 of exposure. Subjection of juveniles to pH 3.0 or 3.5 water resulted in a more immediate or acute response, with no survival after 6 or 12 days, respectively. Adult intermolt *O. virilis* displayed a high tolerance to acidity with complete survival for almost a month at pH 3.5 (Fig. 32). Chronic lethality occurred at pH 3.0 and a very acute response (all dead within 2 days) was observed in pH 2.0 water. Mortality may occur long after initial exposure with a delayed effect becoming apparent only following 10 days for pH 2.6 and 17 days for pH 3.0.

Crayfish of all life stages displayed a similar sequence of symptoms of impending mortality. As death approached, the animal would

Figure 30: Survival of age 2 week S-III hatchling *O. virilis* (5 mm carapace length) to depressed pH for 16 days. Control water was pH 6.8.

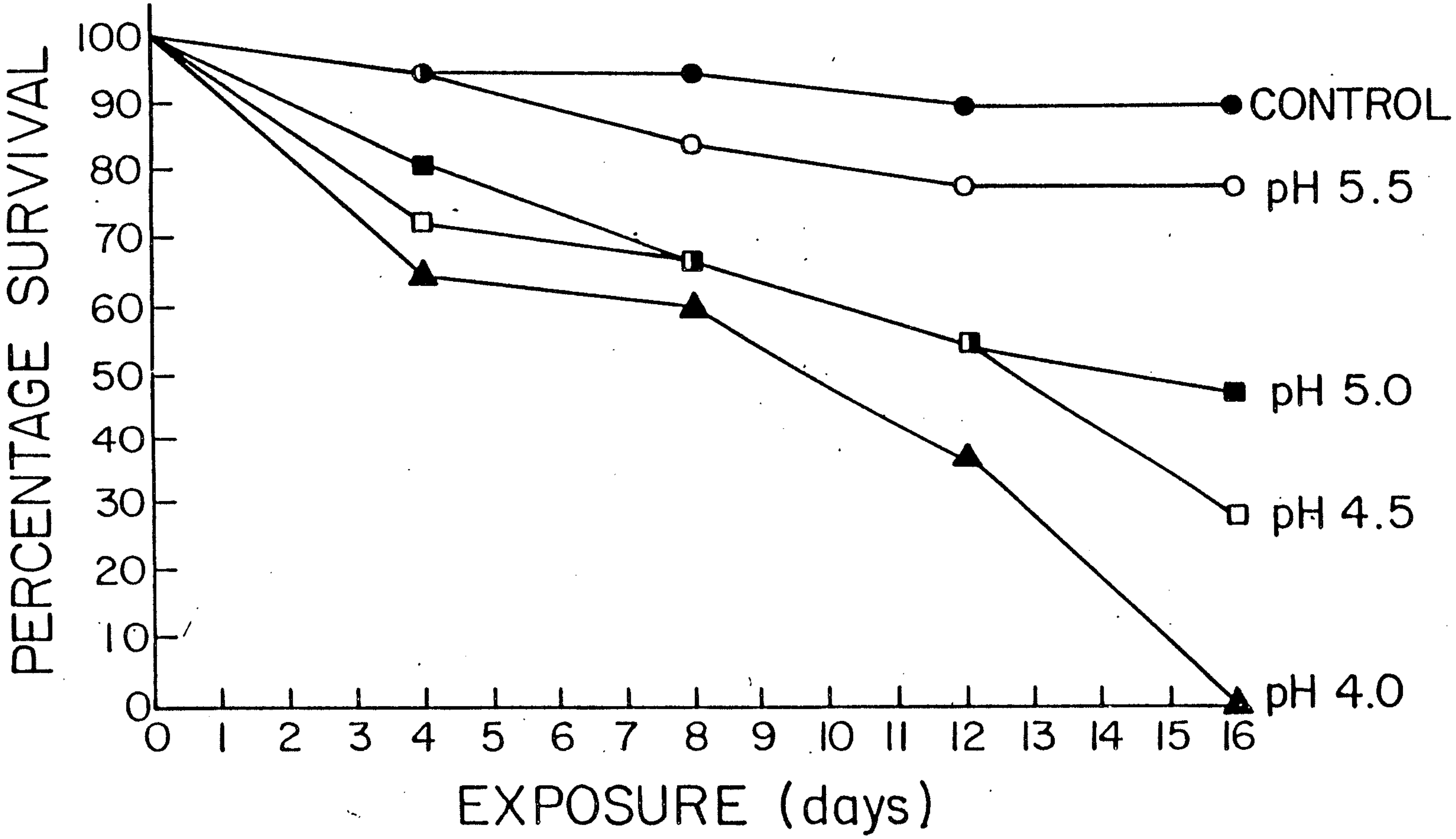

PERCENTAGE SURVIVAL
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
EXPOSURE (days)
CONTROL
pH 5.5
pH 5.0
pH 4.5
pH 4.0

Figure 31: Survival of 2 1/2 month old juvenile _O. virilis_ (10-13 mm carapace length) to depressed pH for 24 days. Control water was pH 6.8.

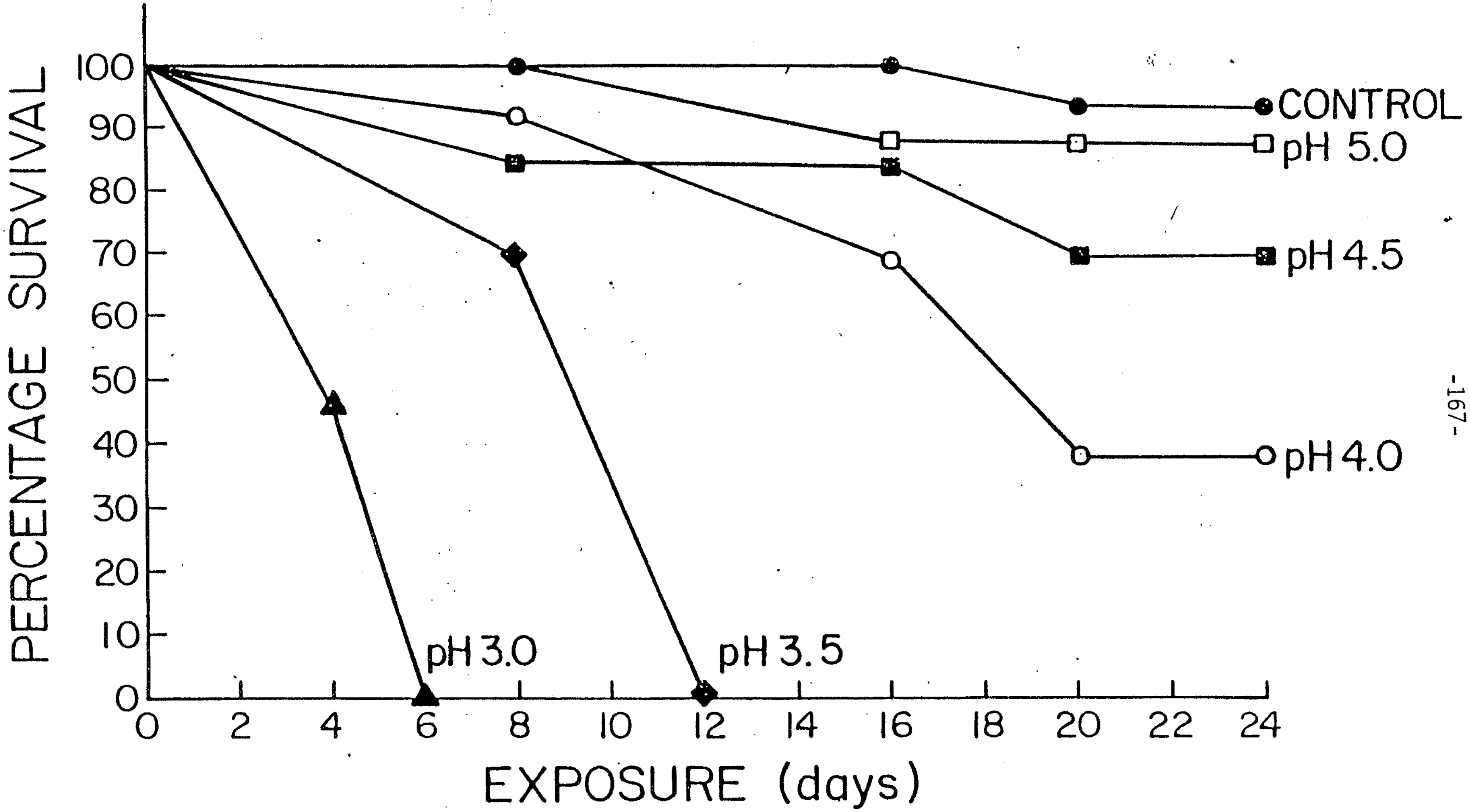
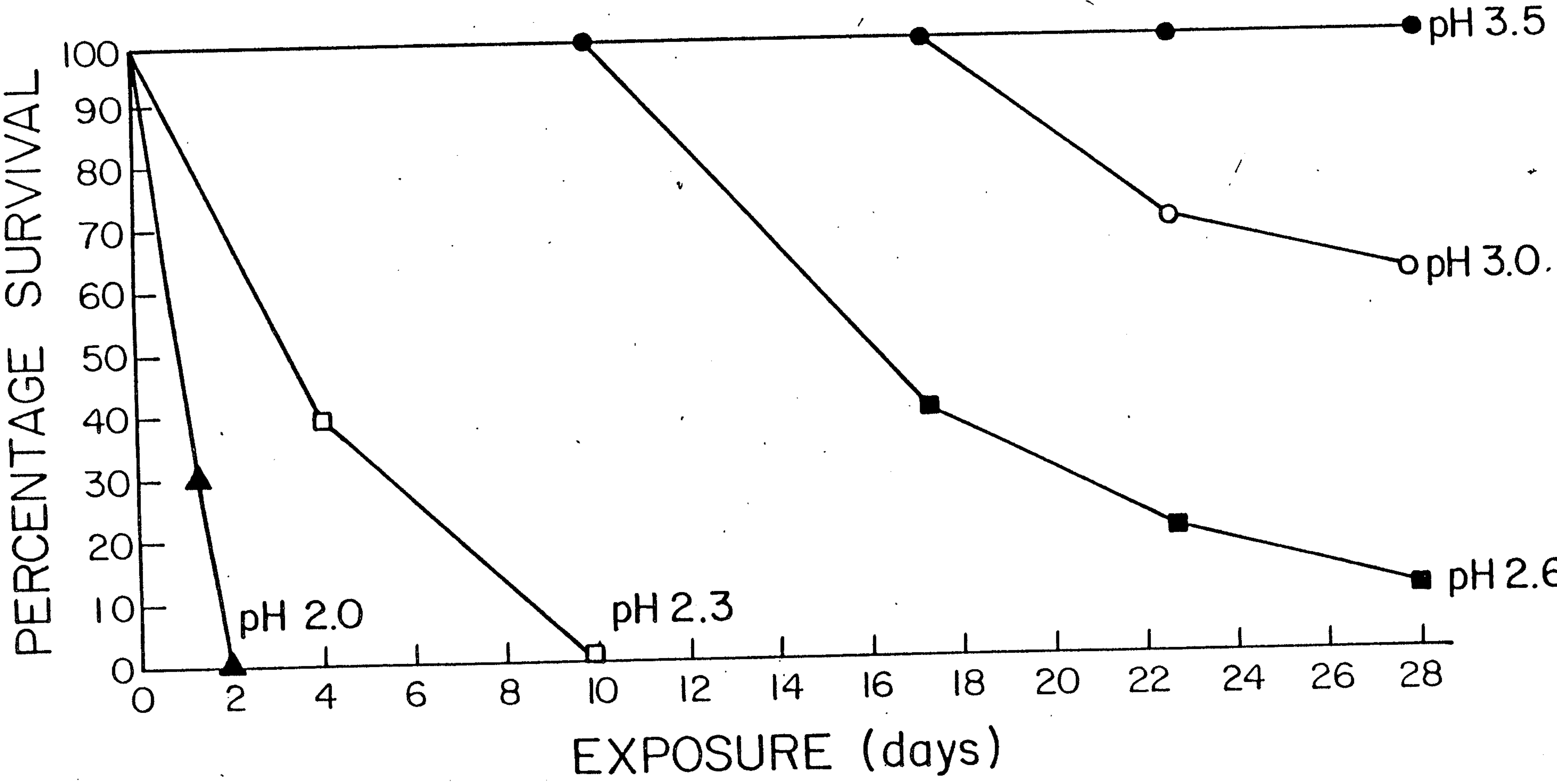

PERCENTAGE SURVIVAL
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
22
24
EXPOSURE (days)
CONTROL
pH 5.0
pH 4.5
pH 4.0
pH 3.0
pH 3.5

Figure 32: Survival of 2-3 year old adult intermolt *O. virilis* (20-30 mm carapace length) to depressed pH for 27 days. Control water was pH 6.8.


pH 3.5
pH 3.0
pH 2.6
pH 2.3
pH 2.0
PERCENTAGE SURVIVAL
0
10
20
30
40
50
60
70
80
90
100
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
EXPOSURE (days)

experience a loss of equilibrium, curling its abdomen underneath the thorax, and lying on either its side or back. Such moribund individuals were sluggish and unresponsive to stimulation with a probe, were incapable of an escape response, and once in this state, became susceptible to cannibalism by other less affected crayfish. Individuals demonstrated sublethal behavioral modifications to low pH before morbidity ensued. A decrease in general locomotory activity became evident and the young were slower to respond to probing. The fright reactions were slower and the distance travelled in attempting to escape was less than for other crayfish in the control aquarium, or the same individual at an earlier time in the treatment aquarium. Loss of equilibrium gradually became more pronounced with the young finishing their fright response on their side or back and having increasing difficulty in righting themselves.

A comparison of the relative tolerances between the three _O. virilis_ life stages to low pH is shown in Figs. 33 and Table 15. Because extreme pH depressions associated with spring melt or summer storm events are episodic in nature, some indication of the duration of resistance times is useful to enable predictions to be made about the fate of organisms during such periods. Each median survival time is statistically distinct ($p < .05$). Once crayfish become moribund they would vary in their long-term inability to compensate to the toxic stress and in the resulting speed at which death occurred. The median effective times (ET50) were calculated by the same procedure as that

Figure 33: Comparative survival of O. virilis life stages in continuous exposure to pH 3.0 water. Young = S-III hatchlings age 2 weeks, 5 mm carapace length (C.L.): Juvenile = young-of-the-year age 2 1/2 months, 10-13 mm C.L.; Adult = age 2-3 years, 20-30 mm C.L. Median survival times for young, juveniles, and adults were 0.66, 3.15, and 30.42 days respectively.

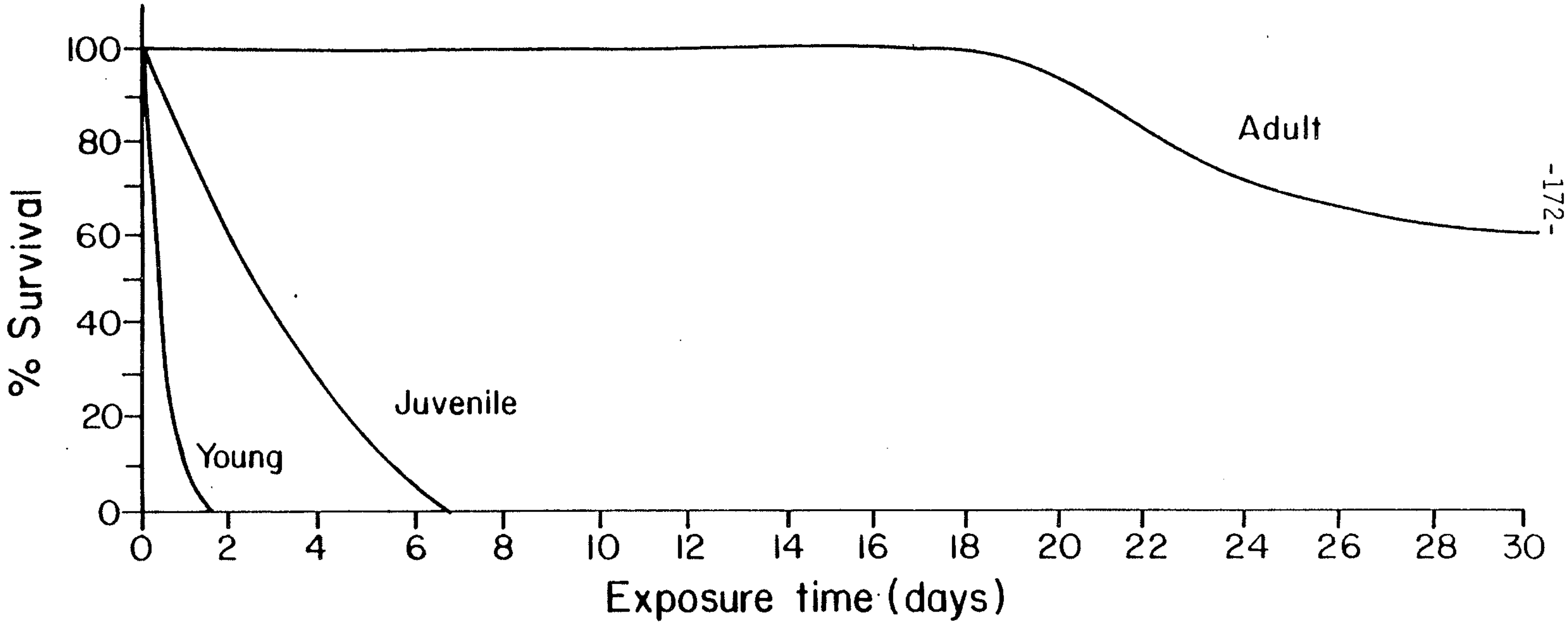

% Survival
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
Exposure time (days)
Adult
Juvenile
Young

Table 15. Median survival times (days) ± 95% C.I. following incipient exposure of *O. virilis* life stages to low pH water. Control water ws pH 6.8

Treatment pH	Young/hatchling	Juvenile	Adult
control	indefinite	indefinite	indefinite
5.5	52.08 ± 8.31	-	-
5.0	16.58 ± 4.80	indefinite(?)	-
4.5	12.83 ± 2.44	60.42 ± 8.56	-
4.0	10.00 ± 1.32	19.17 ± 3.45	-
3.5	--	9.20 ± 1.19	indefinite(?)
3.0	0.66 ± 0.25	3.15 ± 0.60	30.42 ± 9.13
2.6	-	-	15.00 ± 2.40
2.3	-	-	3.83 ± 2.11
2.0	-	-	1.17 ± 0.27

used for the MST values (Litchfield and Wilconox 1949). The adults reached their ET50's at 60-87% (mean 77%) of the time it took them to die, i.e. MST's; ET50's for juveniles were within 76-93% (mean 86%) of their MST values; and once the beginning symptoms of acid toxicity developed, the young would quickly expire, with ET50's 81-97% (mean 90%) of their MST values. Chi^2 testing showed that all eye-fitted LC50 lines provided a satisfactory fit to the experimental results (Fig. 34). For both adults and juveniles, mortality had stabilized by the end of the experiment but, for the young, exposure should have been continued for a few days longer until mortality had completely terminated so that, in this case, the actual incipient LC50 value would be slightly higher (Table 16). Analysis of potency ratios indicated that each LC50 line is statistically different from the other two. Development from a young 2 wk old SIII-hatchling stage to a 2 1/2 mo old juvenile stage, results in an increase of seven-fold in acid tolerance. Continued maturation to a 2-3 yr. old adult crayfish results in a further twenty-five fold increase in tolerance.

Despite exposure to lethal pH values, crayfish did not cease to feed. Detritus feeding by hatchlings was observed at pH 5.0 (day 2) and at pH 4.5 (day 7) and attempted cannibalism was recorded at pH 4.0 (day 2) and at pH 4.5 (day 9). Juveniles were seen to actively consume macerated Gainesburger® even at pH 3.0 (day 2) and at pH 4.0 (day 17) and they attempted to cannibalize moribund individuals at pH 4.0 (day 15). Adult survivors from pH 3.0 treatment would ingest macerated

Figure 34: Concentration-effect lines fitted by eye for calculation of incipient LC50 values for O. virilis exposed to depressed pH.

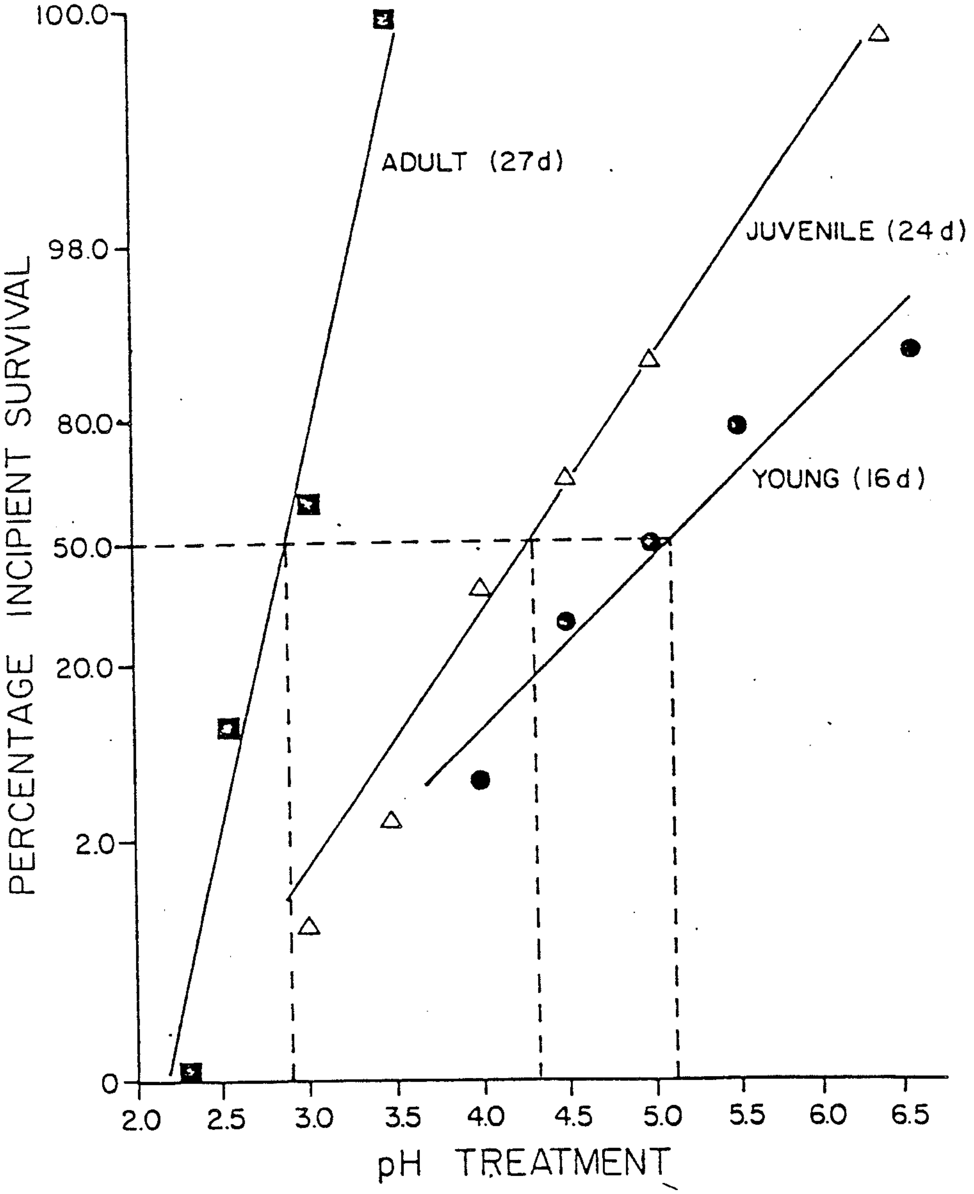

100.0
98.0
80.0
50.0
20.0
2.0
0
PERCENTAGE INCIPIENT SURVIVAL
ADULT (27d)
JUVENILE (24 d)
YOUNG (16 d)
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
pH TREATMENT

Table 16. Median lethal pH ± 95% C.I. and slope function(S) for O. virilis life cycle stages.

Life cycle stage	96 hr LC50		Incipient LC50		
adult	2.35 ± 0.13	S = 1.05	27 d	2.90 ± 0.15	S = 1.07
juvenile	2.95 ± 0.30	S = 1.20	24 d	4.25 ± 0.26	S = 1.15
young-hatchling	3.70 ± 0.34	S = 1.42	16 d[a]	5.15 ± 0.26	S = 1.19

[a] Mortality had not yet stabilized.

Gainesburger following the termination of the experiment (day 27).

Observations on the susceptibility of molting crayfish to low pH were inconclusive. A juvenile died during completion of a molt at pH 4.0 (25 hr) while successful moltings were recorded at pH 6.8 (day 4), pH 5.0 (day 5) and pH 4.0 (day 6). No adults molted during the course of the experiment. Adult carapace rigidity of either dead animals or those surviving after 27 days did not appear different from the pretreatment condition.

3-A.3.2 Recovery From Acid Stress

The data for cumulative mortality of juvenile crayfish in pH 3.0 and 3.5 water is in agreement with previous experimental results (Table 17). The mortality responses of both "transfer" treatments closely approximated those in the regular treatments of similar pH until the time of 50% mortality. The sequence of symptoms of impending mortality was characteristic of that observed in previous experiments.

Transfer of crayfish in the pH 3.0 - control treatment occurred at 48 hr of exposure. Of the remaining 5 juveniles, 3 showed a reduced degree of locomotory activity whereas 2 had lost equilibrium for almost 9 hr and were near death. Within 6 hr of transfer, all 5 crayfish were active and had regained their vigorous escape response. No mortality occurred during the rest of the experiment. In the pH 3.5 - control treatment the beginning symptoms of acid stress became evident at 65 hr with the first death at 78 hr and transfer of survivors to control water

Table 17. Recovery ability of juvenile crayfish from L240 held in control water following exposure to acute lethal pH until 50% mortality of test animals. Transfer of crayfish in the pH 3.0 to control treatment took place at 48 hr and in the pH 3.5 to control treatment at 78 hr. Control water was pH 6.8.

	Cumulative Mortality									
Treatment	20 hr	40 hr	60 hr	80 hr	100 hr	140 hr	180 hr	220 hr	260 hr	300 hr
control	0	0	0	0	0	0	0	0	1	1
pH 3.0	1	4	6	7	9	10	-	-	-	-
pH 3.5	0	0	0	1	1	2	6	9	10	-
pH 3.0 to control	2	4	5	5	5	5	5	5	5	5
pH 3.5 to control	0	0	0	1	2	3	5	5	5	5

at 172 hr. Although 4 of the remaining crayfish were in an advanced state of morbidity, within 6 hr of placement in control water, they had recovered. No mortality occurred for the remainder of the experiment.

3-A.3.3 Behavior of Ovigerous Females

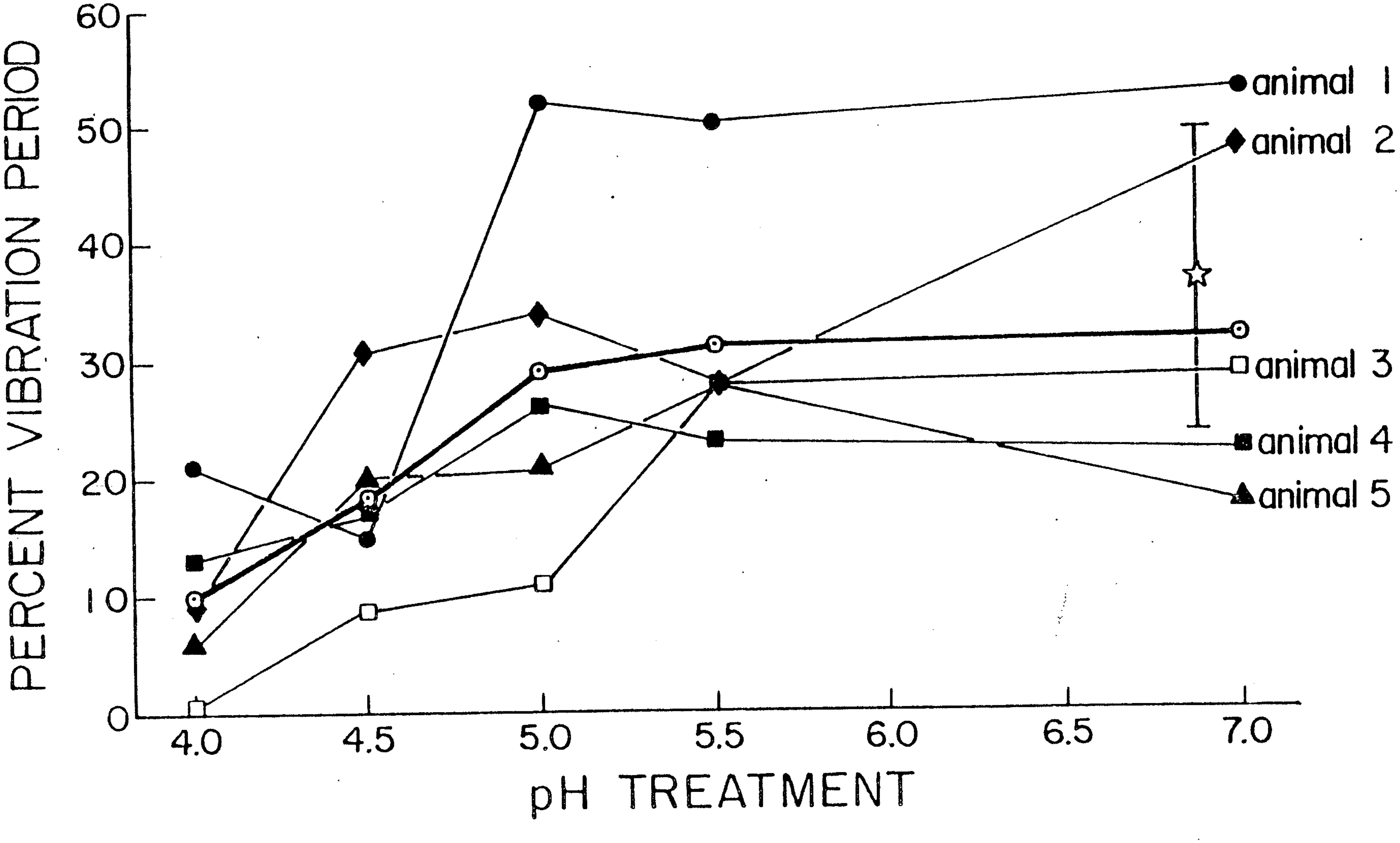
Observations of crayfish behavior within the large holding aquarium, both immediately after capture and two days later, indicated that little stress occurred when individuals were transferred to the testing vessel. Following the 30 min adjustment period after being placed in the testing vessel, individuals became stationary and settled into a set rhythmic pattern of pleopod oscillation. pH, over the range tested, had no effect on the vibration frequency or the duration of the vibration period (Table 18). No change occurred in the duration of the interval period with acidification to pH 5.0. Below this pH the length of time spent stationary with no pleopod beating increased with higher acidity so that a smaller percentage of the test time was spent by each crayfish in egg vibration.

Ovigerous females spent a variable amount of time in pleopod beating (Fig. 35) under control conditions. Acidification to pH 4.0 caused a decrease of about one-third in the total time spent vibrating for animals 1 and 2. Animal 3 experienced an almost complete cessation at pH 4.0. No change occurred with animal 4 and only a slight decrease in percent time vibrating for animal 5. The mean response showed that acidification from pH 7.0 to 5.0 had no effect on the pleopod beating of

Table 18. Influence of pH upon O. virilis pleopod egg vibration. Results of each treatment are means ±S.E. for 5 animals. Temperature was 17.3-20.5°C and oxygen at saturation for all tests. Control water was pH 6.8.

Treatment pH	Vibration velocity (times/min)	Vibration period (secs)	Interval period (secs)
control	69.7 ± 2.6	26 ± 4	60 ± 13
5.5	68.6 ± 3.4	35 ± 3	75 ± 8
5.0	81.1 ± 7.6	25 ± 8	62 ± 9
4.5	74.1 ± 8.7	24 ± 6	106 ± 13
4.0	62.4 ± 2.0	13 ± 3	138 ± 19

Figure 35: Behavioral response of pleopod oscillation of L240 ovigerous crayfish to depressed pH. Heavy line represents mean values. Star denotes mean ± S.D. of 10 animals in holding aquaria.


PERCENT VIBRATION PERIOD
60
50
40
30
20
10
0
4.0
4.5
5.0
5.5
6.0
6.5
7.0
pH TREATMENT
animal 1
animal 2
animal 3
animal 4
animal 5

female crayfish. Mean percentage of total time spent vibrating dropped from 30% at pH 5.0 to 18% at pH 4.5 and to 10% in pH 4.0 water. ANOVA testing found that individual crayfish differed significantly ($p < .001$) in the percent time spent vibrating the pleopods and that the mean vibration percentage was significantly different ($p < .001$) between pH treatments. Further analysis (LSD procedure) showed that vibration percentages at pH treatments 4.5 and 4.0 were significantly lower ($p < .05$) than those at treatments pH 5.0, 5.5 and 7.0 which were not different from each other.

3-A.4 DISCUSSION

3-A.4.1 Comparisons of Relative Tolerance

The susceptibility of the crayfish *O. virilis* to toxic levels of acidity is dependent upon its life cycle stage. Fifty percent mortality among newly hatched SIII young was reached with an exposure time about one-fifth that required for juveniles, which in turn was one-tenth that required for adults. If the pH sufficient to kill 50% of the adult crayfish over an extended period of time is accepted as a guide for setting water quality criteria, mortality of the entire juvenile sub-population would occur within 6 days and all the young would expire within a single day. The earlier developmental young stages (SI and SII) are probably even more sensitive.

Fewer than one-quarter of the crayfish toxicity tests reported in the literature have utilized exposures for prolonged periods of time or attempted to establish lethal thresholds. An important consideration gained from the present study is the need to extend the testing exposure period until treatment mortality has completely stabilized rather than choosing an arbitrary time for termination. The increase in LC50 from 96 hr to the incipient exposure level, as a result of prolonged mortality, substantiates the unreliability of using short-term experiments as a predictive tool in setting standards for water quality.

Laboratory studies have shown that the mode of toxicity for invertebrates, including crayfish, to low pH, is similar to that for fish - a failure to maintain internal ion homeostasis (Shaw 1960; Potts and Fryer 1979; McMahon and Morgan 1981). Acid disturbances may be compensated for in some invertebrates by a change in the relative rates of ion exchange. The characteristic biphasic mortality response of _O. virilis_, as a result of delayed mortality is probably due to the more robust individuals physiologically compensating, until, with time, the electrolyte regulatory mechanisms become inadequate and these crayfish too expire. The age-specific mortality of _O. virilis_ may be as much a function of general body size, as of a gradual increased degree of resistance attributed to physiological maturation. Robinson et al. (1976) showed that the survival time of brook trout is directly related to size at low pH. Small organisms have a greater body and gill surface area per unit weight than do larger animals, which allows detrimental ion fluxes to proceed more rapidly.

Table 19 summarizes literature values of LC50 calculated from

Table 19. Comparison of acute 96 hr and incipient acid toxicity between aquatic organisms.

	ORGANISM	pH Test Range	Duration	Type of Acid	LC50	REMARKS	REFERENCE
Crayfish	*O. virilis* Adult Juvenile Hatchling	2.0 - 2.5 3.0 - 5.0 4.0 - 5.5	4d 4d 4d	H_2SO_4	2.35 2.95 3.70		Present Study
	O. rusticus Adult Juvenile Hatchling	2.0 - 4.4 2.0 - 4.0 2.0 - 4.0	4d 4d 4d	H_2SO_4	2.60 2.80 4.00	- hard water - temperature 4°C - only acute levels tested	Baker (1963)
	O. rusticus Adult	2.0 - 6.0	4d	H_2SO_4	2.51	- hard water	McMahon and Morgan - (1981)
	Parastacoides tasmanicus	1.1 - 3.0	4d	HCl	est 2.00	- exceptional tolerance due to natural acclimatization	Newcombe (1975)
Other Invertebrates	*Gammarus lacustris*	4.0 - 6.0	4d	H_2SO_4	est 5.08	- soft water - temperature & seasonal effects	Borgstrom and Hendrey (1976)
	Hyalella azteca	4.8 - 6.0	4d	H_2SO_4	mean 4.81	- hard water - different strain resistance - O^2 effect	de March (1979)
	Mysis relicta	4.5 - 6.0	5d	H_2SO_4	est 4.50	- ELA soft water - cannibalism effect	Olesiuk (unpub)
Fish[a]	rainbow trout	3.0 - 6.0	4d	HCl	mean 4.21	- hard water - high pCO_2 effect	Lloyd and Jordan (1964)
	rainbow trout 4 mo 18 mo		4d 4d	H_2SO_4	mean 4.20 3.38	- soft water - temp. acclimation effect	Kwain (1975)
	brook trout		7d	H_2SO_4	est 3.5	- continuous flow	Daye & Garside (1975)
	atlantic salmon alviens	2.7 - 4.3	7d	H_2SO_4	est 4.0	- soft water	Daye & Garside (1977)
	bluegill		4d		3.60		EIFAC (1969)
Crayfish	*O. virilis* Adult Juvenile Hatchling	2.0 - 3.5 3.0 - 5.0 4.0 - 5.5	27d 24d 16d	H_2SO_4	2.90 4.25 5.15	- threshold not reached	Present Study
	O. virilis Adult	4.0 - 6.0	19d	H_2SO_4	est 3.70	- ELA soft water - static baths - molting stages effect	Malley (1980)
Other Invertebrates	*Mysis relicta*	4.5 - 6.0	19d	H_2SO_4	est 5.65	- ELA softwater - cannibalism effect	Olesiuk (unpub.)
	caddisfly (2 species)	1.0 - 7.0	30d	H_2SO_4	mean 2.92	- hard water - organisms fed	Bell (1971)
	stonefly (4 species)				mean 4.27	- emergence more sensitive	
	dragonfly (2 species)				mean 4.36		
	mayfly (1 species)				5.38		
Fish	rainbow trout	3.0 - 6.0	15d	HCl	4.5	- hard water - high pCO_2 effect - threshold not reached	Lloyd & Jordan (1964)
	white sucker	3.0 - 4.2	42d	H_2SO_4	4.1	- hard water - continuous flow - HCl not different	Beamish (1972)

[a] Further Fish results are presented in Sry et al. (1981).

laboratory acid tolerance experiments for a variety of invertebrate and fish species (further results for fish can be obtained from Spry et al. 1981). Although defining the effects of highly acid solutions on organisms may have little practical value as most species fail to reproduce at such low pH levels, these measurements are useful because they serve as a basis for comparing the relative acid resistance of species and life stages to acid concentrations that might be experienced during periods of snowmelt.

M. Baker (unpubl. data) conducted a short-term study comparing the acid resistance of three life cycle stages of O. rusticus (adults 30.6 mm C.L.; juveniles 14.7 mm C.L.; hatchlings 6.5 mm C.L.). Results were similar to those obtained in this investigation. The LC50 values for hatchlings and juveniles were within the 95% confidence limits of those for O. virilis and that for adults agreed within the 90% confidence interval. Median survival times for similar pH treatments usually agreed within 95% confidence. McMahon and Morgan's (1981) results for O. rusticus adults are comparable to Baker's findings. Newcombe (1975) found that adult intermolt P. tasmanicus were exceptionally tolerant of acidity with no adverse effects down to pH 2.75, and with a 96 hr LC50 of 2.0. The high tolerance appears to be due to the long-term natural adaptation of this species to waters with an average pH of 4.5 and a minimum of 3.8. Appelberg (1980) reported no abnormal reactions or mortality in adult A. astacus exposed for 79 days to pH as low as 4.5. Due to the physiological alterations in an organism's metabolism and the rapid and non-selective uptake of water, molting is recognized as a period of increased sensitivity to both natural and toxic stresses. Malley (1980) found adult O. virilis in early postmolt stages were more susceptible to acidity, producing a 19 d LC50 of pH 3.70, significantly higher than the present 27 d LC50 of pH 2.90.

Only the work by Jay and Holdich (1977) has implied that adult crayfish can have high acid sensitivity, with exposure to even pH 6.0 proving lethal. Mean survival times of their adult A. pallipes were considerably shorter than those calculated for O. virilis hatchlings in this study. However, the average pH of the control water used by Jay and Holdich (1977) was 7.75, indicating a high carbonate/bicarbonate alkalinity, yet no mention was made of removal of CO_2 via aeration following acid addition. Since Lloyd and Jordan (1964) have shown that a buildup of CO_2 producing respiratory hypercapnia will cause the toxicity of acid to be seriously overestimated, it is possible that high CO_2 concentrations confounded the results of Jay and Holdich's (1977) study (cf. France 1982).

Aquatic organisms differ markedly in their tolerance to low pH (Table 19). The most sensitive species are the small invertebrate crustaceans, mayflies and the young-hatchling stages of crayfish (incipient exposure). Most fish species, dragonflies and stoneflies, and juvenile crayfish appear to be of intermediate tolerance. Fish reach their incipient lethal thresholds in a much shorter time than do invertebrates, i.e. compare Lloyd and Jordan's (1964) results for rainbow trout with the increase in LC50 for Mysis or O. virilis. The organisms displaying greatest tolerance to acidic conditions are caddisflies and adult intermolt crayfish. This sequence of relative tolerance in the laboratory agrees with field observations documenting the disappearance of organisms from acidified lakes. Evidence for the high acid tolerance of adult crayfish is demonstrated by Beamish (1974) and Collins et al. (1981) who found crayfish in a lake near Sudbury at pH 4.5-5.0 despite the fact that several less tolerant fish species had disappeared. There are several other reports mentioning the presence of crayfish in polluted lakes with pH levels below 5.0 that have also lost their fish (Hendrey and Vertucci 1981; N. Yan, OME, pers. comm.).

3-A.4.2 Recovery Ability

Episodes of extreme pH shock accompanying the spring melting of snow or autumn storm events are of a finite duration lasting no more than 14-30 days (Hagan and Langeland 1973; Hultberg 1976). Survival of any species, therefore, depends not just upon its tolerance to the magnitude of pH depression but also upon the time course of toxic stress in relation to the duration of perturbation. Although knowledge of the resistance times (MST) is essential for predicting the ultimate fate of populations to episodic acid pulses, the speed at which an individual goes through the symptoms of impending mortality (ET50) will also be of ecological significance. Moreover, the ability of organisms to recover from a moribund state induced by acid stress, once the pH begins to rise again, will strongly influence the eventual percentage of the population that will be affected.

The present experiment suggests that exposure of juvenile O. virilis to conditions of acid stress does not result in permanent damage. Even individuals in advanced states of low pH induced morbidity revived quickly once placed in neutral water. There was no indication of any delayed long-term effect resulting from acid exposure. The tolerance experiments with adult O. virilis substantiate these conclusions. When adult crayfish in pH 2.3 treatment that had lost equilibrium for over 25 hr and become unresponsive to probing and considered dead, were removed from the acid water for measurement, they quickly regained vigorous activity.

Observations from the literature concerning the ability of fish to recover from acid stress are discordant but probably explainable on the basis of the experimental acidity levels used. Although failure of ion regulation is the primary mode of toxic action at chronically lethal pH levels, the very rapid death at low pH (<3.0) is due to anoxia caused by a deterioration of the gill surface membranes (reviewed in Fromm 1980; Spry et al. 1981). Once mucus has formed over gill surfaces as a result of exposure to pH below about 3.5, fish will not recover even if placed in neutral water in contrast to fish that have lost equilibrium due to exposure to pH values between 3.8-4.5.

McMahon and Morgan (1981) found that following sublethal exposure to pH 3.5, all O. rusticus hemolymph acid-base and ionic parameters returned to their initial values after a 2-4 day recovery period in control water. The recovery of moribund juvenile O. virilis from pH 3.0 exposure and of adults from pH 2.3 water indicates that the crayfish gill is not as susceptible to acid damage as that of fish. This recovery ability of crayfish implies that exposure of such organisms to low pH during snowmelt would cause no permanent physiological damage to the survivors once the pulse had ended.

3-A.4.3 Pleopod Aeration of Eggs

O. virilis displays a more energetic pattern of pleopod oscillation than that shown in studies on P. clarkii tested at the same temperature (Suko 1956). P. clarkii had an average vibration frequency of 22 beats per min, vibration period of 43 sec, and an interval period of 207 sec, with approximately 17% of the total time spent in this behavior compared to 32-37% of the time for O. virilis in control water. It is possible that these contrasts may reflect differences in the length of the egg developmental period (O. virilis - 1 1/2 mo; P. clarkii -4 mo) or perhaps the stage of development. Bechler (1981) recorded an increase in the frequency of pleopod vibration from about 10 beats per min until 14 days prior to hatching, at which time a plateau of 33 beats per min was reached (range of 22-48). These values are considerably lower than the 69.7 ± 5.8 vibration frequency recorded for O. virilis at neutral pH. This difference probably reflects the colder temperatures (12-16°C) used in Bechler's study.

Oxygen consumption increases under exercise or stress. Ress (1962) found that Gammarus could regulate their consumption rate to a critical level below which their O_2 uptake was reduced, by decreasing their pleopod beating rate in low O_2 water. Crayfish are able to regulate their internal state by adjusting their metabolic rate in response to low environmental O_2 concentrations (Wiens and Armitage 1961; Moshiri et al. 1970). It therefore seems possible that ovigerous crayfish could also reduce their pleopod vibration period when exposed to hypoxic acid environments known to strongly influence the uptake and transport of O_2 in both fish (Fromm 1980) and invertebrates, including crayfish (Hiestand 1931; Tyagi 1973). Exercised rainbow trout had a lower resistance to acid than fish at rest (Wood and Graham 1981), perhaps due to an acceleration of branchial ion loss and production of respiratory and metabolic acids or a more rapid build-up of an O_2 deficit. For these same reasons, ovigerous crayfish attempting to maintain rhythmic pleopod vibration for aeration of developing eggs would be expected to be more sensitive to depressed pH than other individuals. The present results suggest that when encountering acidified water, the need of the ovigerous female to satisfy its own increased O_2 demand will override its maternal behavior to the developing eggs. This is in contrast to the crab Carcinus which has been shown to modify its maternal behavior to confer a respiratory advantage to the developing eggs when placed under conditions of hypoxic stress (Wheatly 1981).

3-A.4.4 Implications of Experimental Results for the Survival of Crayfish Populations Exposed to Acidification

The occurrence of crayfish inhabiting the littoral regions of many oligotrophic acid-sensitive lakes has suggested the possibility that they would expire during periods of snowmelt. From results obtained in the present set of tolerance experiments, knowledge of crayfish life history patterns, and data concerning the magnitude and duration of chemical pulses, the response of crayfish populations to acidification can be predicted. Lakes in central Ontario receive between 36-77% of the year's export of H^+ from their watersheds during snowmelt, which reaches its maximum in mid-April and is almost terminated by the beginning of May (Jeffries et al. 1979). ELA lakes are hydrologically similar, with the termination of the spring meltwater pulse coinciding with ice disappearance, generally during the last week of April or first week of May (Beaty 1981). Egg extrusion of all five *Orconectes* crayfish species found in south-central Ontario is synchronous and occurs during ice melting in late April in water temperatures of 5-8°C (Berrill 1978). Because the hatching of *O. virilis* eggs in northwestern Ontario does not occur until late June or early July, the SIII hatchling stage would not be exposed to snowmelt. By the time of autumn storms, these young have increased their acid resistance seven-fold. The absence of inverse thermal stratification at this time allows the acid inputs to be mixed and partially neutralized throughout the entire water column

(Henriksen and Wright 1977). The relatively short duration of these storm events, together with their reduced severity, would probably ensure that the 24 d LC50 for juveniles of pH 4.25 or the pH 4.5 MST of 60.42 days would never be realized. This is probably also true with exposure of these juveniles to snowmelt the following spring. The exceptional pH tolerance of adult _O. virilis_ (no mortality despite one month exposure at pH 3.5) indicates that they will not expire even during the most extreme spring acid inputs. No molting occurs at that time. _O. virilis_ populations existing in small headwater Precambrian Shield lakes are therefore not likely to decline as a result of episodic acid pulses. Nevertheless, the stocking of other crayfish species with sensitivities similar to _O. virilis_ in any lake with an average annual pH below 5.5 could result in eventual population extinction due to mortality of the young life stages.

The most critical life history period for _O. virilis_ will be the egg-laying phase which occurs during springmelt. For those crayfish species such as _A. astacus_ that oviposit in late autumn and carry their developing eggs during all phases of springmelt, it is the effect of low pH upon the developmental eggs that will ultimately determine whether a crayfish population will survive in the long run (Furst 1977b). The mean percentage of total time which _O. virilis_ spent vibrating eggs was significantly lower than normal only at pH levels 4.5 and 4.0. Egg mortality in the L223 population occurred at pH 5.35, a value which did not affect the behavior of ovigerous females in this experiment.

Consequently, low pH will directly affect crayfish recruitment long before modifications of maternal behavior become important.

Hobbs and Hall (1974) have warned of the dangers of extrapolating from a single crayfish species. Species which burrow into the lake bottom are probably more able to tolerate contamination of their habitat than are those species living in open water beneath rocks and debris. Localized sediment buffering (Hongve 1978) may provide favorable refuge to those species which are able to take advantage of this microhabitat. The mitigative mechanisms of acclimatization and avoidance by crayfish to low pH must be considered (cf. 3-B) and finally the possibility of synergistic effects of heavy metals remobilized in acid environments has yet to be investigated.

CHAPTER III

EXPERIMENTAL RESPONSE OF THE CRAYFISH _ORCONECTES_ _VIRILIS_ TO LOW pH

III-B

ACCLIMATIZATION AND AVOIDANCE

3-B.1 INTRODUCTION

The possibility of an altered resistance of organisms to low pH following previous exposure to sublethal acid conditions, is of considerable importance to the current acid precipitation problem. Many authors have attempted to demonstrate such acclimation in fish with both short-term (Lloyd and Jordan 1964; Robinson et al. 1976; Trojnar 1977; Swarts et al. 1978; Daye 1980) and long-term studies (Edwards and Hjeldnes 1977; Mount 1973). All these investigations have failed to produce a correlation between previous pH history and survival times. No comparative studies on resistance adaptation of invertebrates to lowered pH have been performed.

Laboratory studies have shown that the mode of toxicity for invertebrates, including crayfish, to low pH, is similar to that for fish - a failure to maintain internal ion homeostasis (McMahon and Morgan 1981; Shaw 1960; Potts and Fryer 1979). The abundance of certain invertebrate species in acidic waters is dependent upon their ability to alter iono-regulatory affinities to maintain a stable hemolymph NaCl balance (Vangenechten and Vanderborght 1980; Potts and Fryer 1979). Differences exist in osmoregulation between populations of the same species of crayfish inhabiting water of varying ionic concentrations (Wong and Freeman 1976) suggesting that they may be capable of acid acclimation.

The shallow depths inhabited by crayfish during early spring exposes them to the surface runoff of acidic snowmelt. Ovigerous females and yearlings are concentrated between 0-1.5 m and adult males between 1.5 - 3.0 m (Momot 1967a; Capelli 1975). Populations, however, may be able to seek refuge in deeper waters by avoiding the thermally stratified acidic meltwater at this time. Following a decrease of 2 pH units in a Norwegian lake, Groterud (1972) showed that Copepoda escaped this surface stratum of acid meltwater and Muniz and Leivestad (1980) found that brown trout gathered in 'chemical havens' of higher pH during a period when a lake was subjected to such a rapid acid influx. Crayfish are capable of extensive movement (Capelli 1975). Therefore, the possibility exists that they could prolong their existence in lakes receiving acid meltwater by modifying their depth distribution.

Laboratory studies investigating the behavior of fish to water of low pH are numerous (Wells 1915; Shelford 1923; Jones 1948; Bishai 1962; Ishio 1965) but only those works of Hoglund (1961) and Johnson and Webster (1977) were conducted with low carbonate freshwater or concerned with acid pollution. Little work, however, has been done on the ability of invertebrates to avoid acid conditions. Costa (1962) studied Gammarus pulex and Laughlin et al. (1978) monitored the field and laboratory reactions of the blue crab Callinectes sapidus to low pH. Neither of these works can be extrapolated to the current acid precipitation problem.

The purpose of this set study was two-fold: 1) to determine if adult crayfish Orconectes virilis (Hagen) from an experimentally acidified lake have achieved compensatory mechanisms of physiological acclimatization sufficient to prolong their survival at lethal pH levels compared to crayfish from a control lake, 2) to examine the ability of this crayfish to detect and avoid water of low pH at values similar to those that might be expected following the spring melt of acid snow, and to investigate the importance of the previous sensory conditioning of test animals to the interpretation of these behavioral results. Successful acclimatization and/or avoidance, may reduce stress for crayfish exposed to acid pollution.

3-B.2 MATERIALS AND METHODS

Adult (age II, III; 19-30 mm carapace length) and young-of-the-year (8-13 mm C.L.) crayfish were collected from control Lake 240 (L240) and L223 during August and September 1980 with the aid of a diver-operated suction gun designed to prevent mechanical damage to the specimens (Davies and Ramsey in prep.). Crayfish were held for 24 hr in aerated aquaria containing L239 water (acidified to pH 5.3 for L223 animals) prior to testing. Only those individuals that appeared healthy were used for the experiments.

3-B.2.1 Acclimatization

Tests were conducted in 50 L, 65 x 85 cm Plexiglass® aquaria subdivided into 20 compartments with grey perforated PVC plastic sheeting allowing crayfish to be held individually to prevent cannibalism. Lake 239 water was acidified to pH 2.0, 2.3, 2.6, 3.0 and 3.5 with H_2SO_4 and vigorous aeration for 1 hr assured removal of all excess CO_2 resulting from acid addition. Ten adult crayfish (5 male, 5 female) from each lake were randomly placed in each pH treatment aquarium. The experiment was conducted for 27 days because toxicity comparisons based on short arbitrary time periods may be misleading and indicate differences where none really exist on the basis of incipient LC50's (Sprague 1969).

Crayfish mortality and pH were checked 6 times daily for the first week and 3 times daily thereafter. Periodic additions of acid were required to maintain the treatment pH levels. Continuous aeration and a 12L:12D light cycle were provided. Temperature was between 14 and 18°C throughout the experiment. Crayfish were not fed. At day 10 and day 20 approximately 75% of the test water was renewed. Escape response behavior and carapace rigidity were noted and mortalities were removed, weighed and measured.

The pH-mortality data were statistically analyzed by the log-probit transformation procedure of Litchfield (1949). To determine if the two incipient LC50's are significantly different, being indicative of acclimatization, Litchfield's (1949) test for parallelism and potency ratios was performed. Calculation and testing of differences between median survival times (MST) for each pH treatment followed the similar graphic procedure presented in Litchfield and Wilcoxon (1949).

3-B.2.2 Avoidance

Experimental design and presentation of data are adapted from Laughlin et al. (1978). Avoidance reactions were tested in a sealed plywood Y-maze trough providing an interface of sharp gradient throughout the mixing area. Exploratory tests with dye and acidified water showed no change occurred in the quality of water with passage from inlet to outflow. A perforated barrier was present which could be lowered, separating the trough into two arms and the mixing area. All tests were performed with hypolimnion L239 water (pH ca 6.8). Two 170 L tanks were filled with water and one acidified with H_2SO_4 to the desired pH. Water samples measured in an infrared analyzer showed that vigorous aeration of the test tanks for a half hour was sufficient to remove all excess CO_2 resulting from acid addition, thus allowing tests for avoidance of hydrogen ions only. The behavioral effects of sulfate are not known. Water from each tank was pumped to either arm of the Y-avoidance chamber at a flow rate of 1.5 L/min.

Experiments were conducted in a secluded area to minimize external influences on crayfish behavior. Preliminary tests showed no difference in locomotory behavior of crayfish under conditions of moderate illumination or darkness, so all experiments were carried out using moderate light levels. The temperature of test water was 16-20°C and oxygen was maintained at saturation at all times.

Following the procedure of Laughlin et al. (1976), adults were tested individually. A crayfish was placed in the mixing area and following a 5 min adjustment period, the barrier was slowly raised, presenting the animal with a choice of either arm receiving control or acid water. The avoidance reactions of 7 crayfish were tested for each pH treatment. Experiments were run for 15 min with 4 of the 7 individuals. In the case of the remaining 3 crayfish (usually those which displayed the greatest avoidance response) the acid and control inflows were transposed after 15 min and the experiment continued for an additional 15 min to detect if preferential selection of one trough arm was occurring. The number of mixing interface crossings and visits to each arm was counted and timed as well as the relative distance moved in each trip up an arm. Following each test the animal's sex and carapace length were recorded.

Because the percentage time data appear as totals for seven animals, one or two individuals with very strong avoidance reactions may cause polarity and give the impression that all seven are similarly avoiding that particular pH level. To account for this, an avoidance ranking was done on an individual basis, thus weighting the results equally. Arbitrary scores of -2, -1, 0, +1, or +2 representing the range from strong preference to strong avoidance were given to each of the seven crayfish and then totalled for that pH treatment. Maximum avoidance would then be 14, i.e. 7 × 2.

Young of the year (y-o-y) crayfish were tested in groups of 15 for L240 and groups of 12 for L223. After a 5 min adjustment period, animals were allowed 30 min to choose a position in the avoidance apparatus, the barrier was lowered, and counts made of the number in each area. Acid and control inflows were then switched and the experiment continued for an additional 30 min with subsequent counts. Two complete replicates were conducted for each pH treatment. Avoidance was indicated when the total number of crayfish in the control arm both before and after the switch was significantly (Z-test for differences in binomial proportions) larger than the total number in the arm receiving acidified water.

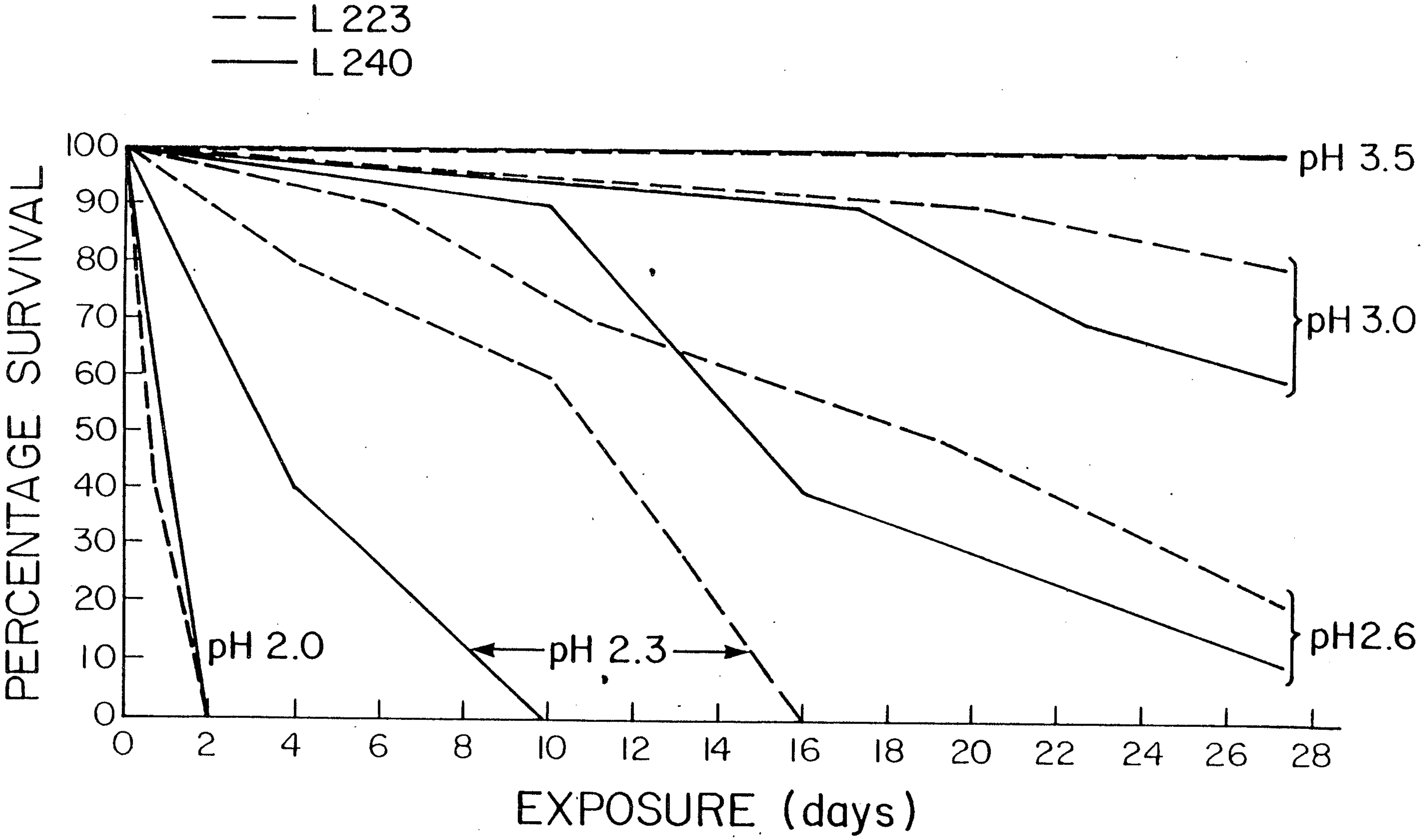
3-B.3 RESULTS

3-B.3.1 Acclimatization

No mortality of intermolt crayfish occurred following one month's exposure to pH 3.5 water while almost instantaneous death resulted from placement in pH 2.0 water (Fig. 36). Survival curves for L240 and the L223 acid exposed crayfish were similar for all treatments except pH 2.3. Statistical analysis of the median survival times (MST) indicated that only at pH 2.3 did crayfish from the L223 population have a significantly longer MST (Table 20). The LC50 values calculated at 96 hrs and after 27 days (incipient level) were not significantly different for crayfish from either the control or the acidified lake (Table 21).

Mortality was not dependent upon sex or size over the range measured. Stressed crayfish would lose their tail-flip response as early as 2 days preceding death. Animals became moribund, lying on their back, with some individuals maintaining this position for over 24 hr

Figure 36: Comparison of survivorship between adult intermolt *O. virilis* (20-30 mm carapace length) from L223 and L240 exposed to depressed pH.



L 223
L 240
PERCENTAGE SURVIVAL
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
EXPOSURE (days)
pH 3.5
pH 3.0
pH2.6
pH 2.0
pH 2.3

Table 20. Median survival times (days) ±95% C.I. and slope function (S). RR = potency testing ratio; * = significant difference ($p < .05$).

pH	L240		L223		RR
3.5	indefinite		indefinite		
3.0	30.42 ± 9.13	S = 1.52	41.67 ± 12.67	S = 1.72	1.37
2.6	15.00 ± 2.40	S = 1.28	16.25 ± 7.75	S = 1.55	1.08
2.3	3.83 ± 2.11	S = 1.86	10.83 ± 1.95	S = 1.35	2.83*
2.0	1.17 ± 0.27	S = 14.0	0.96 ± 0.27	S = 1.48	1.22

Table 21. Median lethal pH ±95% C.I. and slope function (S).
Incipient = 27 day exposure.

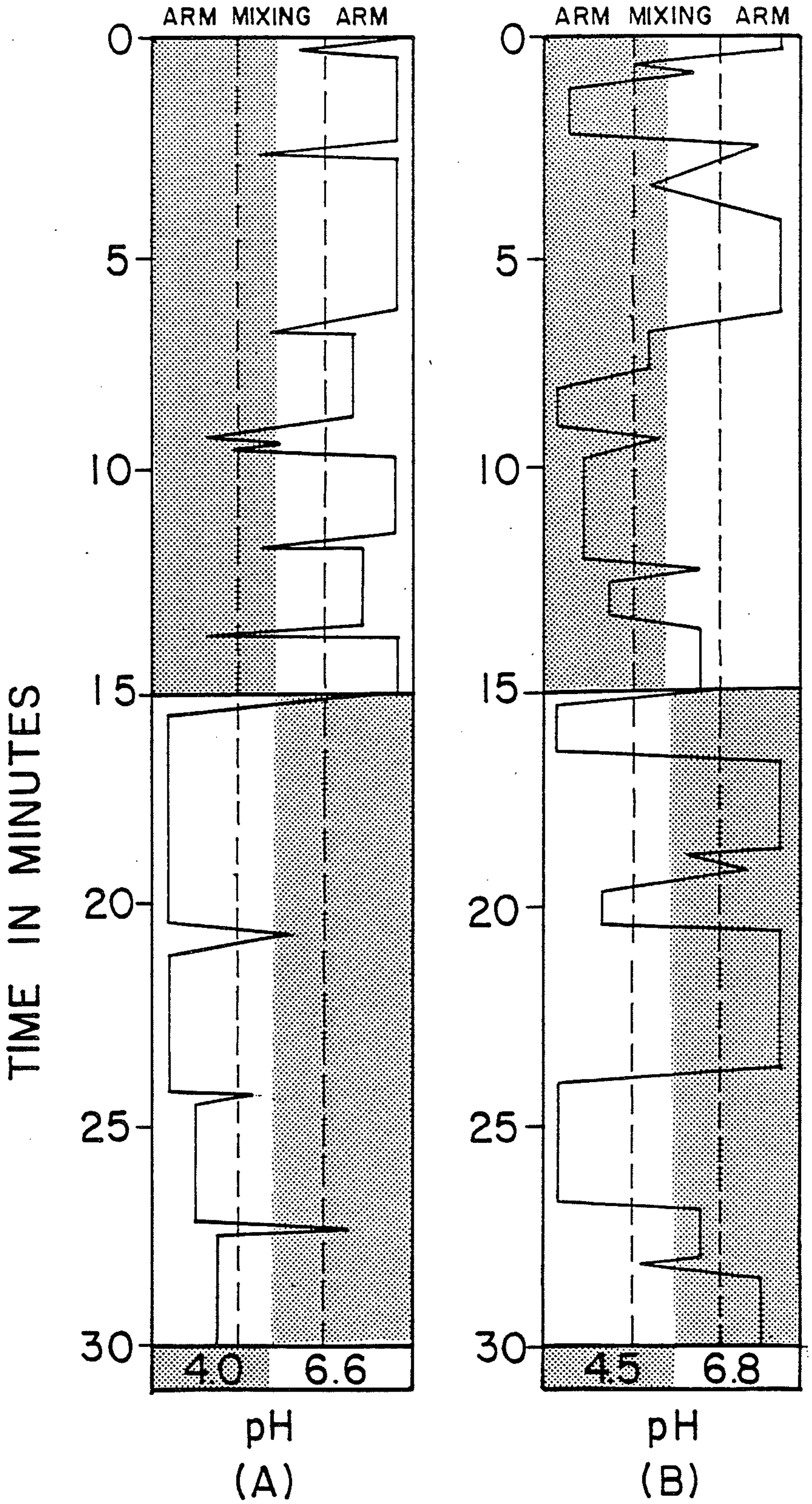
	96 hr LC50		Incipient LC50	
L240	2.35 ± 0.09	S = 1.05	2.90 ± 0.15	S = 1.07
L223	2.20 ± 0.13	S = 1.09	2.80 ± 0.11	S = 1.07

before finally dying. Crayfish at pH 3.5 remained active throughout the course of the experiment. Carapace rigidity of both mortalities and animals surviving after 27 days did not change since the onset of the investigation.

3-B.3.2 Avoidance

The behavior of individual crayfish varied greatly with some animals displaying a markedly greater sensitivy to low pH than others. Highly sensitive individuals spent most of the time far up the control arm near the inflow but later began to explore the trough once more (Fig. 37a). Rapid movement ceased when the acid interface was encountered in the centre of the mixing area. The crayfish would then pause and wave its antennae in large circles, both forward into the acidic water and also back over its own carapace. Sometimes this was sufficient to cause the individual to walk backwards until its head was clear of the acidic water and then turn around and begin movement up the control arm once more. In many cases a very rapid escape response was elicited with the vigorous flicking of the tail until the crayfish was again in the control arm. Occasionally following a pause at the interface, the crayfish would begin a slow exploratory movement forwards into the acid side accompanied by much antennal waving. After one or two body lengths, the animal sometimes reversed its walking direction or flicked its tail. Often a crayfish would stop waving its antennae and continue forward into the acid arm following interface pausing (Fig. 37b). At the higher pH values tested, walking was even more indiscriminate in that there was no indication in crayfish behavior of pausing at the interface, suggesting an absence of acid detection. There was no difference in the avoidance behavior between sexes, but

Figure 37: Schematic representation of reactions of a single adult L240 crayfish to water of different pH in the Y-maze avoidance chamber. Examples of (A) evident avoidance response and (B) indiscriminent response.


ARM MIXING ARM
ARM MIXING ARM
TIME IN MINUTES
0
5
10
15
20
25
30
4.0
6.6
4.5
6.8
pH
pH
(A)
(B)

there was a tendency for smaller crayfish to be more sensitive.

The mean time spent in a visit to the acid arm by adult L240 crayfish decreased with increasing acidity (Table 22). At pH 4.5 and 4.0 the number of visits to the control arm became nearly twice that to the acid arm. As the pH decreased the animals became increasingly agitated and increased the number of interface crossings in an attempt to escape the acid. The percentage of these crossings that were avoidance reversals (rapid change of walking direction or escape reaction) progressively increased from 2.5 to 46.2% as the test pH was lowered. The intensity of crayfish avoidance response was inversely related to pH. Crayfish showed maximal avoidance at pH 4.0 and 4.5 (71.1% and 54.1% of the test time spent in the control arm as compared to 12.7% and 20.5% spent in the acid arm respectively). Marginal avoidance was evident at pH 5.0 with 42.4% of the time in the control arm as compared to 28.4% in the acid arm. Crayfish displayed no avoidance to pH levels 5.5 and 6.0.

Differences in mean time spent in a visit or number of visits between arms for adult L223 crayfish became evident only at pH 4.0 (Table 23). Crayfish from L223 moved less than those from L240. The avoidance response, as shown by the percentage of reversals at the interface or percentage time calculations, was much less than for L240 control animals at the same pH. Maximal avoidance occurred only at pH 4.0 with 59.4% of the test time spent in the control arm compared to 24.0% in the acid arm. Marginal avoidance occurred at pH 4.5 with 40.7% time in control compared to 28.2% in acid. No avoidance occurred at pH 5.0, 5,5 and 6.0. The individual ranking substantiates that the avoidance sensitivity of L223 crayfish is approximately one-half pH unit lower than that of crayfish from L240.

Table 22. Avoidance response of adult Lake 240 crayfish (19-28 mm C.L.) to experimentally acidified test water. The pH of the control water was 6.5-7.0 for all tests. Each test represents results of 7 animals. Interface denotes boundary between acid and control water in the mixing area of the Y-maze testing chamber. Reversals represent percentage of interface crossings that were rapid avoidance of acid water. Individual avoidance ranking is a comparative nonparametric statistic summed for all 7 crayfish as described in text.

Experimental pH	Mean time (secs) spent in a visit to		Total number of visits to		Total number of interface crossings	Percentage reversals	Total time in seconds (%) spent by all crayfish in each area				Individual avoidance ranking
	Control	Acid	Control	Acid			Control	Acid	Mixing area: Control side	Mixing area: Acid side	
6.0	95	134	24	20	40	2.5	2268 (25.2)	2682 (29.8)	2178 (24.2)	1872 (20.8)	0
5.5	118	110	29	26	42	7.1	3421 (38.0)	2858 (31.8)	1034 (11.5)	1687 (18.7)	2
5.0	147	106	26	24	51	19.6	3818 (42.4)	2552 (28.4)	1448 (16.1)	1182 (13.1)	4
4.5	104	66	47	28	60	33.3	4866 (54.1)	1846 (20.5)	1844 (20.5)	444 (4.9)	7
4.0	149	50	43	23	65	46.2	6395 (71.1)	1142 (12.7)	1119 (12.4)	334 (3.8)	10

Table 23. Avoidance response of adult Lake 223 crayfish (19-26 mm C.L.) to experimentally acidified test water. The pH of the control water was 6.5-7.0 for all tests. Each test represents results of 7 animals. Interface denotes boundary between acid and control water in the mixing area of the Y-maze testing chamber. Reversals represent percentage of interface crossings that were rapid avoidance of acid water. Individual avoidance ranking is a comparative nonparametric statistic summed for all 7 crayfish as described in text.

Experimental pH	Mean time (secs) spent in a visit to		Total number of visits to		Total number of interface crossings	Percentage reversals	Total time in seconds (%) spent by all crayfish in each area				Individual avoidance ranking
	Control	Acid	Control	Acid			Control	Acid	Mixing area: Control side	Mixing area: Acid side	
6.0	90	113	22	24	32	0.0	1989 (22.1)	2718 (30.2)	1773 (19.7)	2520 (28.0)	-2
5.5	168	153	19	20	28	3.6	3188 (35.4)	3068 (34.1)	1161 (12.9)	1584 (17.6)	0
5.0	140	137	23	23	33	12.1	3217 (35.7)	3160 (35.1)	1398 (15.5)	1225 (13.6)	1
4.5	111	90	33	28	49	20.4	3666 (40.7)	2539 (28.2)	1995 (22.2)	800 (8.9)	4
4.0	184	114	29	19	38	23.6	5349 (59.4)	2163 (24.0)	1098 (12.2)	390 (4.3)	7

Experimental results with y-o-y crayfish follow the same trends as those of the adults. Lake 240 y-o-y strongly avoided ($p<.005$, $<.025$) pH 4.0 and 4.5 water (Table 24). Significant but marginal avoidance ($p<.05$) of one of the pH 5.0 treatments also occurred. Lake 223 y-o-y demonstrated decreased avoidance behavior (Table 25). Only one pH 4.0 treatment showed strong avoidance ($p<.025$) while the remaining one and a single pH 4.5 treatment displayed marginal avoidance ($p<.05$).

To prevent startling the y-o-y crayfish, close observations of intraspecific behavior were not possible. Densities, however, were low enough to prevent aggressive encounters from occurring. It was noted, as Laughlin et al. (1976) observed, that the majority of individuals comprising those present in the mixing area were usually found on the control side where pH values were nearly identical with those values in the control arm.

3-B.4 DISCUSSION

3-B.4.1 Acclimatization

Acclimation is the physiological alteration of an organism's homeostasis in a compensatory response to a change in a few environmental variables. Exposure is under controlled experimental conditions of generally short duration. Acclimatization occurs through exposure for much longer periods to modifications of the organism's natural habitat. Either case is accompanied by adaptation that enables the organism to better survive in its changed environment.

Adult age II and III crayfish from L223 have been subjected to depressed non-lethal pH conditions throughout their entire life: 5 mo at pH 5.3 in 1980 preceded by 1 yr at pH 5.6 and either 8 mo and birth at pH 5.8 or the 1 yr at pH 5.8 with the preceding 8 mo and birth at pH

Table 24. Distribution of young-of-the year (8-12 mm C.L.) Lake 240 crayfish in avoidance chamber. Control (pH 6.8) and acid water entering each arm were reversed midway through each test and results represent totals. Statistical significance at *p<.05; **p<.025 and ***p<.005 levels.

pH	Number of crayfish In control arm	 In acid arm	 In mixing area	Z value
6.0	10	9	11	0.25
6.0	12	10	8	0.56
5.5	11	12	7	0.00
5.5	16	11	3	1.24
5.0	13	10	7	0.80
5.0	12	6	12	1.69*
4.5	16	7	7	2.39**
4.5	17	8	5	2.39**
4.0	17	7	6	2.68***
4.0	20	6	4	3.67***

Table 25. Distribution of young-of-the-year (9-13 mm C.L.) Lake 223 crayfish in avoidance chamber. Control (pH 6.8) and acid water entering either arm were reversed midway through each test and results appear as totals. Statistical significance at *p<.05 and **p<.025 level.

	Number of crayfish			Z value
	In control arm	In acid arm	In mixing area	
6.0	7	11	6	0.00
6.0	10	8	6	0.64
5.5	10	9	5	0.28
5.5	7	10	7	0.00
5.0	10	9	5	0.71
5.0	13	9	2	1.11
4.5	9	12	3	0.00
4.5	12	6	6	1.79*
4.0	10	4	10	1.91*
4.0	14	5	5	2.62**

6.1. Despite this long period of exposure, acclimatization of adult O. virilis to acidic water as measured by resistance to lethal pH has not occurred for this population. There was no significant indication of an enhanced ability in the L223 crayfish to compensate toward the toxic stress at even the chronic lethal levels. The high acid tolerance of adult O. virilis precluded the operation of ionic compensatory mechanisms induced by exposure to the pH 5.3 water in L223. Further resistance experiments on the more sensitive young life stages to a similar sublethal pH value, as well as physiological investigations of crayfish inhabiting long-term acidified waters, are needed to determine the importance of acclimatization to prolonging crayfish existence in anthropogenically acidified watercourses.

Lake 223 crayfish, however, were not less resistant to lethal acidity than L240 control animals as might have been expected from the results of McMahon and Morgan (1981). They noted that, during the later stages of acid exposure with O. rusticus, an increase in hemolymph calcium levels occurred, suggesting a dissolution of calcium carbonate from the exoskeleton to provide the hemolymph with more bicarbonate buffer. This built-in buffering system was believed to gradually become active as a acclimatizing mechanism against acidosis. Crayfish from L223 already exhibiting greatly reduced carapace rigidities as a result of lower calcium levels might therefore be expected to be more susceptible to pH stress. This clearly did not occur. Further, qualitative carapace rigidity, even after 27 days of exposure, was not noticably reduced from that observed prior to treatment.

Although Jay and Holdrich (1977) note that the distribution of the British crayfish Austropotambius pallipes is strongly influenced by hydrogen ion concentration, inhabiting waters of only a narrow pH range

6.8-8.2 with no acclimatization outside these bounds, there do exist natural populations of other crayfish species that have demonstrated an ability to adapt to waters of abnormally high acidity. Newcombe (1975) reported the occurrence of the Tasmanian species *Parastacoides tasmanicus* in waters with a constantly low pH averaging 4.5 and with a minimum of 3.8. Laboratory tests showed these organisms had a remarkable tolerance of acidity (an LC50 of 2.00 calculated from the presented data). A thriving population of *Cambarus bartoni* exists in Clearwater Lake near Sudbury, Ontario which has had a pH of approximately 4.3 for over thirty years (Norman Yan, Ontario Ministry of the Environment, pers. comm.). Similarly, Collins et al. (1981) recorded that adult *C. bartoni* (28-48 mm C.L.) were abundant in several Ontario lakes acidified from circumneutral levels in the late fifties to a relatively stable value slightly below pH 5.0- since the early seventies. They also collected a single young *O. propinguus* (19 mm C.L.) suggesting reproduction had occurred the previous year at a mean epilimnetic pH of about 4.7. An unidentified species of crayfish has been found in a lake acidified from pH 5.4 - 4.9 over a 20 yr period in the Adirondack Mountains of New York (Hendrey and Vertucci 1980). Reproducing populations of *Astacus* have been found in Swedish lakes having a pH of 5 (Appelberg 1980). Appelberg, however, believes that this phenomenon may be more dependent upon other chemical variables than adaptation to acid per se.

It is not known whether these reports indicate physiological acclimatization or are rather the result of physiologically produced natural selection by which less resistant individuals are progressively removed allowing the remaining subpopulation to be more resistant, eventually leading to genetic adaptation of the crayfish. Only the work of Rahel and Magnuson (1980) on the pH tolerance of fish from three lakes of different acidities, has studied this problem. Their experiments showed that increased tolerance of fish from the most acidic lake was a result of genetic adaptation and not physiological acclimatization. The crayfish tested in the present experiment are from the first generation exposed to acidification in L223, born at a time when there were no adverse effects upon reproduction (2.3.4). The exceptional acid tolerance of adult *O. virilis* precludes the operation of natural selection via differential mortality among the L223 population (2.3.6). Consequently, this study tested for physiological acclimatization by *O. virilis* without the confounding influences of genetic adaptation present in other studies.

3-B.4.2 Avoidance

Hoglund and Hardig (1969) suggested that invertebrates with carbonate shells may display greater avoidance to environmental pH alterations than fish. A comparison with other studies shows that this is not the case for *O. virilis* which only demonstrated strong avoidance below pH 4.0-4.5 in these experiments. However, the intensity of behavioral reaction is dependent not just upon the pH value itself but also upon the substance used to effect this alteration as well as the chemical characteristics of the test water employed. It is important to determine to what extent the effect of pH upon aquatic life is due to its ionized hydrogen and to what extent due to the presence of other molecules.

The addition of a strong acid to natural waters containing bicarbonate will result in a shift in the relative proportion of the dissolved inorganic carbon species. Hydrogen ions react with bicarbonate forming carbonic acid which is further dehydrated to free carbon dioxide and water. Until this buffering process is complete, the initial result of acidification is a temporary increase in the concentration of CO_2 which in time will escape to the atmosphere. Over 90% of the total DIC progressively comes into solution as CO_2 before a depression of pH to 5.5; further acidification below this pH level causes only a neglible rise in pCO_2 values. Of course, the larger the water bicarbonate concentration, the greater the amount of CO_2 evolved upon acidification (cf Hoglund 1961; Hoglund and Hardig 1969). In such water, the concentration of hydrogen ions will only be a small component contributing to the acidity in the early stages of acid addition. Poorly buffered waters on the other hand, will respond immediately to the addition of acid with elevated hydrogen ion concentrations and only small rises in pCO_2.

Previous studies of acid avoidance with crayfish (Powers 1915), <u>Gammarus</u> (Costa 1962) and fish (Wells 1915; Powers 1929; Bishai 1962) used water of very high carbonate concentration. The high levels of "acid" avoidance reported in these studies (below about pH 6.0-6.5) apply to varying concentrations of CO_2 evolved upon acidification and not hydrogen ions. For the same reasons the conclusion of Laughlin et al. (1978) that "pH (hydrogen ions) was the primary factor involved in the avoidance of blue crabs (significant avoidance at pH 6.0 and below)" is spurious. The test water was naturally pH 8.1-8.2 and following acidification no bubbling was provided to remove excess CO_2. The relative importance of the CO_2 effect can be seen in Table 2 in Laughlin et al. (1978). Actual avoidance of pH 4.6 runoff collected from the polluted site (and having a chance to equilibrate with the atmosphere) was measured as only 57.3% of the test time spent in the control arm;

avoidance to laboratory induced pH 4.6 acidification (with no CO_2 stripping) showed 89.0% of the test time spent in the control arm. Avoidance of experimentally acidified water between the pH range 5.5-6.0 was similar to that found with field water at pH 4.6., the difference of over one pH unit being presumably due to the presence of elevated CO_2 in the former case.

In order to experimentally predict the avoidance of organisms to acid snowmelt, it is essential that the test water utilized be chemically similar to that found in the natural situation. Studies using high carbonate waters that generate large amounts of CO_2 have little, if any, relevance to the current acidification problem (France 1982). Experiments with fish in water of 8-20 ppm HCO_3^- (Hoglund and Hardig 1969; Hoglund 1961) or 4.21 ppm HCO_3^- (Johnson and Webster 1977) produced avoidance reactions only at pH values below 5.0, comparable to the results obtained in the present study with crayfish in which soft water from L239 (8.11 ppm HCO_3^-) was used and additional care taken to bubble off all excess CO_2 following acid addition.

Observations of species distribution in relation to acidic pollution in actual environmental situations are rare. Both copepod zooplankton and fish exhibited a marked habitat selection by avoiding thermally stratified acidic meltwater and seeking refuge in the profundal zone (Groterud 1972; Muniz and Leivestad 1980) or a less polluted tributary (Leivestad et al. 1976). Hall et al. (1980) experimentally acidified a natural stream to pH 4.0, producing an immediate downstream movement of invertebrates and fish (concentrations of Al were simultaneously elevated). Blue crabs sampled from three oceanic bays were consistently more abundant in the control area than in areas receiving acidic runoff as a result of clearcutting activities

(Laughlin et al. 1978). Crayfish have been found to occur abundantly in a stream pH 6.2-7.2 but were absent from two acid areas (pH 3.1-3.7; pH 4.3-5.6) in contact with industrial sulfuric acid effluent (Hobbs and Hall 1974). Warner (1971) found that the crayfish Cambarus sp. was never collected from waters with pH values below 4.5 that had been heavily polluted by acid mine drainage. Similarly, three species of crayfishes were not found in stream waters acidified below pH 4.0 (Schwartz and Meredith 1962).

3-B.4.3 Importance of Previous Sensory Conditioning to Avoidance Behavior

The possibility of a hydrogen ion conditioning factor in which individual animals show different behavioral reactions depending upon the quality of the environment from which they were obtained, has received little attention. Jewell and Brown (1924) attempted to explain the anomaly of fish living and reproducing in acidic waters in which other individuals of the same species demonstrated avoidance to when tested in the laboratory. The authors criticized earlier works which, being of short duration, ignored the factor of environmental acclimatization: "the behavior of animals from one habitat cannot justify conclusions as to the behavior of the species as a whole, nor can the pH selected by animals from one habitat be regarded as the pH 'optimum' for the species..." (Jewell and Brown 1924). Experiments by Hall (1925) and Brown and Jewell (1926) investigating the behavior of fish sampled from waters of varying acidity showed that when given a choice, fish select not necessarily an "optimum" pH, but rather the hydrogen ion concentration to which they have individually become accustomed.

Lake 223 crayfish have been under acidic conditions for their entire lives, and the intensity of their avoidance reaction is approximately one-half pH unit lower than that for L240 animals. There is even a slight tendency for L223 individuals to demonstrate a preference for pH 6.0 water over the control (pH 6.8) alternative. It seems that the acid sensory mechanism present in L223 crayfish has become conditioned to the pH 5.3 lake water, with behavioral distress gradually becoming evident either above or below this level.

In realistic situations, crayfish in lakes receiving pulses of acid snowmelt would have been exposed to the gradual year-round acidification of the lake throughout their entire life and thus have become slowly sensorially acclimatized to depressed pH conditions. Avoidance would then follow the pattern shown by L223 individuals and not those from the control L240. This suggests that behavioral experiments utilizing animals from unacclimatized populations may produce avoidance results for species survival with limited ecological relevance.

3-B.4.4 Implications of Failure of Physiological Acclimatization for Fishery Management

Furst (1977a) documented an unsuccessful restocking attempt due to depressed pH and, in one case, a 96% population mortality resulted from the cessation of liming by fishery biologists during a single month period (Abrahammsson 1972b). Consequently, Scandinavian fishery managers are interested in obtaining information about other crayfish species with the ability to adapt to and live in low pH waters (A. Stellan Karlsson, Simontrop Hatcheries, Sweden, pers. comm.).

Physiological acclimation of hatchery crayfish to low pH prior to stocking could conceivably reduce mortalities in those water bodies subject to rapid and drastic springmelt inputs, and provide a means of developing populations capable of surviving such acid conditions. An alternative approach could be the stocking of "naturally" acclimatized crayfish taken from other lakes that have themselves been receiving acid pollution.

The outcome of Svardson's (1974) survey of crayfish distribution in Swedish waters suggests that acidification may be taking place too rapidly to allow for either genetic or physiological adaptation to act as a protective measure against eventual population extinction. The results of the present study finding no acclimatization in an O. virilis population subjected to an acidification rate of 0.25 units per annum (higher than rates of 0.03-0.10 recorded in some affected areas) implies that a management practice of stocking crayfish following collection from such an "acclimatized" population into another acid polluted lake, will not be beneficial for successful maintenance of the new population in the second more seriously affected basin. The reported occurrence of crayfish inhabiting lakes with naturally or long-term lowered pH values has led Fürst (1977b) to suggest that it is only these populations that may be able to constitute a foundation for reviving crayfish populations in other recent anthropogenically acidified basins. Further investigations into this area and hatchery acclimation studies at more acidic levels than the pH 5.3 value of L223 would be rewarding to crayfish fishery managers.

3-B.4.5 Implications of Experimental Results for the Survival of Crayfish Populations Exposed to Acid Pollution

Experimental results show that no physiological resistance adaptation occurs in adult O. virilis collected from a lake exposed to acid pollution. Juvenile and yearling O. virilis are considerably more sensitive to acid than adults (3-A.3.1) and it is probable that there is also an absence of acclimatization to low pH in these younger developmental life stages as has been demonstrated to occur with fish (Daye 1980). Consequently, a crayfish population subjected to the gradual decrease of pH in lakes receiving acid precipitation, will not develop an increased tolerance with time, but rather, will still maintain an unaltered susceptibility to the periodic and increasingly extreme acid inputs commonly coincident with the spring melting of snow.

Although physiological survival acclimatization has not occurred in the L223 crayfish population, behavioral tests suggest that these individuals have developed a sensory adaptation to the lowered pH in their environment. Hoglund (1961) noted a correlation between tolerance and avoidance reactions to pH. The present work indicates that crayfish have the ability to detect and avoid acidic water at values that would prove lethal, suggesting that these organisms could escape mass mortality during the period of springmelt by seeking refuge in the profundal zone. However, crayfish in experimental L223 have experienced serious reproductive difficulties at lake pH levels as high as 5.6 (2.3.4) toward which no avoidance was demonstrated. This inability of O. virilis to avoid sublethally harmful acidity levels could have serious effects upon the population. The long-term survival then of this species in lakes receiving acidic runoff seems doubtful if based solely upon an inferred ability of physiological acclimatization or behavioral modification of distribution induced by hydrogen ions.

Two factors not tested in the present study, which could effect crayfish are the rate of pH decrease and the presence of heavy metals.

Creaser (1930) suggested that in unbuffered waters the rate at which a sudden change of pH occurs may be more important to behavioral alteration than the actual magnitude of the change. The velocity of pH alteration is controlled by the meteorological and geomorphic conditions present in each watershed. Probably even more important is the actual rate of lake acidification. It is possible that the annual experimental drop of 0.25 pH units in L223, higher than that observed in several affected areas, is too rapid to allow the population to physiologically and certainly genetically, adapt, which might occur in other less rapidly affected lakes. Secondly, spring acid melt events are often accompanied by elevated levels of heavy metals that have accumulated in the polluted snow (Hagen and Langeland 1973). Crayfish have been shown to actively avoid water contaminated with heavy metals (Chaisemartin et al. 1976; Maciorowski 1978) but whether the additional influence of lowered pH is additive, synergistic or antagonistic is not known. Clearly much more research is required before the ambition of Jeffries et al. (1979) in regard to changing speculation about the ecological effects of acidic snowmelt to the useful knowledge needed to assess its impact, is reached.

CHAPTER IV

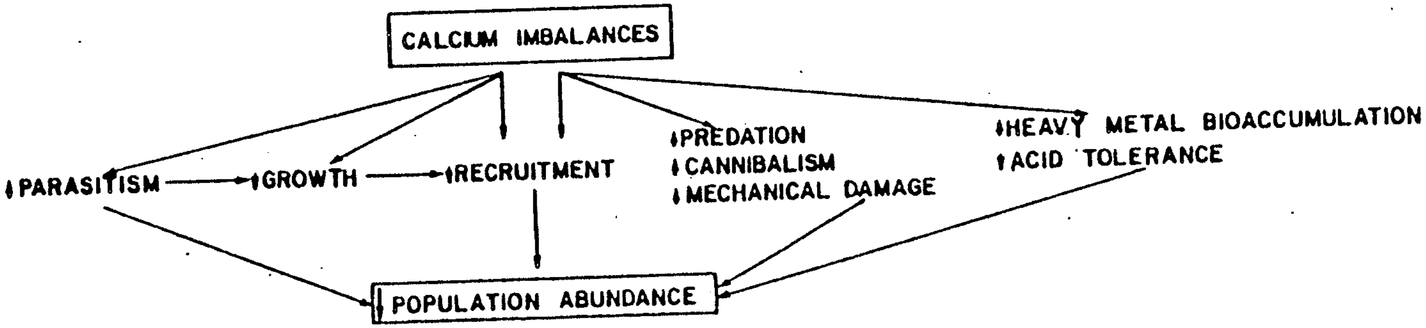
GENERAL SUMMARY

1. Reduced carapace rigidity indicates that the L223 population is undergoing decreases in postmolt exoskeleton calcification. Carapace Ca^{++} content was significantly lower in L223 crayfish (mean % dry wt ± 95% C.I. of 13.90 ± 0.89) than in animals from the three control lakes (19.82 ± 0.55; 20.34 ± 1.14; 22.18 ± 0.84).
2. Despite severe molt related problems in exoskeleton hardening and alterations in diversity of food, the timing of molting events and growth of _O. virilis_ has not been affected by acidification to pH 5.35.
3. Incomplete cuticular hardening of the glair-cement compound forming the egg capsule membrane and stalk has resulted in a lack of secure pleopod egg attachment causing the L223 population to suffer recruitment failure. On an egg production basis, the % reproductive impairment in the control populations averaged 3.2 ± 1.8 (95% C.I.) while levels of 18.7, 36.2 and 29.4 were obtained during 1979-81 in L223.
4. Lake 223 crayfish responded to acidification-related increases in trace metals with an increased bioaccumulation of Mn (L223 value of 240 $ug.g^{-1}$ dry wt compared to control mean of 61 $ug.g^{-1}$ dry wt) and possibly Hg (L223 value of 0.52 $ug.g^{-1}$ dry wt compared to control mean of 0.26 $ug.g^{-1}$ dry wt).
5. The level of _Thelohania_ sp. parasitism in the L223 population was 1.7% in 1979 and increased to 6.5% during 1980 with a constant

incidence of <1.0% in the control lakes.

6. The indirect sublethal interaction of H^+ with a single element -Ca^{++} may strongly influence the eventual success or extinction of crayfish exposed to cultural acidification (Fig. 38).
7. Development from a 2 week old hatchling stage (incipient 16 day LC50 of 5.15) to a 2 1/2 month old juvenile (incipient 24 day LC50 of 4.25) resulted in an increase of seven-fold in acid resistance; continued maturation to a 2-3 year old adult (incipient 29 day LC50 of 2.90) resulted in a further twenty-fold increase in resistance.
8. Juvenile crayfish can recover from severe acid stress.
9. A significant decrease in % time spent aerating the eggs through pleopod vibration was observed, although this only occurred at pH values well below those in L223 that caused egg mortality.
10. Acclimatization of adult crayfish to acidic water, as measured by resistance to low pH during month-long exposures, did not occur for the L223 population.
11. Adult and yearling crayfish from a control population avoided water pH of 4.5 and below. No avoidance was noted above pH 5.0. Crayfish from L223 had a noticeably reduced avoidance response (i.e. individuals have developed a sensory adaptation to lowered pH), significant at only pH 4.0, with no avoidance above pH 4.5.

Figure 38: Hypothetical interrelationships of life history variables regulating the abundance of crayfish populations exposed to acidification.


CALCIUM IMBALANCES
↓PARASITISM
↓GROWTH
↓RECRUITMENT
↓PREDATION
↓CANNIBALISM
↓MECHANICAL DAMAGE
↓HEAVY METAL BIOACCUMULATION
↑ACID TOLERANCE
↓POPULATION ABUNDANCE

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APPENDIX A

GROWTH OF THE CRAYFISH ORCONECTES VIRILIS IN SMALL OLIGOTROPHIC CANADIAN SHIELD LAKES IN THE EXPERIMENTAL LAKES AREA, NORTHWESTERN ONTARIO

by

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ABSTRACT

Crayfish growth in the Experimental Lakes Area (ELA) was investigated by analysis of size-frequency distributions, molt increment data and the calculation of instantaneous growth rates and mean size at onset of sexual maturity. *Orconectes virilis* growth at ELA is slower than other published rates for this species (only 27-38% of that reported for other areas) due to the oligotrophic nature of the softwater lakes, the poor nutritional quality of allochthonous inputs into these basins, and the short growing season. Rate of growth varied both between different study lakes and between different years for ELA populations. Relationships to lake trophic status, as measured by phytoplankton production or chlorophyll-a concentration, explained >70% of the variation in growth rate and >85% of the variation in maximum size of crayfish among lakes. Higher temperatures and a longer growing season during 1980 increased growth 12% over that of the preceding year. Growth variation in this species is due to alterations in the magnitude of growth increment applied to a fixed frequency of molts.

Crayfish population abundance in oligotrophic ELA lakes is suggested to be regulated through the density-independent control of growth and reproduction by environmental factors. Densities never reach levels high enough to cause competition for food or habitat resources, or create density-dependent feedback loops, all of which have been previously shown to occur in other crayfish populations. The extremely low densities of *O. virilis* in several ELA lakes strongly influences reproductive capacity and promotes the operation of the "Allee-effect" component of population control.

INTRODUCTION

An assessment of growth is essential in the study of life history patterns. Differences in growth between crayfish populations from proximal localities has received little attention (Hopkins 1966; Momot 1967; Morrissy 1975a; Flint and Goldman 1977). The purpose of this study was threefold: 1) to analyze and compare the growth of crayfish at ELA with previous studies on _O. virilis_ from other regions in N.W. Ontario (Weagle and Ozburn 1972; Momot 1978) and northern Wisconsin (Momot and Gowing 1977); 2) to determine the natural variability of growth among four ELA basins as related to environmental variables; and 3) to ascertain the relationship of growth rate to population abundance among the study lakes. Previous workers have shown that fish yield can be predicted from phytoplankton production, biomass or chlorophyll-a concentration (Oglesby 1977; Jones and Hayer 1982). A good relationship has also been demonstrated between phytoplankton production or biomass and macrobenthic standing crop or biomass (Dermott et al. 1977; Davies 1980). A relationship between crayfish abundance and lake productivity, however, has not been previously attempted.

MATERIALS AND METHODS

Description of collection sites

Orconectes virilis is a non-burrowing crayfish species with a characteristic habitat preference for rocky bottomed lakes and streams within the Canadian Precambrian Shield (Crocker and Barr 1968).

Morphometric surveys of L239 and L240 showed that approximately 70-80% of the littoral zone extending to a depth of about 5 m and representing 15-17% of the total lake surface area is composed of broken rock shelf, large boulders (50-100 cm diam.), and coarse gravel (3-10 cm diam.) (Stockner and Armstrong 1971; Schindler et al. 1973). Flint and Goldman (1977) have shown that crayfish growth can be strongly influenced by differences in number and mean size of stones. Extreme care was therefore taken to ensure that the collection sites chosen among the four study lakes were of uniform physical characteristics so that between-lake differences in growth would not be related to habitat type. The geomorphology of the collection sites in the present study corresponds to Capelli's (1975) 'type-A substrate' (mostly rocks and gravel; high shelter potential) found to be most suitable for crayfish growth and abundance.

Methods of collection

Problems involved with the capture of ovigerous females in seclusion or elusive young-of-the-year crayfish directly by hand collection (the previously accepted diver technique) were alleviated with the aid of a diver-operated suction gun (Davies and Ramsey, in prep.). *Orconectes virilis* is most active at night (Capelli 1975) when individuals emerge from their rock crevasses into the open to feed and move freely about, thus allowing their capture by divers. All collections were therefore conducted at least one hour after sunset.

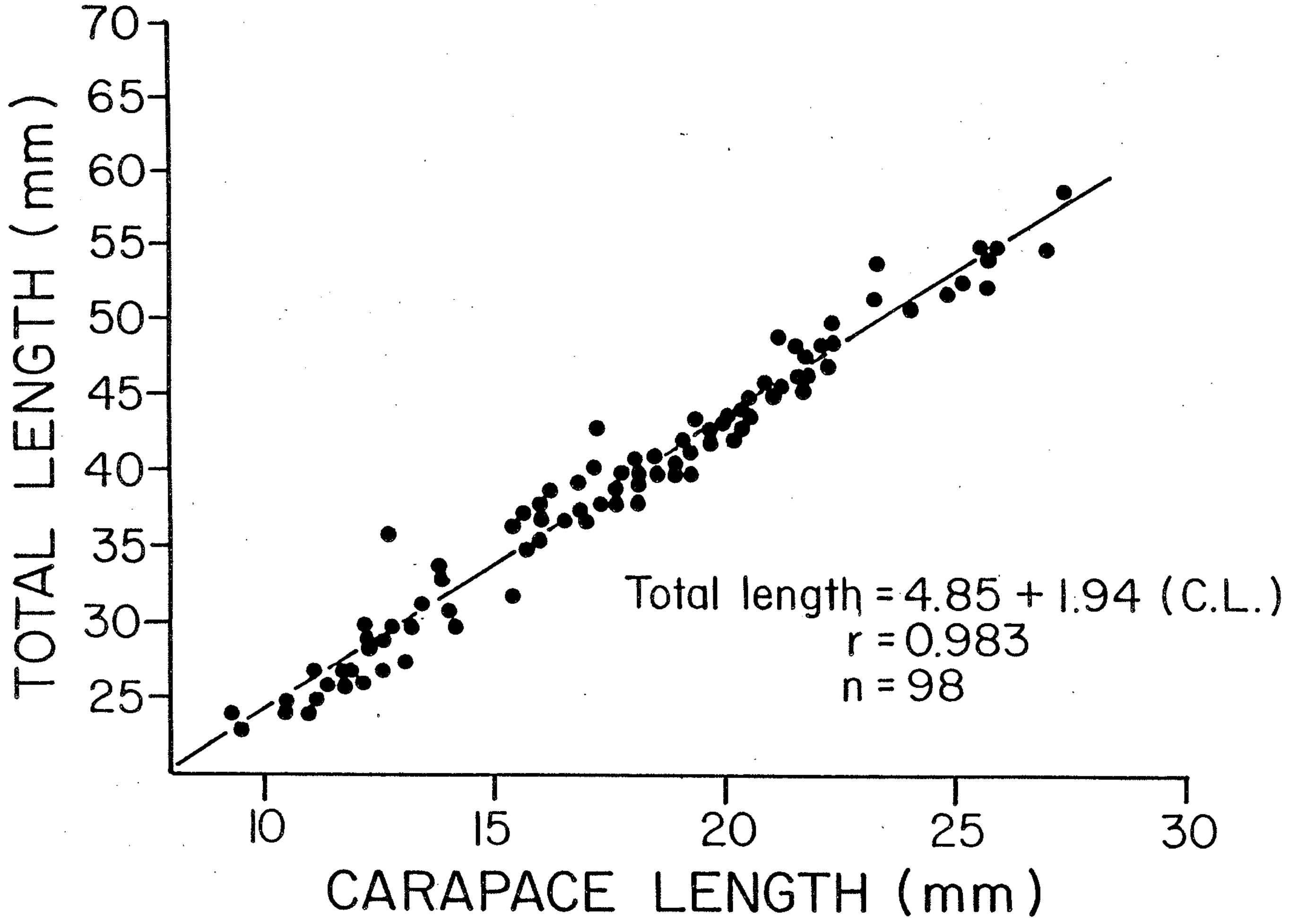
Crayfish were sampled over the depth range 0-6 m.

Data recorded from collected specimens included (1) carapace length measured with vernier calipers to the nearest 0.1 mm from tip of the rostrum to posterior edge of the carapace (usually about half total length; Fig. 1); (2) occasionally total length measured to the nearest 0.1 mm, and weight in grams, of animals retaining all appendages; (3) sex characteristics, pleopod form I or II in males, presence of eggs or cement gland development in females; and (4) subjective measurement of exoskeleton rigidity and/or molting stage assessment. Crayfish were usually returned to the sampling area within 24 hr of capture. Handling mortality did not occur and crayfish returned to the lake were in good condition.

Growth analysis

Growth of age classes was assessed by analysis of population length-frequency distributions (Fig. 2). Momot (1967) studied field growth rates of *O. virilis* and found that, while young-of-the-year (age 0) and yearlings (I) form distinct groups, there was an overlap between age II and III individuals producing an accuracy of 80% in their correct ageing from inspection of the size-frequency data. Cumulative frequencies were plotted on probability paper to enhance the discrimination of size data into discrete components (Cassie 1954) (Fig. 3) permitting the assignment of mean size values and corresponding standard deviations to each age class. Results for both sexes were

Figure A.1: Linear regression of total length vs. carapace length.



TOTAL LENGTH (mm)
70
65
60
55
50
45
40
35
30
25
10
15
20
25
30
CARAPACE LENGTH (mm)
Total length = 4.85 + 1.94 (C.L.)
r = 0.983
n = 98

Figure A.2: Length-frequency distributions of crayfish collected over rocky substrate in L223 during summer 1979. Positioning of roman numerals indicates mean age-group size calculated by the probit plot method of Cassie (1954).

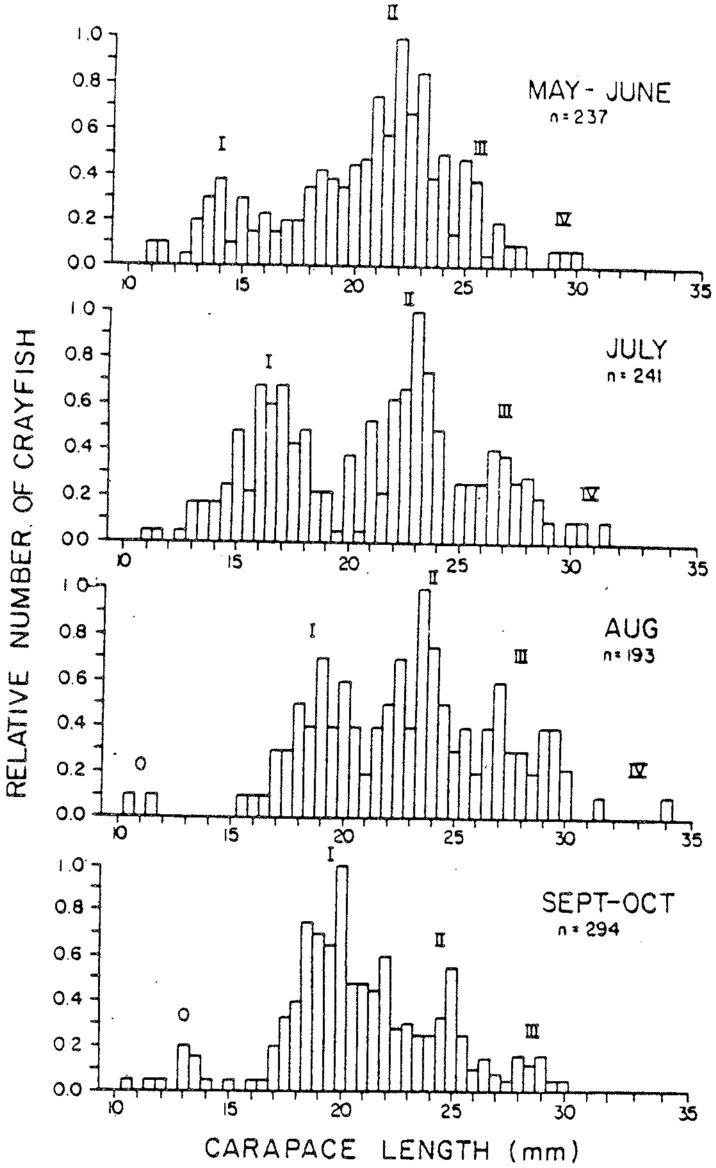
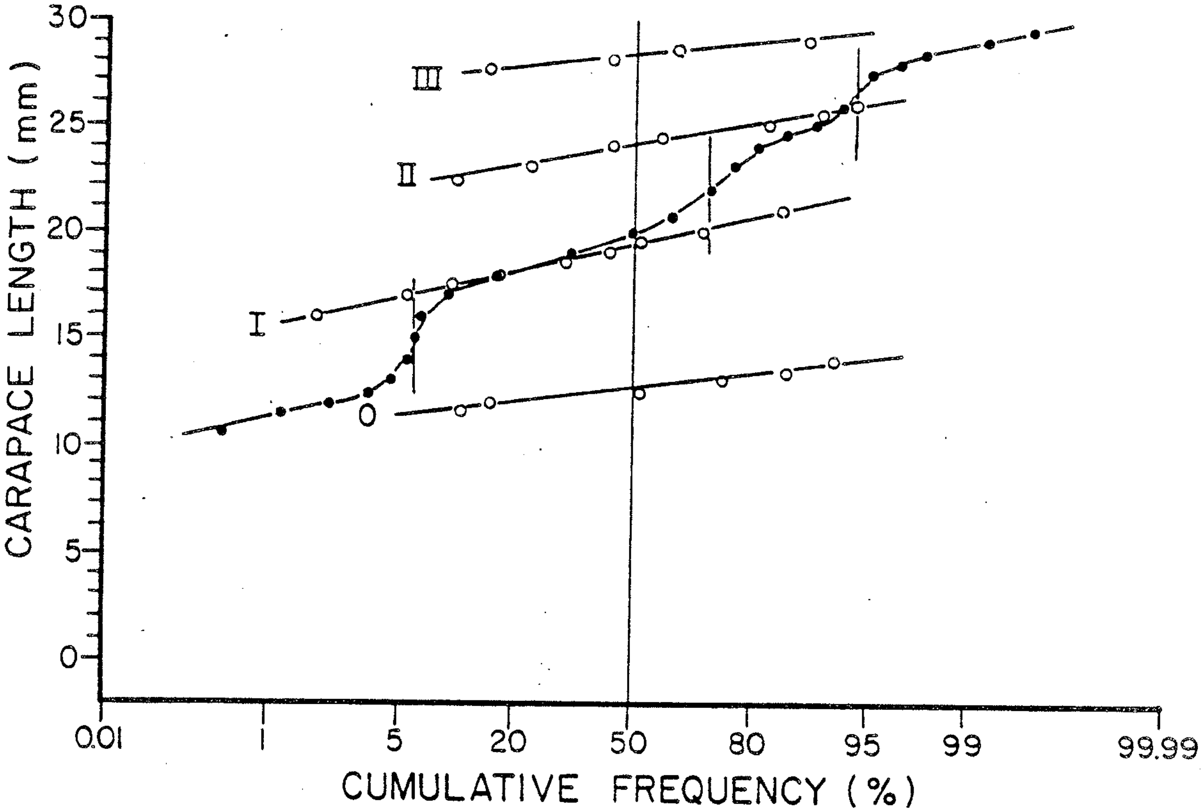

MAY - JUNE
n=237
I
II
III
IV
JULY
n=241
I
II
III
IV
AUG
n=193
O
I
II
III
IV
SEPT-OCT
n=294
O
I
II
III
0.0
0.2
0.4
0.6
0.8
1.0
10
15
20
25
30
35
RELATIVE NUMBER OF CRAYFISH
CARAPACE LENGTH (mm)

Figure A.3: Probability plot of length-frequency distribution of the October-September collection in L223 following Cassie (1954). The symbol represents actual observed cumulative percentage distribution and delineates the distribution of individual size-classes following discrimination. Mean lengths are where the lines cross the 50% frequency level and standard deviations are determined by the difference between the mean and the 15.87 or 84.13 per cent level on the lines.


CARAPACE LENGTH (mm)
30
25
20
15
10
5
0
0.01
1
5
20
50
80
95
99
99.99
CUMULATIVE FREQUENCY (%)
III
II
I
0

combined because preliminary separation of sexes demonstrated the same pattern as the conjoined results (Hopkins 1966).

Data on growth increment per molt was obtained by collecting animals nearing ecdysis from one lake (L240) and retaining them in laboratory aquaria. Only results from those crayfish that molted within a 5 day period were used.

Values of mean carapace length at the beginning and end of the growing season (late May to mid September) were converted to weight units by the length-weight relationships determined for each lake (e.g. Fig. 4). The instantaneous growth rate (G) was calculated for all age groups from each of the four study lakes after Ricker (1975):

$$G = \log_e (W_t/W_0)$$

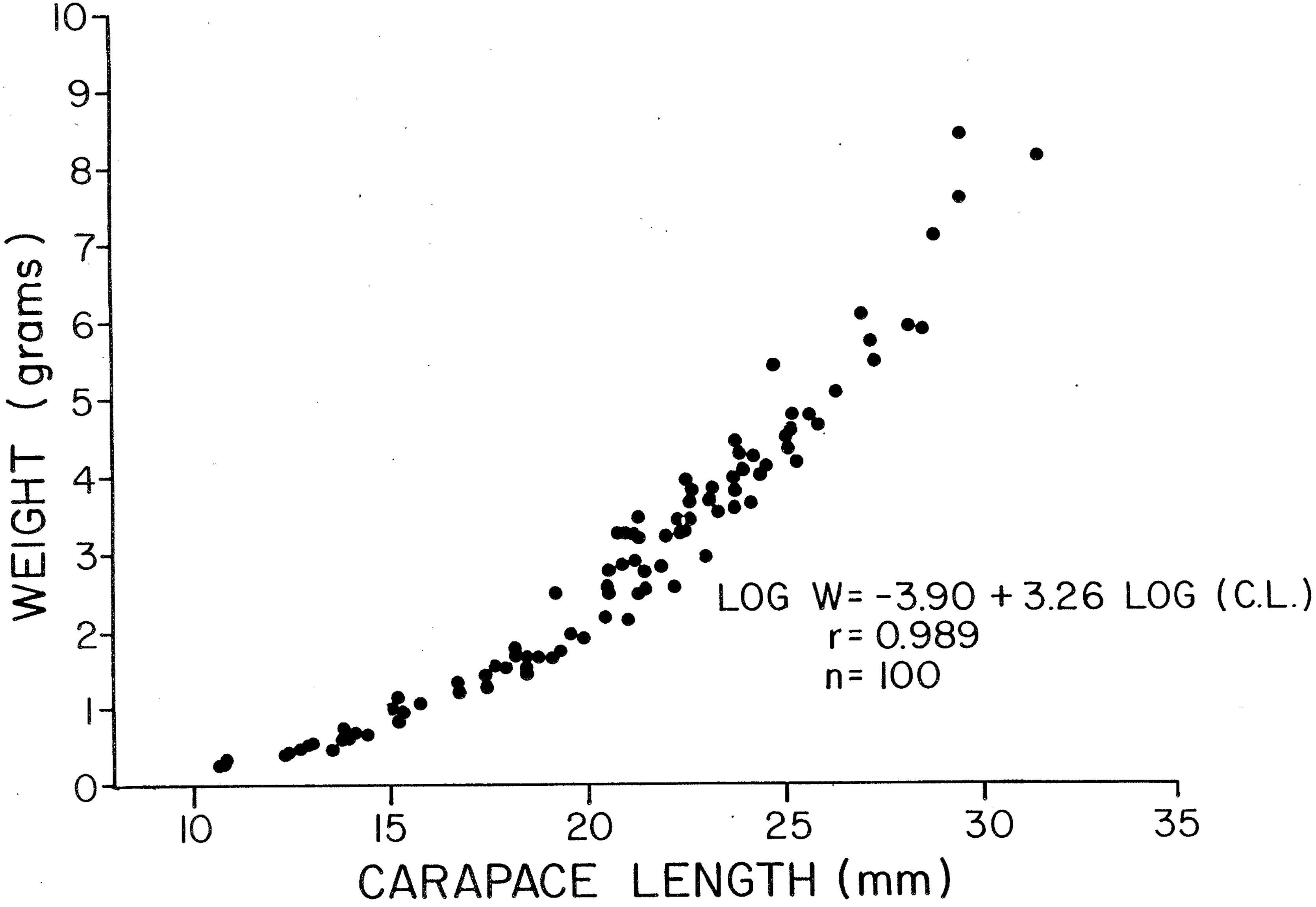
where G is the instantaneous growth rate (g unit time^{-1}), W_0 is the initial weight (g) and W_t the final weight. Growth was also expressed as the relative percentage increase in size (R) such that:

$$R = (l_2 - l_1)/l_1 \times 100$$

where l_1 is the initial carapace length (mm) and l_2 the final carapace length at the end of the growing season (Ricker 1975).

To confirm growth rate differences between the four _O. virilis_ populations, use was made of the relationship between crayfish size and maturity. Plotting cumulative percent of ovigerous females or form I males against carapace length on probability paper permited the calculation of mean size at onset of sexual maturity. According to this technique, a population exposed to environmental factors that are

Figure A.4: Length-weight relationship for age I-IV *Orconectes virilis* collected from L223 during August 1979.


WEIGHT (grams)
0
1
2
3
4
5
6
7
8
9
10
CARAPACE LENGTH (mm)
10
15
20
25
30
35
LOG W= -3.90 + 3.26 LOG (C.L.)
r= 0.989
n= 100

unfavorable to growth, will decrease its average size at onset maturity (SOM) relative to another unaffected population (Wenner et al. 1974).

The percentage of age I males and females that attained sexual maturity during the 1979 growing season was calculated by comparing information from the probit plots of cumulative length-frequencies and mean size at maturity. The percentage of unfertilized mature females (individuals with obvious cement glands and in the process of resorbing ovarian eggs) was obtained from spring 1980 collections.

Relationship to lake productivity

To relate crayfish growth rate to population size, two independent estimates of relative population abundance in the four study lakes were obtained. In 1979, mean catch per unit effort was calculated from at least 5 dives. In mid-July 1980, when the majority of the crayfish were in the intermolt condition and most active because of high water temperatures, 20 minnow traps baited with macerated white suckers were set throughout the SCUBA collection areas. To eliminate catch bias due to size and sex dominance, only the number of adult males caught per trap was compared (Capelli 1975). Capelli showed that the catches of adult males was strongly correlated($r = 0.95$) with diver-determined densities and could be used as an index of relative abundance of crayfish between lakes.

To obtain a wider range in lake productivity, as measured by chlorophyll-a concentration (Fee 1979), crayfish were sampled from several other lakes (L302, Roddy Lake) within or nearby (Lake-of-the-Woods, Clay Lake, Falcon Lake) the ELA.

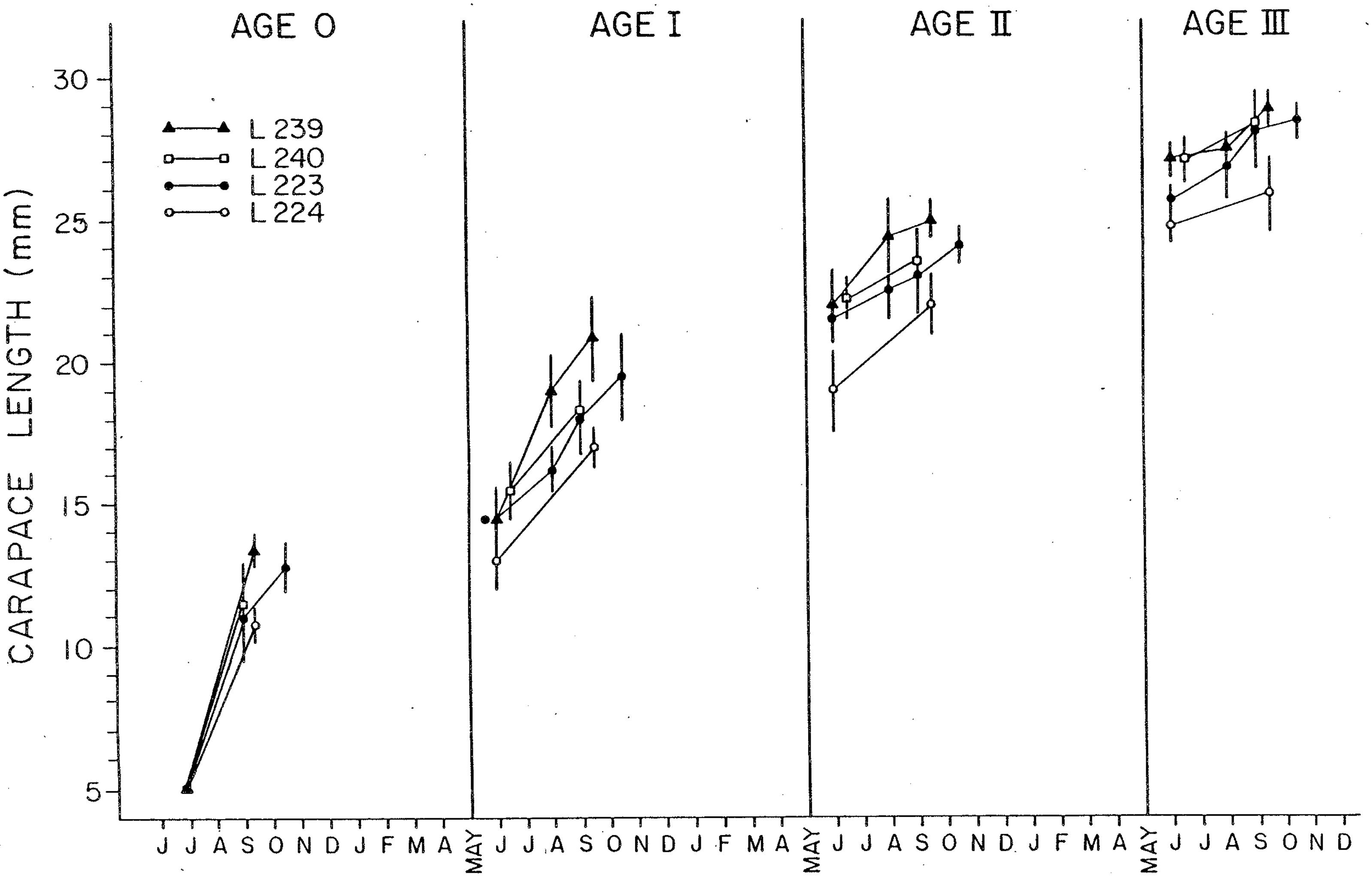
RESULTS

Young O. virilis hatched at a carapace length (C.L.) of 4-5 mm in all lakes (Fig. 5). By the end of the first growing season, the young-of-the-year (y-o-y) crayfish ranged in size from 10-15 mm C.L. (x = 12.3 mm). Age I crayfish were 16-22 mm (x = 18.3 mm) in October at the end of the second years growth. Age II crayfish reached a size range of 21-26 mm (x = 23.7 mm) in their third year and 25-31 mm (x = 27.9 mm) by the end of the fourth year (age III). A few age III crayfish survived the winter and appeared in the succeeding summer collections as age IV animals (e.g. Fig. 2) but all expired before their fifth summer was complete. The largest crayfish collected from L239 was an age IV male with a C.L. of 35.45 mm (7.0. mm T.L., 11.60 g wet wt). The largest age IV males sampled in the other study lakes were 33.8 mm in L223, 33.60 mm in L240, and 29.10 mm in L224.

There was a significant difference (ANOVA test) in the mean size of crayfish between lakes for each age group. Invariably, crayfish from L239 were the largest, while those from L224 were the smallest. Mean carapace lengths for L240 and L223 crayfish were intermediate with L240 individuals being slightly but not significantly the larger of the two.

No increase in size occurred during the 6 month winter period. SCUBA collections in L223 on Oct 15-22, 1979 produced the following mean

Figure A.5: Growth of Orconectes virilis age-groups in the four study lakes in the Experimental Lakes Area, northwestern Ontario. Symbols indicate mean sizes ± standard deviations of crayfish collected during summer 1979. A size of 20 mm C.L. denotes the approximate lower boundary of sexual maturity.


AGE O
AGE I
AGE II
AGE III
CARAPACE LENGTH (mm)
30
25
20
15
10
5
L 239
L 240
L 223
L 224
J J A S O N D J F M A
MAY J J A S O N D J F M A
MAY J J A S O N D J F M A
MAY J J A S O N D

sizes: age 0 - 12.8 mm, age I - 19.5 mm, age II - 24.1 mm, and age III - 28.4 mm (Fig. 5). When sampled on May 18-19, 1980 there was no significant difference ($p > 0.05$; T-test) in the mean length for the equivalent L223 age components: age 0 crayfish, now represented as age group I - 12.4 mm, age II - 19.5, age III - 24.5, and age IV 28.9 mm. This served as a check on the validity and precision of the data collection and analytical methodology, and also implied that size differences observed between lakes were real.

The growth increment between males and females was not significantly different ($p > 0.05$; student's T-test). The mean percentage growth increment decreased with age: 14.8% for age 0 crayfish, 11.0% for age I, 6.6% for age II and 2.6% for age group III (Fig. 6). The average growth increment per molt for L240 crayfish was was calculated at 1.5 mm for age-group 0, 1.7 mm for age I and II animals, and 1.4 mm for the age III class (Fig. 7). The number of molts completed by y-o-y crayfish was calculated using the formula by Van Deventer (1937):

$$Z = (x-y/\text{increment}) + 2$$

where Z represents the number of immature molts, x and y are means of the size group at the beginning and end of the season, and the value 2 denotes the number of molts while still attached to the maternal female. Age 0 O. virilis completed approximately 7 molts during their first growing season. Similar analysis showed that immature age I crayfish molted 3-4 times and mature age II and III individuals molted

Figure A.6: Growth increment per molt as a percentage of original carapace length in L240 crayfish. Open squares represent mean % growth increment for the various age-classes.

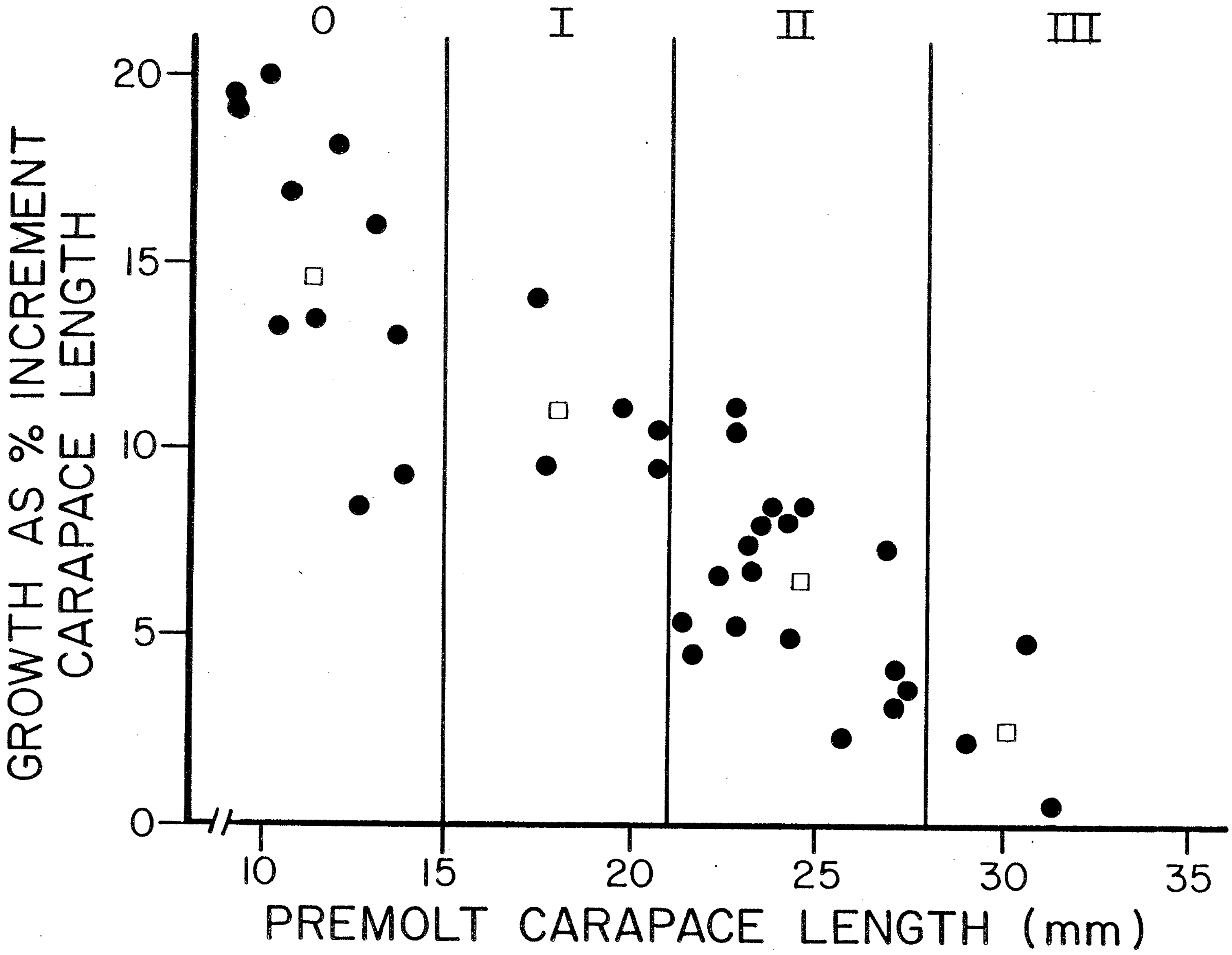
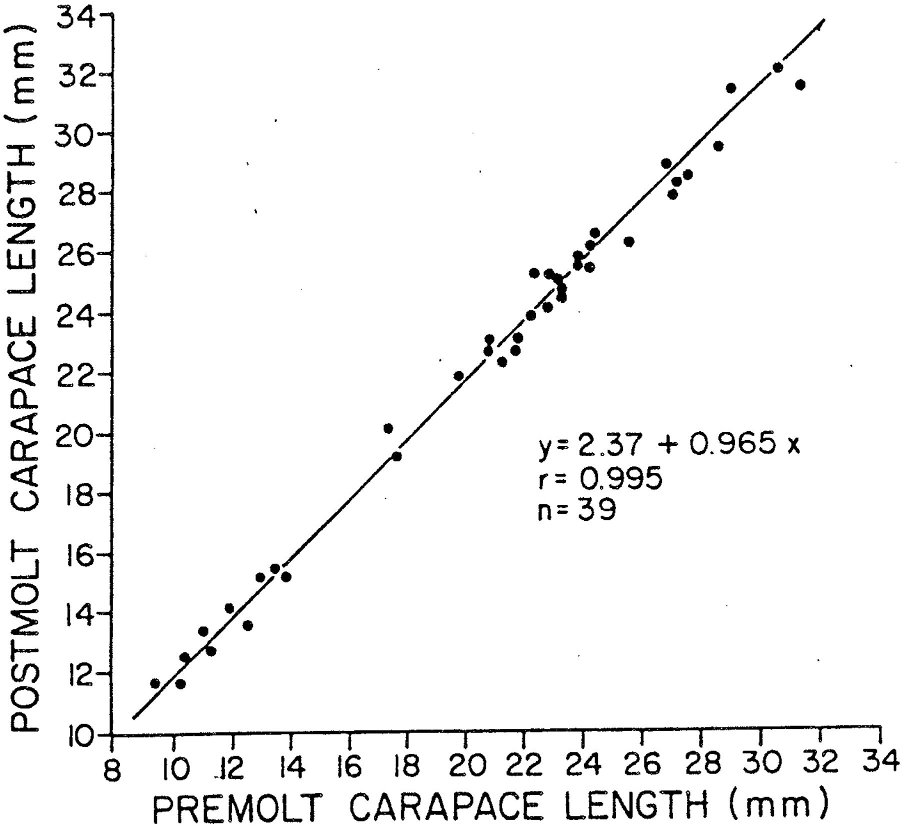

O
I
II
III
GROWTH AS % INCREMENT CARAPACE LENGTH
0
5
10
15
20
PREMOLT CARAPACE LENGTH (mm)
10
15
20
25
30
35

Figure A.7: Linear relationship between premolt and postmolt carapace length of L240 crayfish.


POSTMOLT CARAPACE LENGTH (mm)
PREMOLT CARAPACE LENGTH (mm)
34
32
30
28
26
24
22
20
18
16
14
12
10
8
10
12
14
16
18
20
22
24
26
28
30
32
34
y= 2.37 + 0.965 x
r= 0.995
n= 39

1-2 times depending on their sex.

Annual growth rates declined with age in all lakes and in both years (Table 1). The L224 population had the slowest average overall growth rate, L223 and L240 crayfish intermediate growth, with the L239 population displaying the highest rate of growth. There was an average 12% increase in crayfish growth during the 1980 season over the preceding year. Lake 224 crayfish had the largest year-to-year variation, approximately 15%; crayfish from L239 showed the smallest increase in growth rate between years, about 8%; and L223 and L240 crayfish were intermediate.

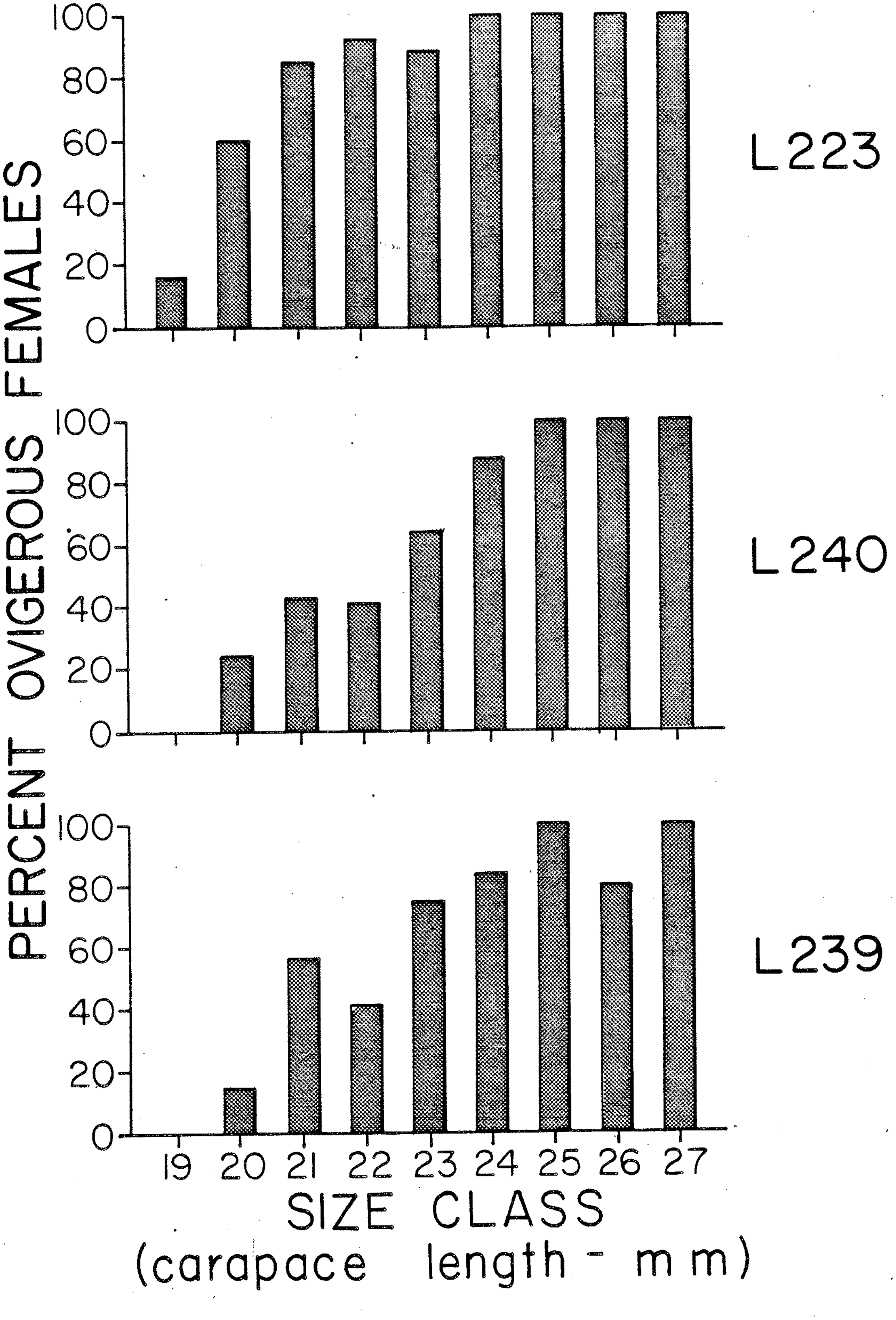
At ELA almost all crayfish greater than 25 mm C.L. were mature (Fig. 8). The percentage of mature crayfish within a particular size class varied among the lakes. Results from SOM index comparisons are similar to those obtained from growth analyses (Table 2). Female _O. virilis_ reach maturity at a size 1-2 mm less than those for the males. The fastest growing population in L239 has the largest SOM's while the slow growing L224 population displays the smallest SOM values. Size at maturity indices are lowest in 1979, again suggestive of a growth retardation in that particular year.

Mean crayfish sizes were related to mean annual primary production (r = 0.85, 0.95, 0.94, and 0.99 for age groups 0 to III, respectively). As expected, correlations were highest for age-group III crayfish whose present size reflects the cumulative effects of four years growth. Sizes of y-o-y on the other hand would be more closely correlated to

Table 1. Growth rates of *Orconectes virilis* at ELA during summer 1979 and 1980. G = instantaneous growth rate ($\log_e w_2 - \log_e w_1$); R = relative % growth rate ($l_2 - l_1/l_2 \times 100$).

		L224		L223		L240		L239		Average	
Year	Age	G	R	G	R	G	R	G	R	G	R
1979	0	1.16	144.4	1.34	157.8	1.29	173.3	1.47	197.8	1.32	168.3
	I	0.27	23.1	0.34	27.6	0.32	24.3	0.49	44.1	0.36	29.8
	II	0.19	10.5	0.11	8.4	0.11	9.2	0.17	13.6	0.15	10.4
	III	0.05	3.6	0.13	9.7	0.07	6.0	0.08	7.0	0.08	6.6
	Total	1.67	181.6	1.92	203.6	1.80	212.8	2.20	262.4	1.92	215.1
1980	0	1.22	155.5	1.39	166.7	1.37	184.4	1.53	211.1	1.38	179.4
	I	0.37	28.0	0.43	35.7	0.28	23.3	0.36	29.7	0.36	29.2
	II	0.18	15.3	0.22	17.1	0.21	16.7	0.32	25.7	0.23	18.7
	III	0.11	10.9	0.16	11.8	0.16	12.2	0.18	14.5	0.15	12.4
	Total	1.88	209.7	2.20	231.3	2.02	236.6	2.39	281.0	2.12	239.7

Figure A.8: Percentage variation of sexual maturity with size in ELA crayfish populations for (A) ovigerous females and (B) form I males, collected during spring and fall 1979 respectively.


PERCENT OVIGEROUS FEMALES
100
80
60
40
20
0
L223
L240
L239
19 20 21 22 23 24 25 26 27
SIZE CLASS
(carapace length - mm)

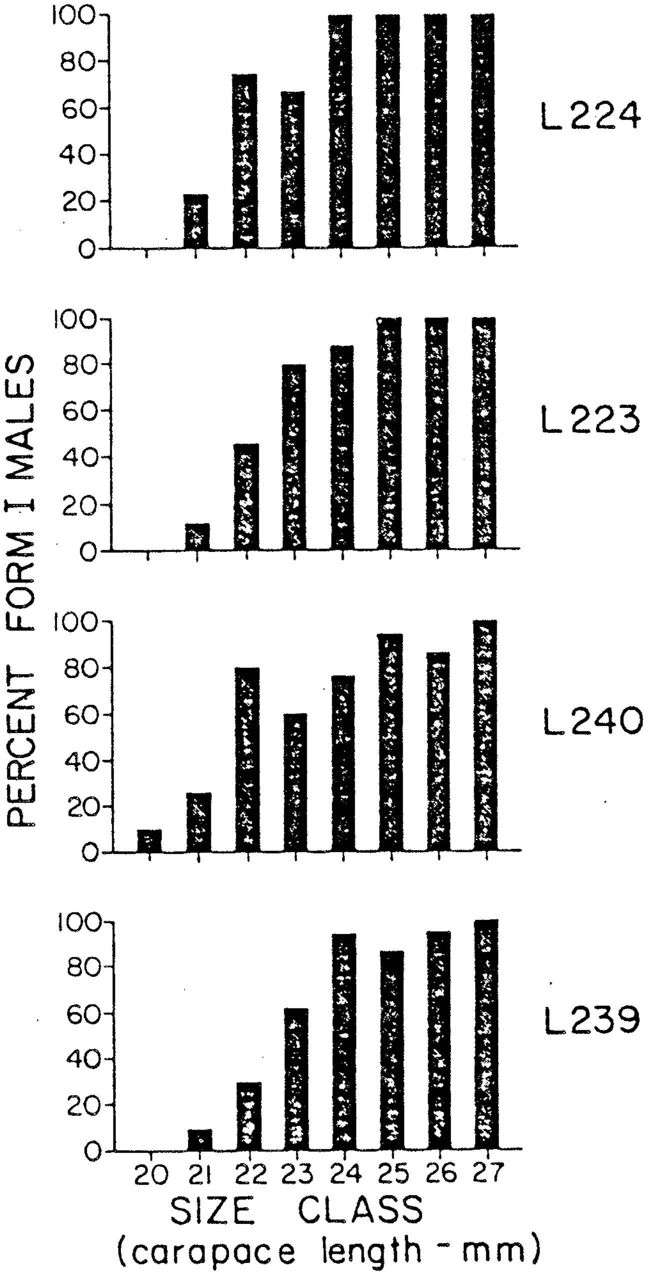

PERCENT FORM I MALES
100
80
60
40
20
0
L224
L223
L240
L239
20 21 22 23 24 25 26 27
SIZE CLASS
(carapace length - mm)

Table 2. Mean carapace length (mm) at onset of sexual maturity for ELA *Orconectes virilis* male (form I condition) and female (ovigerous condition) crayfish as calculated by the probit plot method of Wenner et al. (1974). Standard error about the mean averaged ±0.22 mm. Values represent males collected during the autumn of the year listed and females sampled the following spring.

	1978		1979		1980	
Lake	M	F	M	F	M	F
L239	-	22.0	22.6	21.6	23.6	-
L240	-	21.4	22.2	20.8	23.1	21.3
L223	22.4	20.1	22.2	19.8	22.8	20.7
L224	-	19.2	21.5	-	22.0	-

primary production during that particular year rather than a four year averaged value. The average rate of growth was also related to the phytoplankton production (r = 0.84). Unfortunately, due to the small sample size (n = 4) only the relationship of age III mean size to algal production was significant at the P = 0.05 level. To alleviate this statistical problem and to obtain a wider range in lake productivity, crayfish were sampled from several other lakes of increasing mesotropy. The relationship to average lake chlorophyll-a concentration explains 87% of the variation in maximum crayfish size among the ELA lakes (Fig. 9).

DISCUSSION

Comparison with other geographic areas

O. virilis populations at ELA exhibit the slowest growth recorded for this species at any location (Table 3). Growth rates at ELA were only 27-38% of those reported for other O. virilis populations. The greatest difference occurs for age-group I crayfish (ELA growth is only 17-22% that of other areas), followed by a range of 25-44% for age III, and then growth of 34-63% and 37-58% of that for other areas for age 0 and III, respectively.

There was no significant difference in the growth increment between sexes in O. virilis, similar to other crayfish species (Hopkins 1967; Flint 1975; Pratten 1980). The calculation of average growth increment per molt for L240 crayfish was 0.5-1.1 mm less than that

Figure A.9: Relationship of maximum size of collected <u>Orconectes virilis</u> to average annual epiliminon chlorophyll concentration in the ELA lakes. Data for Kenora Harbour (Lake-of-the-Woods), Falcon Lake, and Clay Lake (open circles) were not included in the calculation. Chlorophyll data for ELA from Fee (1979; 1980) and for other nearby glacial Canadian shield lakes, pers. comm. from J. Donnets, OMNR, Kenora; Man. Dept. of Mines and Resources; and J. Rudd, Freshwater Institute.

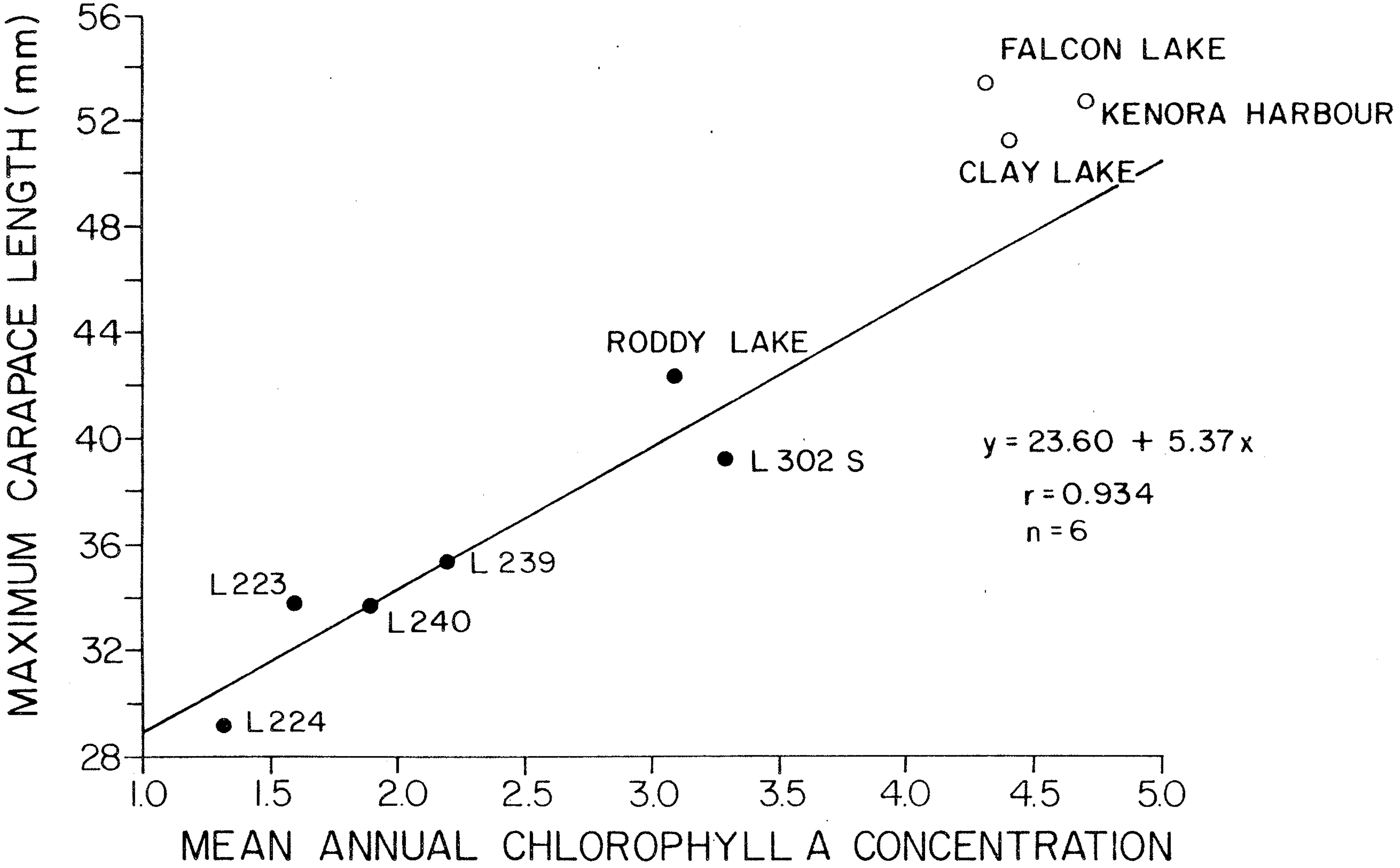

MAXIMUM CARAPACE LENGTH (mm)
56
52
48
44
40
36
32
28
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
MEAN ANNUAL CHLOROPHYLL A CONCENTRATION
FALCON LAKE
KENORA HARBOUR
CLAY LAKE
RODDY LAKE
L 302 S
L 239
L223
L240
L224
y = 23.60 + 5.37 x
r = 0.934
n = 6

Table 3. Comparison of carapace length ranges and mean lengths (values in mm) for *Orconcetes virilis* age groups from the present study with those of other geographic areas.

Study	Location	Site	Age 0	Age I	Age II	Age III
Aiken (1967)	Alberta	stream	8	17 - 24	30 - 35	36 - 42
Weagle & Ozburn (1972)	N.W. Ontario	stream	4 - 14	14 - 28	28 - 31	31 - 44
Momot (1967)	Michigan	marl lakes	14.5	28.6	34.0	38.8
Momot (1978)	N.W. Ontario	marl lakes	18.0	30.5	36.0	41.7
Present study	ELA	oligotrophic lakes	4 - 16 12.3	14 - 23 18.3	20 - 27 23.7	24 - 32 27.9

obtained for a population of O. virilis with substantially larger individuals (Weagle and Ozburn 1972).

The close agreement between the results obtained by growth increment analysis and the change in size observed within each age component in the L240 crayfish population during 1980 supports Flint's (1975) statement that growth calculated from the statistical analysis of population frequency distributions, can truly represent crayfish growth patterns.

The smallest ovigerous female (18.35 mm) and form I male (19.15 mm) are considerably smaller than those found for other O. virilis populations (Weagle and Ozburn 1972; Momot 1967) but similar to ranges recorded for O. propinquus (Van Deventer 1937; Capelli 1975).

Molting in ELA lakes was delayed compared to populations from Michigan (Momot 1967), New Hampshire (Aiken 1965) and southern Ontario (Crocker and Barr 1968; Berrill 1978) but was approximately synchronous to populations from Alberta (Aiken 1967) and N.W. Ontario near Lake Superior (Weagle and Ozburn 1972). Young-of-the-year crayfish from L240 molted about 7 times during their first growing season. Weagle and Ozburn's (1972) calculation of 5 age 0 molts has been criticized by Aiken who believed 7 occurred. Immature age I O. virilis at ELA had 3-4 molts, supportive of Weagle and Ozburn's findings. The timing of molting for immature crayfish at ELA could not be accurately determined although a detailed chronicaling of these events for O. virilis in N.W. Ontario is presented in Weagle and Ozburn (1972).

Growth comparison within ELA

Crayfish growth in the oligotrophic Precambrian Shield lakes of ELA is regulated by extrinsic environmental variables. Substrates and temperature regimes are similar among the study lakes and thus cannot be identified as the source of between-lake variation in growth. Ionic concentrations, including Ca^{++}, differ between ELA lakes but are not considered to exert an effect upon crayfish growth (Capelli 1975). A relationship does exist, however, between crayfish growth and the trophic status of ELA lakes.

Although crayfish can rely upon filter-feeding of algae for survival and growth (Budd et al. 1979), analysis of stomach contents and observations of feeding habits suggest that crayfish growth at ELA is more dependent upon several factors indirectly associated with the production of phytoplankton. The remains of chiromonids, an important contributor to benthic communities at ELA, were always found in y-o-y and juvenile crayfish guts. Davies (1980) found that phytoplankton production explained more than 97% of the variation in the average biomass of chironomid emergence among selected ELA lakes. Diatoms, a significant component of ELA periphyton communities (Stockner and Armstrong 1971), and recognized as an important food source to crayfish in oligotrophic lakes (Flint and Goldman 1975; Capelli 1975), were found in all adult O. virilis stomachs. It was common to see individuals of all sizes crawling about on large boulders, actively scraping and ingesting the extensive growths of epilithic algae. Epilithic algal biomass in Lake Tahoe varied in relation to localized differences in phytoplankton productivity (Abrahamsson and Goldman 1970). Periphyton production and standing crop were higher in L239 than L240 (Schindler et al. 1973) and a general relationship between ELA lake trophic status (enriched eutrophic basins unsuitable for crayfish, excluded) and areal periphyton standing crop may be expected. The predominant food resource for ELA crayfish appears to be a mixture of decaying autochthonous and allochthonous organic matter. The amount of detrital food reserves of autochthonous origin can certainly be expected to be related to the productivity of the basin. For example, suspended carbon values in L302S are much greater than those in L224 (Prokopowich 1979).

A direct relationship between productivity and decapod growth has been demonstrated in a few studies. Newman and Pollock (1974) related large differences in rock-lobster growth within a small area to variations in the amount of benthic biomass available and suitable for food, which in turn was dependent on primary productivity. Using transplantation experiments Svardson (1949) showed that A. astacus growth was regulated by intake of food within lakes of similar thermal regimes and Andrews (1907) found that the rate of growth in young crayfish depended directly upon the food supply in laboratory experiments.

Molting in O. virilis ceases when water temperatures decline below 10°C (Aiken 1967) so no overwinter growth occurs. Van Deventer (1937), Abrahamsson (1966) and Pratten (1980) found variations in crayfish growth dependent on year-to-year climatic differences. The 1979 spring season was one of the coldest on ELA record with ice-out not occurring until May 13, compared to the exceptionally hot spring of 1980 with ice-out on April 25. In 1979 the growing season (above 10°C) was 140 days whereas in 1980 crayfish had 164 growing days (K. Beaty, unpubl. data). The growing season in 1978, a more typical ELA year, was 155 days. The average 12% increase in rate of crayfish growth during 1980 over that of the preceding year can, in part, be attributed to the two extremes in average spring temperatures and the resulting approximate two-week advancement of molting events. In addition, the autochthonous production of phytoplankton is an important quantitatlve indicator for crayfish diet at ELA. The average annual coefficient of variation for phytoplankton production for ELA lakes is about 20% with the lowest variability occurring in lakes with the longest residence times (Fee 1980). The order of increasing epilimentic production variability from L239-L223-L240-L224 corresponds to the increasing magnitude of the crayfish year-to-year growth variation among the four study lakes.

The size at which _O. virilis_ become sexually mature is a sensitive index for discriminating growth differences between ELA lakes as well as year-to-year differences within a lake. These results, together with findings of Flint and Goldman (1977) support the use of SOM for assessing size differences between discrete crayfish populations where accurate estimates of growth rates are not available.

There was no significant difference ($p > 0.05$; test for covariance) in the length-weight relationship between sexes in any lake, a result consistent with other studies on O. virilis (Momot 1967) and attributed to a countering of the positive allometric growth of male crayfish with the increased abdominal width of the females (Flint 1975a). Comparisons of length-weight relationships among discrete populations are commonly used in fishery management and were suggested by Romaire et al. (1976) to reflect the relative fitness or health of crayfish in response to different environmental conditions. There was no significant difference ($p > 0.05$; test for covariance) in the length-weight regressions among the ELA study lakes. Slope coefficients ranged from 3.0-3.3, indicating a slight allometric form of growth. The close similarity of these ELA growth equations to that of a much faster growing population (Momot 1967) indicates that this relationship has little relevance as a descriptive tool to help identify growth differences between O. virilis populations.

Mechanism of growth variation

Three independent factors limit crayfish growth in the ELA region. Certainly the short and cooler growing season will restrict growth in such northern populations (Momot et al. 1978), but much more important though, is the overriding effect of lake productivity. Growth in ELA populations is food limited, so even lakes with similar temperatures within a small local area can display wide differences in growth. The large sizes obtained by crayfish in Roddy Lake or Kenora harbour are comparable to values tabulated by Momot et al. (1978) for more southerly populations. The seasonal differences in growth rate between the two extreme years (ca 12%) are much less than growth variations between lakes (ca 30% between L224 and L239 populations). Thirdly, a major food for crayfish in such oligotrophic lakes with limited macrophytes is allochthonous plant detritus from the surrounding watersheds. _O_. _virilis_ of all age groups at ELA consume a large amount of decaying terrestrial organic matter in the form of wood fragments, pine needles, and pollen grains (France, unpubl. data) which could provide them, via cellulose digestion, with trace elements essential for growth (Momot et al. 1978). Detailed characteristics and the importance of terrestrial influxes as food sources in several _O_. _virilis_ ponds have been described (Jones and Momot 1981). The ELA region is characterized by a dense boreal subclimax forest surrounding all lakes to within a few meters of the shore with dominant riparian species including jack pine, black spruce, juniper, balsam fir and tamarack. Crayfish display preferences for certain leaf species and only eat pine needles as a last resort (Mason 1974). Momot et al. (1978) found that young _O_. _virilis_ would not consume pine needles but would rather scrape the material clean of periphytic algae, resulting in reduced growth. The present results substantiate the hypothesis of Momot et al. (1978) that lakes with a large predominance of conifer detritus will be less suitable for crayfish.

The growth rate of decapod Crustacea can be regulated by changes in the frequency of molting or in the magnitude of growth increment per molt. Conditions of low temperature or food intake can reduce the storage of substances and uptake at ecdysis, thereby decreasing the growth increment. A comparison of the results from this study with other life history data indicate that *O. virilis* display a "type I" growth strategy pattern (Wenner et al. 1974) in which growth variation is due to alterations in the magnitude of growth increment applied to a fixed frequency of molting events.

Relationship of growth to population regulation

This study suggests that *O. virilis* population abundance at ELA is regulated through density-independent control by environmental factors. Capelli (1975) attempted to explain variations in the density of *O. propinquus* among oligotrophic lakes in the climatically similar region of northern Wisconsin. A general correlation between substrate suitability and crayfish numbers was noted, yet substrate alone did not determine abundance because many lakes with type A substrate yielded only few crayfish. He concluded that although substrate probably determined some upper limit for a potential crayfish population in a given lake, some other unmeasured factor(s) determined to what extent that potential was realized. In areas of oligotrophic lakes such as ELA, where crayfish growth is extremely restrained and closely associated with sexual maturity, small differences in growth will exertsubstantial influences upon reproductive capacity and hence population size.

At ELA, a correlation exists between growth rate and the percentage of age I crayfish that attain sexual maturity (Table 4). Because of greatly reduced growth, only 5% of L224 crayfish exceed the approximate lower boundary of sexual maturity size (ca. 20 mm C.L.) during their second season of growth (Fig. 5) compared to nearly three-quarters of the age I L239 population. The majority of recruitment into the L224 population therefore occurs through reproduction of the age II class after an additional years growth. Due to high mortality rates age group II is numerically only one-third to one-half that of the age I size class. The effective breeding population in L224 then is much smaller than those present in L223 and L240 which in turn are less than that in L239. Cole (1954) and Lewontin (1965) modelled the sensitivity of populations to changes in life history parameters and found that the age at first reproduction, particularly within short-lived species (such as crayfish), was the single most important factor affecting rate of population increase.

The size of the breeding population will directly determine the number of successful copulatory pairings between receptive individuals. Crayfish secrete no known sexual pheromone attractant (Mason 1970), instead mating is dependent simply upon the random meeting of mature animals (Weagle and Ozburn 1972). Although much of crayfish activity during the breeding season is devoted to procurring a mate, low winter temperatures may restrict natural pairings by decreasing movement

Table 4. Relationship of lake productivity and <u>Orconectes virilis</u> growth rate to reproduction and relative population abundance in the ELA region.

Lake	Epi primary production ($g\ C \cdot m^{-2} \cdot yr^{-1}$)[a]	Relative growth rate (total % increase)	% age I sexually mature		%mature females unfertilized	Mean ovarian egg count	Relative abundance (catch per hr effort)	Number adult males trap	Number Crayfish/m^2[b]
L239	16.9	262.4	F 80 M 65	73	3.3	98.1	65 ± 6	5.5	1.97
L240	15.7	212.8	F 70 M 50	60	9.1	88.3	51 ± 4	3.3	1.19
L223	15.8	203.6	F 65 M 35	50	11.5	88.7	53 ± 6	3.3	1.17
L224	12.4	181.6	F 10 M 0	5	18.8	77.7	33 ± 5	1.7	0.59

[a] Fee (1980).

[b] Calculated from modified Fig. 9 in Capell (1975): number/m^2 = $-0.01 + 0.36$ (number adult males/trap) $r = 0.95$, $n = 5$.

(Capelli 1975; Flint and Goldman 1977). This may be especially true for northern populations such as those at ELA with a 6-8 month winter season. Capelli (1975) hypothesized that in sparser crayfish populations, the distance between individuals might severely limit the frequency of contact during the winter producing a greater chance that a female had not mated at any given time. This was demonstrated to occur for O. virilis populations at ELA (Table 4). Due to the linear relationship between size of maternal female and number of eggs brooded faster growing populations will proportionally produce a larger recruitment of young (Table 4). These results combine to indicate a direct relationship between crayfish growth and population density at ELA supportive of some earlier Swedish research (Svardson 1949).

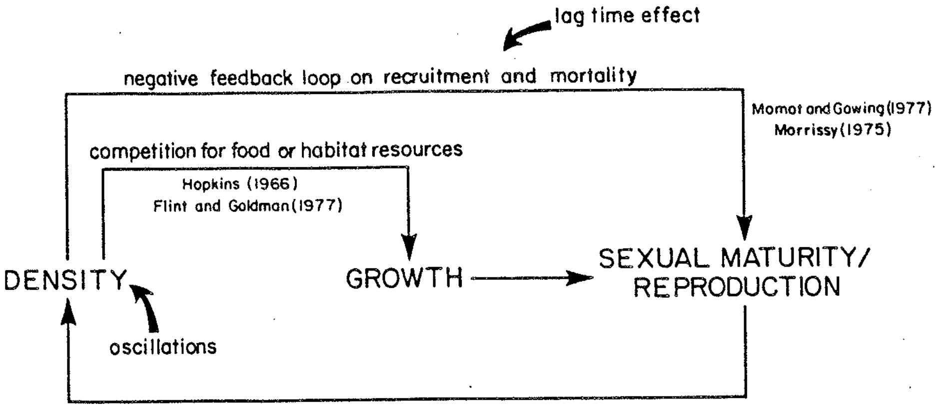
Goldman and Rundquist (1977) compared crayfish densities between two lakes of markedly different trophic status and found that increased lake productivity does not necessarily imply there will be increased crayfish abundance when such populations are limited by substrate rather than by food quality or quantity. Due to the presence of the marginally suitable "type-B" substrate (Capelli 1975) in L302S, crayfish abundance was low compared to the other type-A substrate study lakes. Therefore, even though the high growth rate in L302S had the potential for producing a large population size, the overriding factor of habitat insuitability prevented such an abundance level from being realized.

Densities of O. virilis populations at ELA are the lowest reported in the literature. Previous studies have dealt exclusively with only populations of high density (up to 27.5 m^{-2}) and, not surprisingly, have found an inverse relationship between density and growth due to competitive food shortages (Hopkins 1966; Morrissy 1975; Abrahamsson 1966, 1972). In the ELA region, where growth is food-limited and crayfish densities are low (0.59-1.97 m^{-2}), a reciprocal condition occurs where growth rate controls, rather than is controlled by, population density.

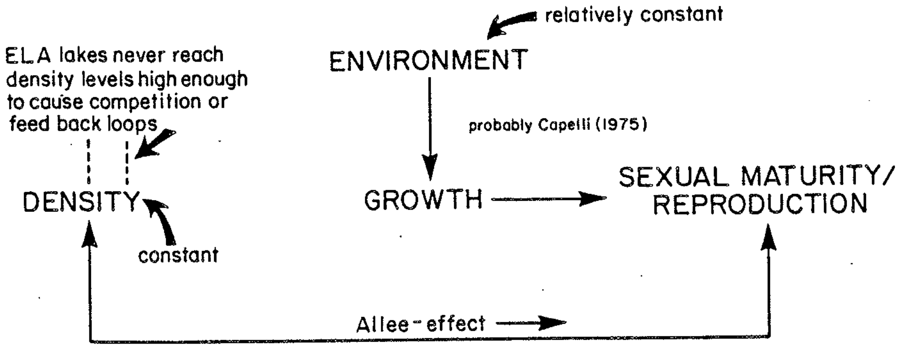
Previous crayfish studies (Fig. 10-a) have proposed density-dependent population control through competition for food (Hopkins 1966) or substrate (Flint and Goldman 1977) resources, or the operation of intrinsic feedback loops on recruitment (density of female brood stock, spawning frequency, fecundity, egg resorption) and/or mortality rates (Morrissy 1975; Momot and Gowing 1977). Momot et al. (1978) and Momot and Gowing (1977) state that population homeostasis is characteristic of the dense (1.9-6.1 m^{-2}) O. virilis populations in small Michigan ponds with stability due to a short lag between change in population density and subsequent adjustment in fecundity. Despite this statement, their data indicates a four-fold variation in population size from one year to the next and in one case, a 74% decrease over a two year period. This is contrary to the situation at ELA where population density is truly stable with no more than a 10% yearly variation in detailed population estimates for L223 (Davies, in prep.).

Poole (1974) concluded that on the whole populations are so strongly influenced by density-independent factors that rarely do they

Figure A.10: Conceptual representation of regulation mechanisms of crayfish population abundance. (A) density-dependent or intrinsic population control; (B) density-independent extrinsic environmental control, characteristic of ELA *Orconectes virilis* populations. Further details of terminology are explained in text.


lag time effect
negative feedback loop on recruitment and mortality
Momot and Gowing (1977)
Morrissy (1975)
competition for food or habitat resources
Hopkins (1966)
Flint and Goldman (1977)
DENSITY
GROWTH
SEXUAL MATURITY/
REPRODUCTION
oscillations

(A)


relatively constant
ENVIRONMENT
ELA lakes never reach
density levels high enough
to cause competition or
feed back loops
probably Capelli (1975)
DENSITY
GROWTH
SEXUAL MATURITY/
REPRODUCTION
constant
Allee-effect

(B)

reach a size where density-dependent factors become important. White (1978) advanced that most animals live in a variably inadequate environment where the single most important factor limiting their abundance is a relative shortage of suitable food. He further demonstrates that although without resource shortage there can be no competition, there can be shortage without competition such that food is so scarce in the environment as to be limiting regardless of competitive pressures. The lipid reserves in O. virilis have been shown to decrease dramatically during midsummer, suggesting that food is a limiting factor for crayfish in certain systems (Armitage et al. 1972). Crayfish densities at ELA, controlled by basic limnological conditions limiting growth, are believed to never reach density levels high enough to cause competition for habitat or food resources, nor create self-regulating feedback loops (Fig. 10-b). The relative constancy of the environmental influence on growth ensures a stable population size free of fluctuations evident in other intrinsically controlled populations of crayfish. It is possible that in oligotrophic systems such as ELA, species capable of regulating their growth and hence population size in relation to the low rate of food production, may actually be at an overall competitive advantage. Regulation of population abundance in O. virilis at ELA is not truly density-independent, in that some minimum threshold density is required before social interactions will lead to successful reproduction. This condition in which reproductive rates decrease rapidly in times of low density, negative feedback loops develop under high density levels, and population growth is optimum at only intermediate densities, is known as the Allee effect (Odum 1975) and is probably a general characteristic of all crayfish populations.

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