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Variation In The Shell Morphology And Growth Rate Of Lampsilis Radiata, A Freshwater Mussel

Robert Charles Bailey

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VARIATION IN THE SHELL MORPHOLOGY AND GROWTH RATE OF *Lampsilis radiata*, A FRESHWATER MUSSEL

by

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Submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

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ABSTRACT

I measured a correlation between the habitat of a freshwater mussel, Lampsilis radiata siliquoidea (Barnes 1823), and its shell morphology and growth rate. I then tried to determine the source of morphological and growth rate variation (environmental or genetic), and whether or not the phenotype/habitat correlation was due to differential adaptation.

Morphometric and annual ring analysis of mussels from Inner Long Point Bay, Lake Erie indicated that L. radiata from more exposed, sandier areas of the bay were faster growing and had thicker shells than those from less exposed, muddy areas. Variation among exposure areas in allozyme phenotypes of two gene loci (PGM and PGI) showed little evidence of genetic divergence. Statistically significant heterogeneity among exposure areas in PGM genotypes was small relative to that between mussel populations in Balsam Lake (in the Trent-Severn watershed) and the lower Great Lakes. Heritabilities of glochidia shell dimensions were low (<20%), providing little evidence of past or present disruptive selection of shell dimensions. Substrate preference was displayed by the mussels in experimental ponds. L. radiata preferred a finer, more heterogeneous substrate over a coarse sand, and larger mussels showed a stronger substrate preference than smaller individuals. Such habitat preference could influence the breeding structure of the population near the

borders of habitat areas.

A laboratory experiment using L. radiata from Inner Long Point Bay showed that the optimal shell morphology for burrowing depended on the substrate the mussel was placed in. Less obese, thinner-shelled mussels were better burrowers in sand; the converse was true in mud. The pattern of optimal burrowing morphologies found in the laboratory experiment did not accurately predict morphological variation in the natural population. This could be because of i) a poor fitness surrogate (burrowing), ii) lack of important environmental variation in the experiment (e.g. turbulence), or iii) a non-adaptive pattern of morphological variation in the natural population.

In summary, variation in the morphology and growth rate of L. radiata is caused by phenotypic plasticity. The observed correlation between shell phenotype and habitat may represent differential adaptation, but an experiment where other fitness correlates are measured, and more realistic environmental variation is incorporated, is needed to test this hypothesis.

ACKNOWLEDGEMENTS

I will begin by thanking Roger Green, who provided unfailing spiritual, intellectual, and material support for this project, and never made me feel "supervised". Paul Handford and Shiva Singh, as members of my committee, were always ready to exchange ideas and "straighten me out". Shiva added considerable logistic support of my electrophoretic adventures.

Additional scientific stimulation has come, over the years, from Dave Ankney, Keith Somers, Scott Hinch, Paul Hebert, and Ray Alisauskas in particular, and the Collip Community in general.

Greg Goff introduced me to Long Point Bay. Field work in the bay was simplified by loan of a boat from the Canada Centre for Inland Waters (Burlington) in 1984 and 1985, docking facilities provided by the Ontario Ministry of Natural Resources (Simcoe), and lodging periodically provided by the Long Point Bird Observatory. Norm's Marina provided a boat and assistant (thanks Robert) in 1986. Other technical help in the field came primarily from Scott Hinch, along with Cindy Walker, Vicki Young, Shirley Lim, and Rob Roy. Laboratory and pond work was simplified by the efforts of Vicki Young, Cindy Walker, Karen Watkinson, Scott Hinch, Roger Frappier, Helene Dupuis, Ken Carver, Bob Keith, Ann Overweel, and Rick Harris. The experimental ponds were generously loaned by Miles Keenleyside. Paul Hebert introduced me to cellulose acetate electrophoresis and gave

me access to his genetic data. Shirley Lim drafted all the good figures.

Administrative help from Marge Moulton and Callie Cesarini was invaluable. Jane Sexsmith made writing this thesis easier by her endless help with my "other job".

Lastly, I would like to thank A., B., and especially C. Bailey, not "because they made it all possible", but because they gave me a reason to walk away from all of this when I had to - a key to sanity, and perhaps quality, I think.

In memory of James Lindon Booth (1953-1986), who died while looking for knowledge more significant than anything in these pages.

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1. INTRODUCTION

Studies in evolutionary ecology often proceed from an observed or predicted correlation between a species' habitat and a particular subset of its morphological, physiological, or behavioural traits (an ecocline; Huxley 1938). Two examples are the observed variation in shell colour and banding in Cepaea in relation to microclimate (Bantock and Ratsey 1980) or predation (Harvey et al 1975), and the theoretically predicted variation of life-history traits in relation to environmental stability (i.e. "bet-hedging"; Murphy 1968; Schaffer 1974; Stearns 1976). The present study examines an ecocline often observed in freshwater mussels (Bivalvia : Unionacea).

For over seventy years, biologists interested in freshwater mussels have observed correlations between habitat and shell characteristics (e.g. Wilson and Clarke 1914; Ortmann 1920; Ball 1922; Baker 1928; Brown et al. 1938; Eagar 1948; Agrell 1949; Dell 1953; Cvancara 1963; Harman 1970; Green 1972; Clarke 1973; Cvancara et al. 1978; Kat 1982; Stern 1983; Hinch et al. 1986). In particular, a relationship between water movement, hereafter called "exposure" (flow rate in rivers and streams, wind and current induced turbulence in lakes) and shell morphology has often been observed, although usually only crudely quantified. Proximate and ultimate factors causing the correlation have been proposed, but never adequately tested. The present

study began with the measurement of a correlation between exposure in a lentic habitat and the shell morphology and growth rate of Lampsilis radiata siliquoidea (Barnes 1823), a unionid mussel. Using electrophoretic and heritability data, as well as the results of habitat selection and burrowing experiments, I will propose an explanation for the observed correlation.

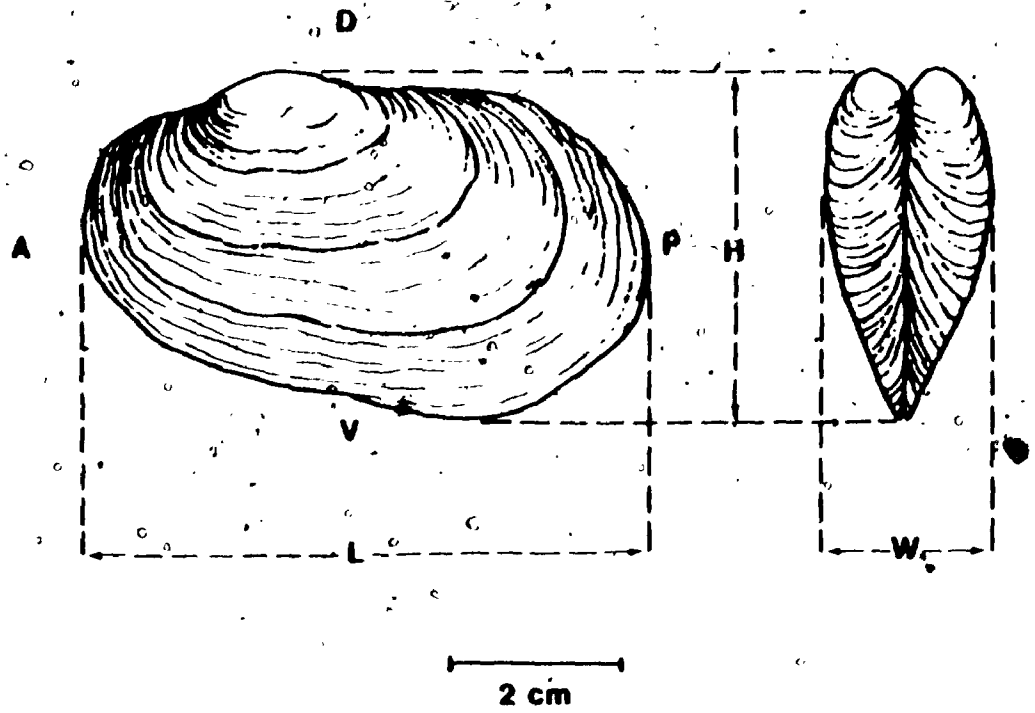
1.1 Historical Observations and Hypotheses

Ball (1922), Eagar (1948), and Tevesz and Carter (1980) reviewed observations of correlations between habitat and shell form in the Unionacea. Although exceptions exist, three relationships have often been noted :

1. Shell size and thickness increase with increasing exposure.
2. Obesity (W/L, Fig. 1) and relative height (H/L, Fig. 1) of the shell decrease with increasing exposure.
3. Shells with straight ventral and arched dorsal margins (Fig. 1) are found in areas of high exposure, while curved ventral margins and an expanded posterior end are typical of clams from slower moving water.

Most studies have found the above relationships in comparisons of upstream and downstream lotic habitats (e.g. Ball 1922; Ortmann 1920), or by comparing river and lake forms (e.g. Grier 1920, Clarke 1973). Within lakes, increasing exposure has been associated in some studies (Brown et al. 1938; Green 1972; Clarke 1973) with smaller size. This is contrary to the trend of larger size in faster

Figure 1. Dimensions and orientation of a mussel as defined in this study (L = length; H = height; W = width; A = anterior; P = posterior; D = dorsal; V = ventral).


D
A
P
H
V
L
W
2 cm

waters described above. However recent evidence indicates that with "moderate" variation in exposure within a lake, three mussel species show more or less the expected covariation of water movement and shell form (Hinch et al. 1986; Hinch and Bailey 1987).

Those postulating mechanisms for these correlations have necessarily considered two questions :

1. Is the morphological variation proximally caused by environmental or genetic variation?
2. Is the morphological variation an adaptive response to environmental conditions?

Most freshwater mussels have an obligate parasitic stage as larvae ("glochidia"). After being ejected from brood sacs on the gill of the female, the glochidia attach to the gills or fins of fish (Clarke 1973). Ortmann (1920), and later Kat (1984a), speculated that the development of local (morphological) races may be limited to mussels with relatively narrow-ranging fish hosts. Wide-ranging hosts would facilitate panmixis by increasing gene flow among mussel populations in different geographic areas. Hinch et al. (1986) proposed that gene flow between habitat types may be reduced by the habitat preferences of the fish hosts. They implied that the habitat specificity of the fish, rather than their actual range, could be important in aiding reproductive isolation of mussel sub-populations with differentially adapted shell morphologies. Both the Ortmann (1920) and the Hinch et al. (1986) hypotheses presuppose that morphological variation in the shell is only enabled by

genetic divergence. They predict that a more or less parallel evolution of shell morphology has occurred in all mussel species showing similar form/habitat correlations.

Eagar (1978), on the other hand, argued that the shell form of freshwater mussels is cued by the environment in which the post-parasitic juvenile mussel drops from the fish. He felt that the adaptive morphological variation found among mussels of a given species indicates the potential for a well-adapted shell regardless of the habitat to which a juvenile is transported by the fish host. Eagar (1978) clearly implied that there is adaptive plasticity carried in the genotypes of many mussel species. Therefore, no reproductive isolation of populations (as first proposed by Ortmann 1920) would be necessary to explain morphological adaptation to different habitats by the mussels.

There is another genetic mechanism which would explain correlations between shell form and habitat without requiring reproductive isolation. Mean heterozygosity in some marine bivalves is positively correlated with growth rate (reviewed in Singh and Green 1984). After random dispersal of juveniles into different habitats, selection could favor faster-growing heterozygotes in more turbulent waters for the reasons proposed above. One would then expect a greater degree of heterozygosity in adults in these habitats, and maintenance of genetic variability because of "patches of heterosis" within a panmictic population.

Whether or not the observed morphological variation represents genetic divergence, the question of adaptation

must also be considered. Do the observed relationships between shell morphology and habitat reflect differential morphological adaptation, or do they just describe non-adaptive patterns of variation?

Many have proposed that smaller, more obese shells in quiescent waters simply reflect a non-adaptive response to poorer growing conditions. Food supply may be reduced (Ball 1922; Stansbery 1970; Kat 1982), although this has never been measured. Filtration of water for feeding and respiration may be affected by high silt concentrations in slow-moving waters (Ball 1922; Kat 1982). Ellis (1936) found that mussels in muddy water had their valves closed 75-95% of the time, while those in silt-free water were closed less than 50% of the time. He also found that heavy siltation killed most mussels kept in experimental tanks. Kat (1982) argued that the net intake of energy would be reduced on muddy substrates because clams would require more energy to maintain proper filtering position. Freshwater mussels usually burrow into the sediment anterior end first until at least half of their shell is buried. This leaves their posterior end, with the incurrent and excurrent siphons, exposed.

Wilson and Clarke (1914) suggested that larger, flatter shell forms are better adapted to burrowing in the coarse substrates of fast current areas, while smaller, more obese shells maintain a clam's buoyancy in soft substrates. This was rejected by Ball (1922), who didn't feel that a one or two percent difference in obesity would affect shell

buoyancy. He recalled collecting highly obese shells in sand and gravel areas of the lower Ohio River. Eagar (1977) showed that dorsal arching of Margaritifera margaritifera shells, often more pronounced in individuals from fast water, is positively correlated with shell "density" (shell weight divided by volume displaced by the two valves). The advantage of a heavier shell in fast waters is clear. Lighter shells, with straighter dorsal edges, would be better adapted for movement over softer substrates in quieter waters. Eagar (1978) also claimed that more obese shells allow for a greater volume of soft tissue, thereby improving the "metabolic and functional activity" of mussels in quieter waters.

Stanley (1970) considered the functional morphology of the entire Class Bivalvia. He felt that shell 'streamlining' (i.e. low obesity) is an adaptation for fast burrowing in bivalves occupying shifting, coarse- grained substrates, and that thicker shells in such habitats provide structural integrity and stability. In soft, fine-grained substrates, thin shells prevent sinking into the sediment (Stanley 1970). Stanley's 'adaptive hypotheses' are clearly similar to those proposed by Wilson and Clark (1914) specifically for the Unionidae.

1.2 Structure of the Thesis

This study consisted of three components. The first was the quantitative description of a relationship between exposure and shell form of Lampsilis radiata siliquoidea in Inner Long Point Bay, Lake Erie. A comparison of growth rate at different life stages among groups of animals from different levels of exposure was also made using measurements of annual rings on the mussel shells.

The second component of the study considered the cause (environmental or genetic) of the observed morphological variation : do mussels in different habitats of the bay appear to be genetically isolated, particularly with respect to shell characteristics? This question was approached by using both allozyme analysis and an estimate of the heritability of shell size and shape in "pre-juvenile" L. radiata. Additionally, an experiment was carried out to determine whether or not L. radiata shows substrate preference, and if this preference depends on shell morphology. Endler (1986), Jones and Probert (1980) and Maynard Smith and Hoekstra (1980) have discussed how varying habitat selection by different phenotypes in a heterogeneous environment helps maintain genetic variation.

The final component of the study concerned adaptation : does the observed relationship between shell morphology and habitat represent differential adaptation? As a test of the adaptedness of shell form in different habitats, a fitness surrogate was defined (burrowing rate), and an aquarium experiment was carried out using individuals of varying

morphology placed on varying substrate types. The experiment indicated whether or not the "optimum" shell morphology (in the narrow sense defined here) depended on the substrate. I then compared the results of the experiment with the previously collected field data to see whether or not the morphological variation observed in nature mimics the experimentally determined optima.

Given the evidence I collected concerning the two major questions (cause of variation, adaptation), a mechanism underlying the initially observed correlation will be proposed. If there was no evidence of either genetic divergence or differential adaptation in different habitats, the observed correlation just represents "heterostasis". The mussel is responding phenotypically to its environment, and proximal hypotheses for variation in shell form such as those suggested by Kat (1982) would be appropriate to consider. Genetic divergence with no evidence of differential adaptation may represent either genetic drift or correlation with another trait under selection (Gould and Lewontin 1978). Evidence for differential adaptation of the shell form and growth rate to different habitats, coupled with evidence of genetic divergence among mussels in the different habitats, would support a model of divergent selection (as proposed by Hinch et al. 1986). Differential adaptation with lack of genetic divergence would be evidence of adaptive phenotypic plasticity, as suggested by Eager (1978), or perhaps the "patches of heterosis" model (Singh and Green 1984).

2. MATERIALS AND METHODS

2.1 Study Organism

Lampsilis radiata siliquoidea (Barnes, 1823), hereafter referred to as L. radiata, is a unionid belonging to the Lampsilinae subfamily (hookless glochidia, posterior part of outer demibranchs used as marsupia, well developed hinge teeth, and long breeding seasons; Clarke 1981). It is found in most aquatic habitats, usually on gravel or sand bottoms but occasionally on mud (Clarke and Berg 1959). L. radiata ranges from Quebec to Alberta in the Canadian Interior Basin (Clarke 1973), in the Mackenzie River system north to Great Slave Lake, in the Great Lakes drainage from Lake Superior to Lake Ontario (where intergrades with L. radiata radiata begin to occur), and in the upper Ohio-Mississippi drainage from New York to Minnesota and Arkansas (Clarke 1981). It is thought to be a long-term breeder (gravid from August to the following July) whose glochidial hosts include Pomoxis nigromaculastus (Lesueur) (black crappie), Pomoxis annularis Rafinesque (white crappie), Stizostedion canadense (Smith) (sauger), Stizostedion vitreum (Mitchill) (walleye), Perca flavescens (Mitchill) (yellow perch), Ambloplites rupestris (Rafinesque) (rock bass), Lepomis macrochirus Rafinesque (bluegill), Micropterus salmoides (Lacepede) (largemouth bass), Micropterus dolomieui Lacepede (smallmouth bass), and Morone chrysops (Rafinesque) (white bass) (Clarke 1981). It is dioecious, with prominent sexual dimorphism in shell morphology (the female is relatively swollen in the posterior

dorsal area of the shell [Clarke 1973]).

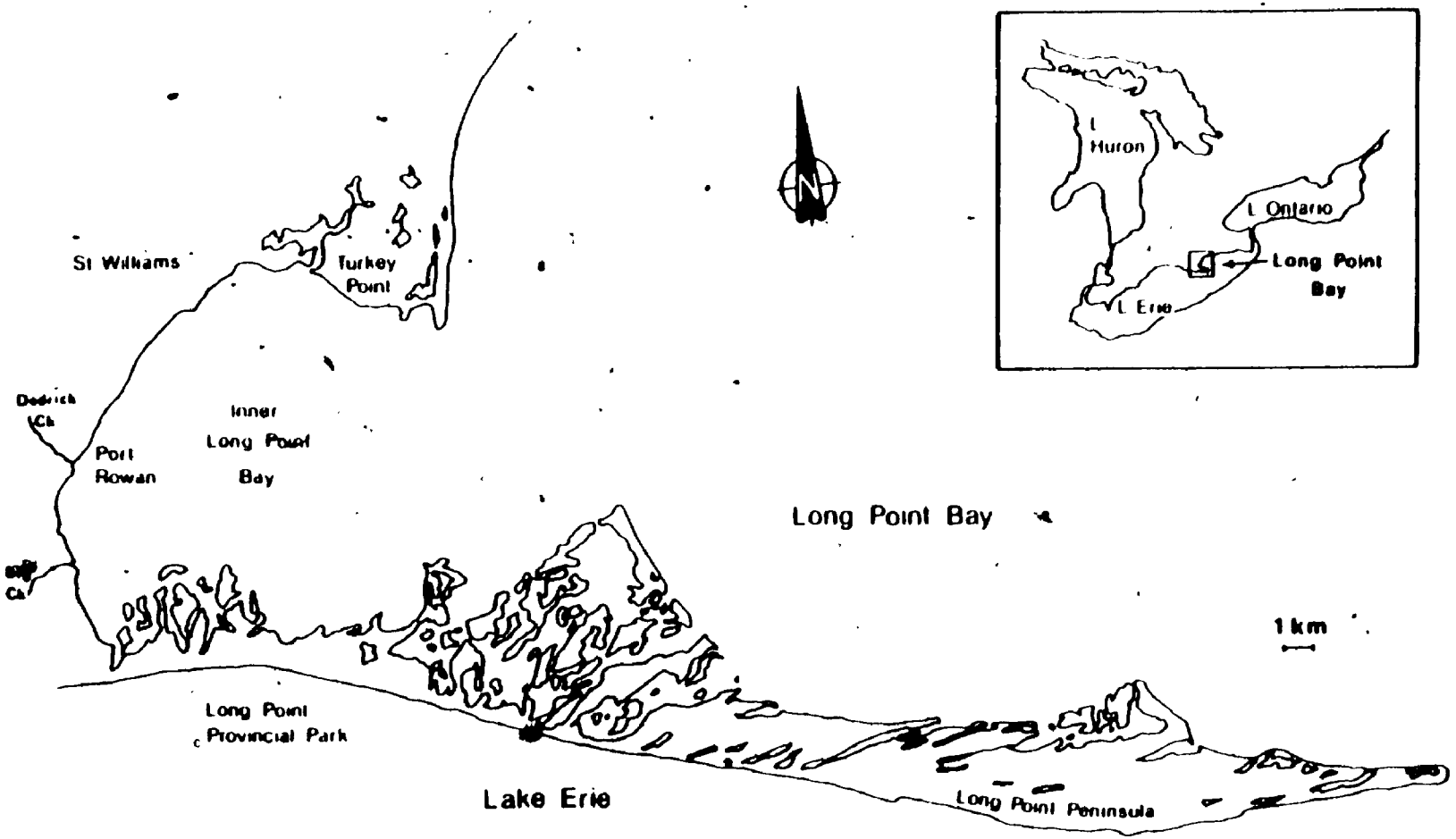
2.2 Study Area

Inner Long Point Bay, hereafter referred to as IB, is at the western extreme of Long Point Bay, which is defined by a dynamic sand spit pushing eastward into Lake Erie from its northern shoreline (Fig. 2). The bedrock surrounding IB is the Middle Devonian Norfolk Formation of light-brown, resistant limestones which create a gently sloping, even terrain down to the lakeshore (Heathcote 1981). This is covered in the direct vicinity of the bay by a clay plain which is about 10m deep and causes poor drainage. Big Creek is the primary inflow into the bay, draining a watershed of 730 km^2, while nearby Dedrick Creek drains 81km (Leach 1981). Together their mean daily discharge equals about 1% of IB's volume (Leach 1981). The bay covers an area of approximately 75km^2. Smith (1979) described the bottom of IB as mud to about 2km off the western shoreline, followed by sandy loam and sand as he moved eastward, out of IB. Water quality in IB has been described as "the best in nearshore Lake Erie" with "oligo-mesotrophic status" (Heathcote 1981; Heathcote et al. 1981; Gregor and Rast 1979).

2.3 Variation in the Benthic Habitat

In June, 1984, 10 transects averaging 5km in length were sampled by boat in IB. At every km along a transect the boat was anchored and a sampling site was established. The transects were chosen in an effort to equally allocate the

Figure 2. The study area.


St Williams
Turkey Point
Port Rowan
Inner Long Pond Bay
Long Point Bay
1 km
Long Point Provincial Park
Lake Erie
Long Point Peninsula
L Huron
L Ontario
Long Point Bay
L Erie

sites to near (<2km) and offshore areas. Altogether, 41 sites were sampled.

At each site a plastic, circular sampling frame (i.d.=1m) was used to sample three systematically chosen subsites within 5m of the boat. At each of these subsites I used SCUBA to measure depth, hand- collect all mussels in the frame area, collect and bag a sediment sample, and collect a water sample in a Pyrex stoppered bottle. All samples were placed on ice in a cooler immediately.

Within 12 hours the mussels were cleaned and identified (using Clarke [1981]; verified by J.M. Topping [Nat. Mus. Canada]) and the water samples were processed. The pH was measured with a Fisher Accumet pH meter (Model 156). Alkalinity and calcium were determined titrimetrically, as described in APHA (1978). The sediment analyses were done over a two month period; samples were kept frozen until analysis. Loss on ignition (LOI) of the sediments was estimated by drying a sample at 80C, cooling, weighing a small (<25g) subsample, ashing at 550C for one hour, cooling, and reweighing. LOI was the proportion of weight lost during the ashing process. Log-log regression of post-baking on pre-baking weight with several preliminary samples showed no effect of the weight of sample used on the LOI obtained, at least within the range of sample weights I analyzed. Sand content was the percentage of a dried, 250g subsample of sediment which did not pass through a 75μ (No. 200) sieve after wet sieving. No sediments contained particles larger than coarse sand.

After pooling the subsite data (i.e. averaging data from the three subsites to obtain summary data for each site), the major environmental gradient in IB with respect to the variables measured was determined using a principal component analysis (PCA) on the covariance matrix of the log-transformed (except for pH) environmental data (alkalinity, calcium, pH, depth, %sand, LOI).

2.4 Correlation Between Habitat and Shell Form and Growth

The survey data showed that L. radiata was the most common unionid species in IB, and morphometric analysis of the L. radiata collected in the initial survey showed that its shell morphology was correlated with exposure. Therefore, in September, 1984, L. radiata were collected at three new sites in each of three areas of IB. Based on maps of the previously collected environmental data, these sites appeared to span a range of high, medium, and low exposure in IB; this was verified by collecting and analysing sediment samples at each of these new sites. I collected and analysed 193 male L. radiata (73 high; 77 medium; 43 low exposure). The females collected were excluded from further analysis because L. radiata is sexually dimorphic (Section 2.1) and there were too few females in the sample to properly characterize and compare their morphology and growth patterns either among exposure groups or with the males.

The shells were cleaned, air dried, and weighed with a Mettler PC4400 electronic balance. The length, height, and width (Fig. 1) of the shells were then measured with

Mitutoyo 500-115 Digimatic Calipers. Canonical variates analysis (CVA; Reyment et al. 1984), as implemented in SAS PROC CANDISC (SAS Institute Inc. 1982), was used to compare the shell morphology of the three groups of mussels. Scatter plots showed allometric relationships among the variables, so log transformations of all variables were used in the CVA.

To compare growth rates among the three groups, a Walford plot analysis of consecutive annual ring measurements (McCuaig and Green 1983) was done. I used analysis of covariance to compare the Walford plot regression lines for mussels from each exposure level. Also, as an independent comparison of growth in the first year of life, the length of the first annual ring was measured and compared among the three groups using one-way ANOVA.

2.5 Allozyme Variation

I used cellulose acetate electrophoresis (Sargent and George 1975) to measure allozyme frequencies of two enzymes (Phosphoglucomutase [PGM], Phosphoglucose Isomerase [PGI]), each of which had one clearly scorable, polymorphic locus. The recipes and specific procedures for screening these enzymes are described in Hebert (1986). Other enzymes were run according to recipes given in Hebert (1986) or modified from Shaw and Prasad (1970), but they were found to be either monomorphic (Malate Dehydrogenase [MDH]), of poor resolution (Leucine aminopeptidase [LAP], Hexokinase [HEX]) or not detectable in the tissue examined (Aldehyde dehydrogenase [AO], Lactate dehydrogenase [LDH], Glucose-6- phosphate

dehydrogenase [G6PDH]).

I analyzed allozyme variation in L. radiata collected in the September, 1984 intensive sample (Section 2.4). A small piece of foot tissue was cut from each individual and placed in a 3mL centrifuge tube. Two drops of distilled water were added, followed by sonication in an ice bath using a Kontes Micro-Ultrasonic Cell Disrupter. The sample was then centrifuged at 15000 rpm for 15 minutes, quick frozen at minus 20C, and subsequently stored at minus 70C until shortly before analysis. Although the samples were stored for as long as two years, comparison of results from the oldest samples with those from freshly collected mussels showed no difference in activity with the enzymes studied.

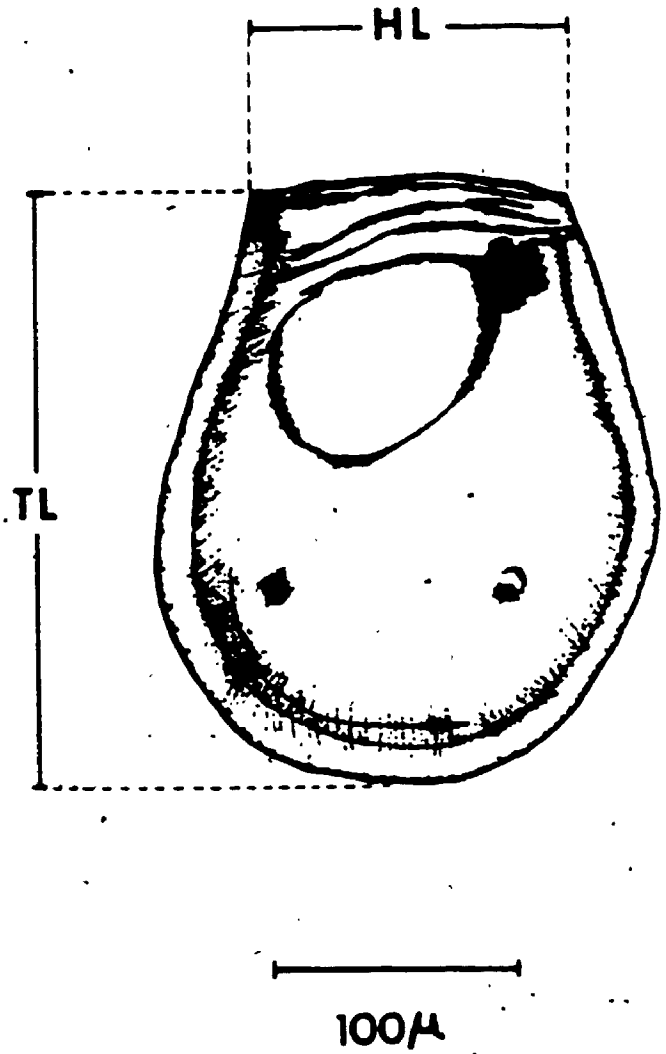
For PGM and PGI I compared genotype frequencies for each of the three habitat groups (high, medium, and low exposure) using contingency tables. I also compared the genotype frequencies with those expected under Hardy-Weinberg equilibrium, and evaluated linkage between the PGM and PGI loci within the three areas. Similar data from L. radiata collected in Lake St. Clair and the Detroit River (part of the Lower Great Lakes waterway), as well as in Balsam Lake (44°35'; 78°50'; part of the Trent-Severn waterway) were obtained from P.D.N. Hebert (University of Windsor). These data were also examined for indications of heterogeneity in genotype frequencies among sites more geographically separated than the different exposure levels in IB.

2.6 Heritability of Glochidia Size and Shape

In September, 1986, I used SCUBA to collect 15 female L. radiata from several sites covering the full range of exposures in IB. The females were gravid and the glochidia in all females were at the same developmental stage : ready to parasitize fish. The total length (TL; Fig. 3) and hinge length (HL; Fig. 3) of fifty glochidia from each female were measured using a Leitz Diavert Inverted Microscope connected to a Bioquant Hipad Digitizer and Apple][e Computer.

Nested ANOVA (using SAS PROC NESTED [SAS Institute Inc. 1982]) was used to estimate the proportion of the phenotypic variance of TL and HL due to differences among families (i.e. glochidia within a given female). If a group of glochidia within a given female is comprised of full sibs, this proportion actually gives an upper bound to one half the heritability of TL and HL (Falconer 1981). It is an upper bound because differences among families will include maternal effects and dominance deviations, both of which will usually increase the similarity of individuals in a given family. Confidence limits for these heritability estimates were computed using Snedecor and Cochran (1980 : p. 245-246). Total, "genetic" and "environmental" correlations between total length and hinge length were also computed using the method described in Falconer (1981). The "genetic" correlation also includes maternal effects.

Figure 3. Measurements made on the glochidia (TL = total length; HL = hinge length).

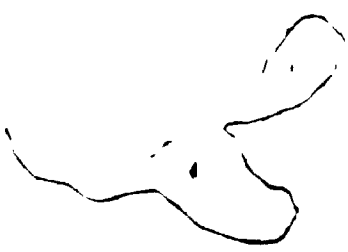

HL
TL
100μ

2.7 Habitat Preference Experiment

In June, 1985, 100 *L. radiata* were collected from each of low and high exposure areas in IB. These mussels were transported to two artificial ponds on the campus of the University of Western Ontario. Each pond was approximately 5m wide and 9m long, with a depth of about a metre, and with equal areas of sand and mud sediment on the bottom (Fig. 4). Both sediments were obtained from Southwinds Sand and Gravel, London, Ontario. The sand used was "golf course sand"; the mud was from silt deposits created by the wastewater from the washing of crushed gravel. Percent LOI (Section 2.3) was nil for both substrates. Results of particle size analyses using wet sieving and hygrometer analysis (Bowles 1978) are shown in Figure 5.

A number was etched onto each mussel's shell to identify individuals. Fifty mussels from each exposure area were then placed at randomly generated coordinates on the bottom of each of the ponds on June 12, 1985. Four months later (October 9, 1985) 151 mussels were recovered over a two day period with SCUBA and the ponds were then drained. Eight additional mussels were found the following day, and 23 were recovered from the dry ponds the followwing spring. 1985) the mussels were collected, their final position recorded, and their shells cleaned, dried, weighed, and measured (length, height, width). The habitat from which each mussel was originally collected in the field ("Source") and the initial substrate in which it was placed in a pond, as well as the interaction between these two factors, were tested as

Figure 4. The habitat preference experiment.



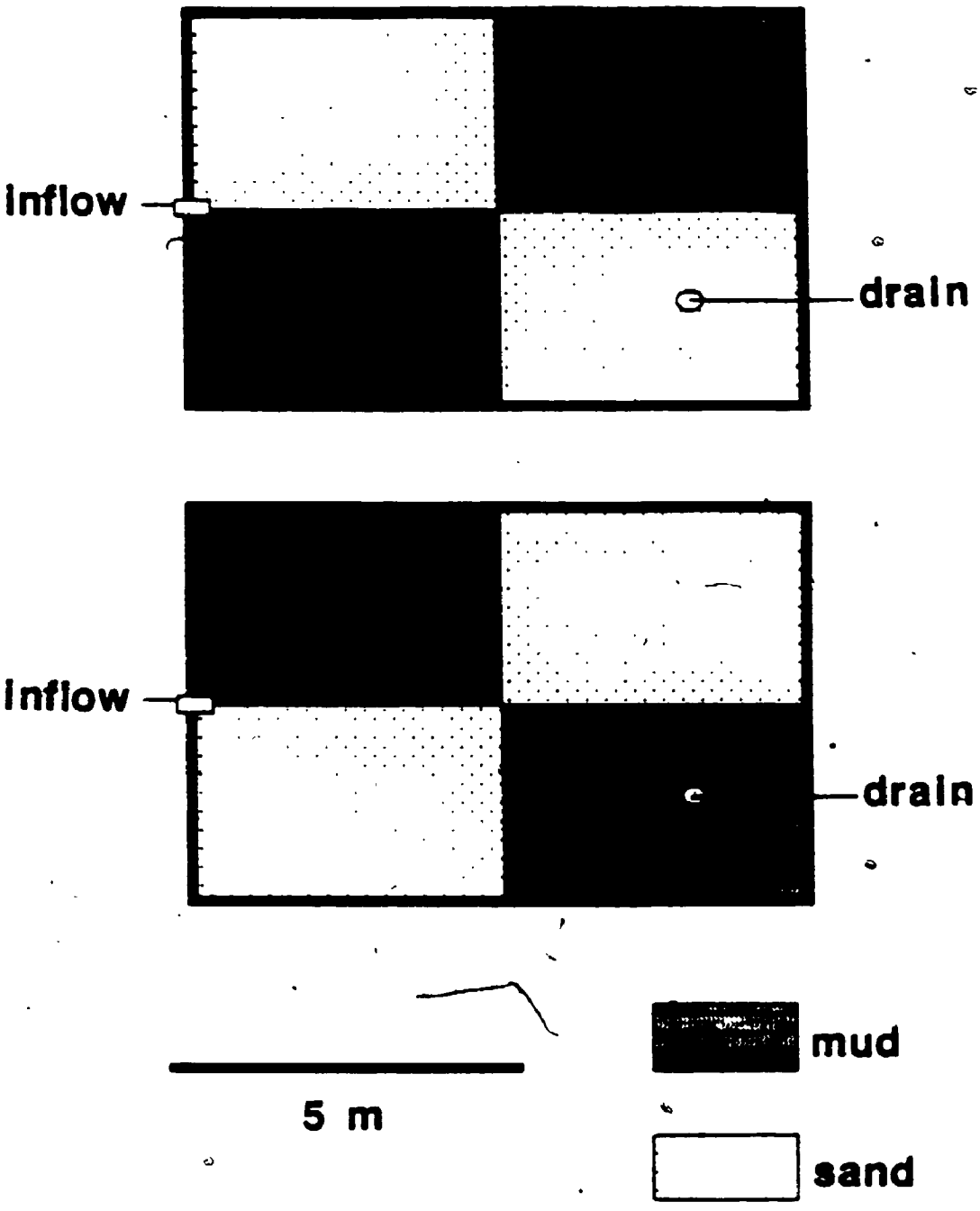
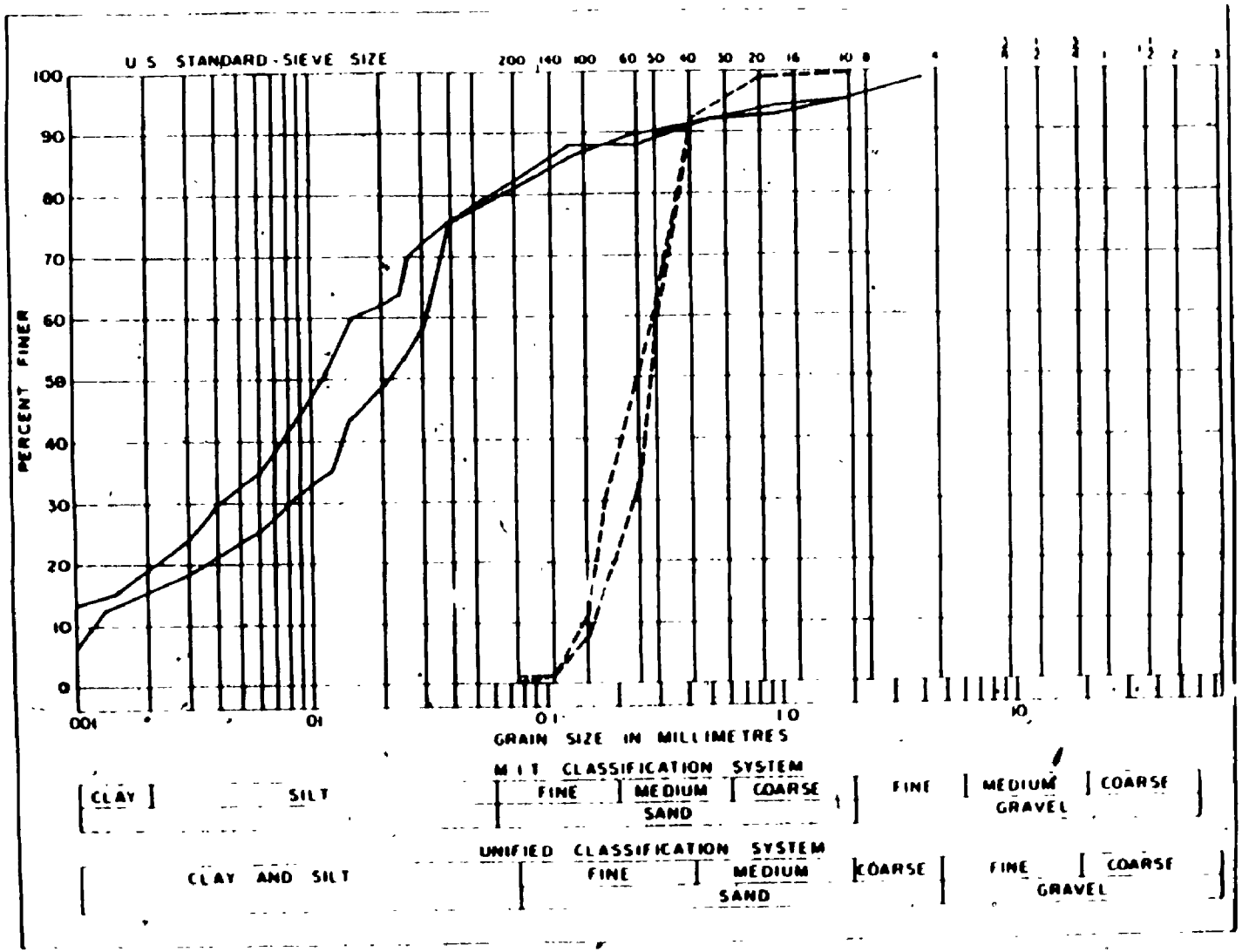

inflow
drain
inflow
drain
5 m
mud
sand

Figure 5. Particle size distribution of substrates used in the habitat preference experiment (---- = sand; ——— = mud).


U S STANDARD - SIEVE SIZE
200 140 100 60 50 40 30 20 16 10 8 4
PERCENT FINER
100
90
80
70
60
50
40
30
20
10
0
001
01
01
10
10
GRAIN SIZE IN MILLIMETRES
M I T CLASSIFICATION SYSTEM
CLAY
SILT
FINE
MEDIUM
COARSE
SAND
FINE
MEDIUM
COARSE
GRAVEL
UNIFIED CLASSIFICATION SYSTEM
CLAY AND SILT
FINE
MEDIUM
COARSE
SAND
FINE
COARSE
GRAVEL

predictors of the mussel's final substrate "choice" using a loglinear model (Fienberg 1980). To aid in interpreting choice differences due to the source of the mussels (habitat of collection in IB), the shell morphology of the mussels from each of the two areas in IB (high and low exposure) was compared using CVA of log-transformed weight and linear measurements.

2.8 Burrowing Experiment

Male L. radiata collected in September, 1986, were numbered and placed in holding jars in a Min-o-cool Model MT-700 700L tank, with a DI-100 Refrigeration/Aeration Unit maintaining a constant temperature of 18C. There was a 10/14 hour light/dark cycle in the laboratory where the tank was kept. Four burrowing areas were constructed in the tank, consisting of two sandy and two muddy substrate "pens" (Fig. 6). Both the sand and the mud were collected from IB, and they differed noticeably in texture after being put in the tank; the mud was unconsolidated, loose material while the sand became quite hard packed. Particle size distribution, determined as described for the pond sediments, reflected the difference in the two substrates (Fig. 7).

After a one month acclimation period in the tank the burrowing experiment was started. A number was etched onto the shell of each of the 48 mussels available for the experiment. For each experimental run they were picked at random from holding jars in the tank and twelve mussels were allocated to each of the four burrowing areas. Each mussel

Figure 6. The burrowing experiment.

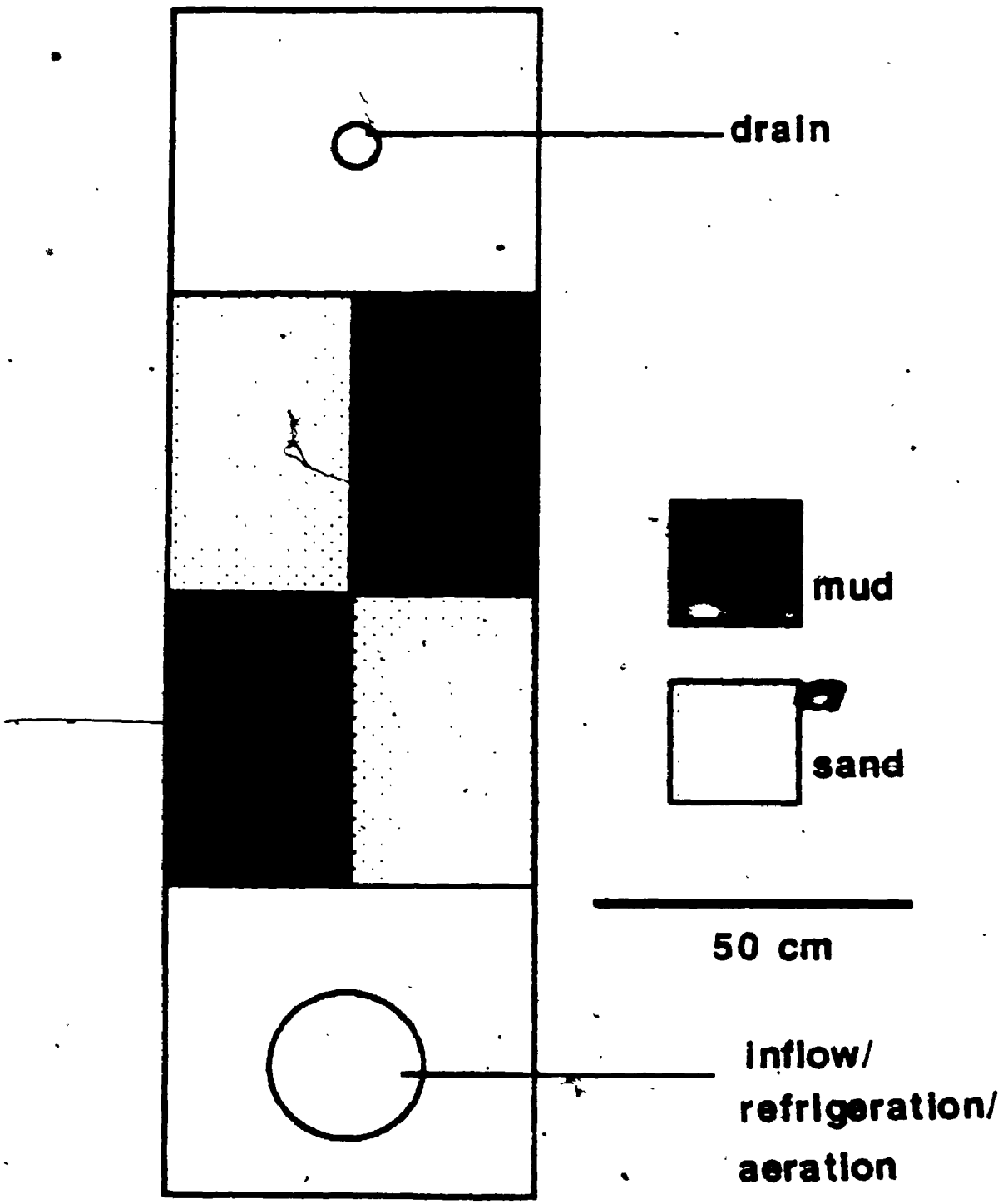
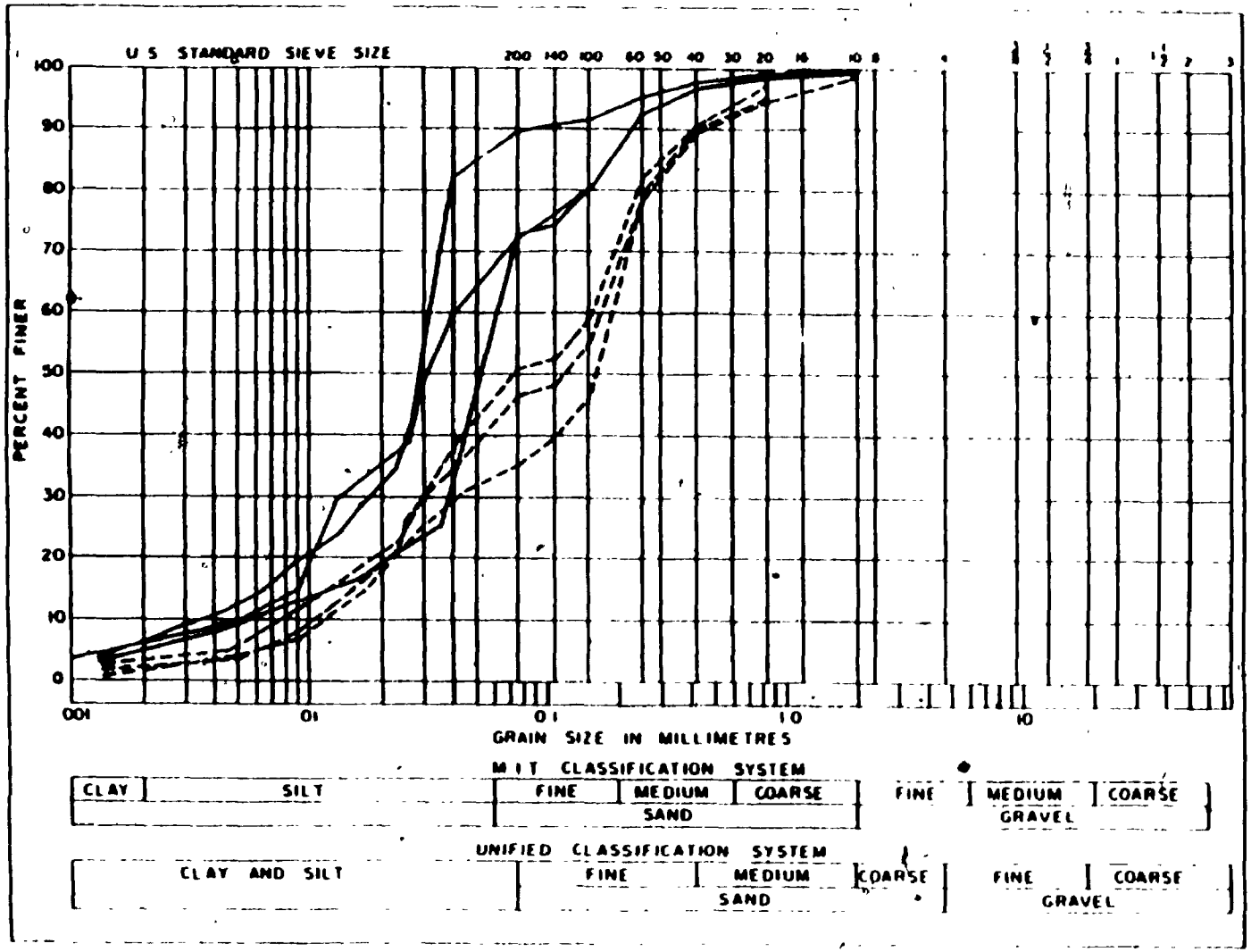

drain
mud
sand
50 cm
inflow/
refrigeration/
aeration

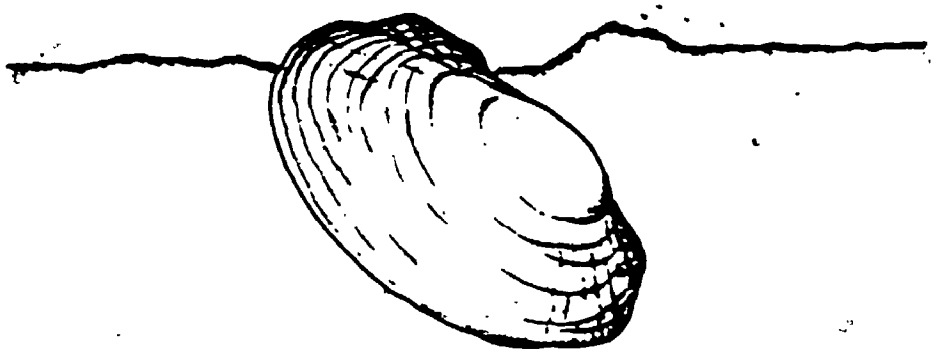
Figure 7. Particle size distribution of substrates used in the burrowing experiment (--- = sand; —— = mud).


U S STANDARD SIEVE SIZE
200 140 100 60 50 40 30 20 16 10 8 4 1/4 1/2 3/4 1 1 1/2 2 3
PERCENT FINER
100 90 80 70 60 50 40 30 20 10 0
001 01 0 1 1 0 10
GRAIN SIZE IN MILLIMETRES
M I T CLASSIFICATION SYSTEM
CLAY
SILT
FINE
MEDIUM
COARSE
SAND
FINE
MEDIUM
COARSE
GRAVEL
UNIFIED CLASSIFICATION SYSTEM
CLAY AND SILT
FINE
MEDIUM
COARSE
SAND
FINE
COARSE
GRAVEL

was placed in normal filtering position, its anterior end in the sediment with less than a quarter of the shell (the posterior end) above the sediment-water interface (Fig. 8). After 48 hours each mussel was removed from its burrow and placed on its side on the sediment directly beside its former burrow. Daily observations were made and a record was kept of the mussels which redug their burrows and resumed normal filtering position. When approximately half of the mussels had reburrowed, or after six days (whichever came first), the mussels were returned to the holding jars. Later that same day they were randomly placed in new burrows and the procedure was repeated.

After repeating the experiment six times, the mussels were cleaned and dried, and weight and linear measurements were made as described previously. I then used PCA (SAS PROC PRINCOMP; SAS Institute [1982]) of the (log-transformed) shell measurements to create four uncorrelated variables (the component scores) which described variation in the shell morphology of the mussels. These PC scores, along with the substrate into which a mussel was placed for a given experimental run, were used as predictors of whether or not a mussel reburrowed during the experiment. Logistic regression (Fienberg 1980), as implemented in SAS PROC CATMOD (SAS Institute 1982), was used for the analysis. "Experimental run" was also included as a predictor to account for differences among the runs in the proportion of animals burrowing. Interactions between substrate and the morphological variables were included in the model to test

Figure 8. Typical position of a mussel (after Tevesz and McCall 1979).



4 cm

for evidence of different "optimal burrowing morphologies" in the two substrates. Because animals were re-used in the six runs of the experiment, the significance levels obtained must be considered "liberal". A more appropriate split-plot analysis for logistic models such as this has yet to be developed (Fienberg 1980).

3. RESULTS

3.1 Variation in the Benthic Habitat

The initial benthic survey indicated that substrate characteristics of IB were much more variable among the sites than either water chemistry or depth (Table 1). This was also indicated by the PCA (Table 2), which showed only one meaningful gradient in the environmental data : mucky (low % sand, high LOI) to sandy (high % sand, low LOI) sediments. I interpreted this as a gradient of "exposure", or wind and current induced turbulence in the benthic environment. Quartiles of the scores of sites along this gradient show that there is a trend of more exposed sites as one moves out of the bay (Fig. 9)

L. radiata was the most commonly found unionid among all sites visited in IB (present in 23 of 41 sites), and it had the highest average density (0.58 mussels/m^2) of the ten species of Unionidae recorded (Table 3).

3.2 Correlation Between Habitat and Shell Form and Growth

The locations of the nine new sites where L. radiata were intensively sampled are shown together with the original survey sites in Figure 9. It is clear that although these new sites did represent a large amount of variability on the "exposure" axis, I did not sample mussels from "very high" exposure areas in my intensive sample (i.e. none of the nine new sites fell in the upper quartile on the exposure axis). Several sites in "very high" exposure areas were visited, but

Table 1. Means, ranges, and coefficients of variation of environmental variables measured in Inner Long Point Bay in June, 1984 (n = 41 sites).

	Mean	Range	C.V. (%)
Alkalinity (mgl^{-1})	2.34	1.89-2.99	13
Calcium (mgl^{-1})	39.4	32.7-50.0	12
pH	8.4	8.2-9.0	2
Depth (m.)	2.53	1.17-3.60	22
% Sand	50.0	2.4-99.2	68
% LOI	2.5	0.3-6.3	70

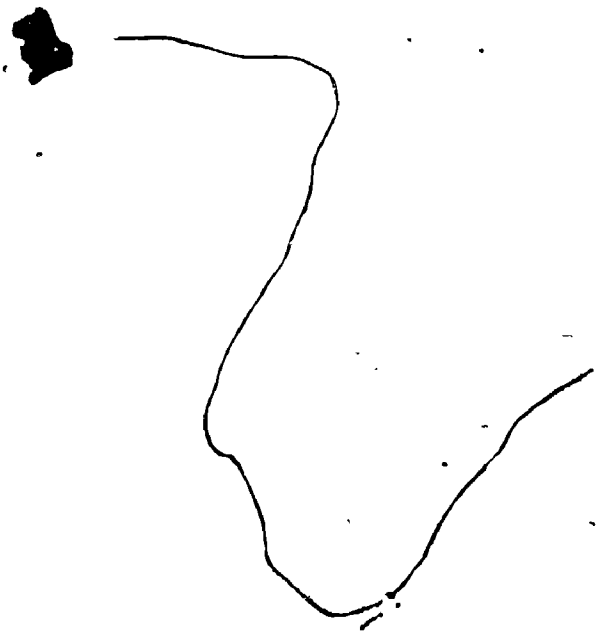


Table 2. Principal component analysis of log-transformed (except for pH) environmental data summarized in Table 1. Structure coefficients are simple correlations between the PC scores and the original variables.

	PC 1		PC 2	
	Eigenvector	Structure	Eigenvector	Structure
Alkalininity	-0.08	-0.72	-0.04	-0.09
Calcium	-0.07	-0.71	-0.04	-0.10
pH	0.06	0.41	-0.12	-0.20
Depth	-0.06	-0.28	0.16	0.18
%Sand	0.80	0.98	0.58	0.19
%LOI	-0.58	-0.94	0.78	0.32
Eigenvalue	1.16		0.08	
% Accounted for	87		6	

Figure 9. Quartiles of general survey and intensive sample sites on the exposure gradient (□ = very high; ▲ = high; ○ = medium; ● = low; L1, L2, L3 = low exposure intensive sample sites; M1, M2, M3 = medium exposure intensive sample sites; H1, H2, H3 = high exposure intensive sample sites).

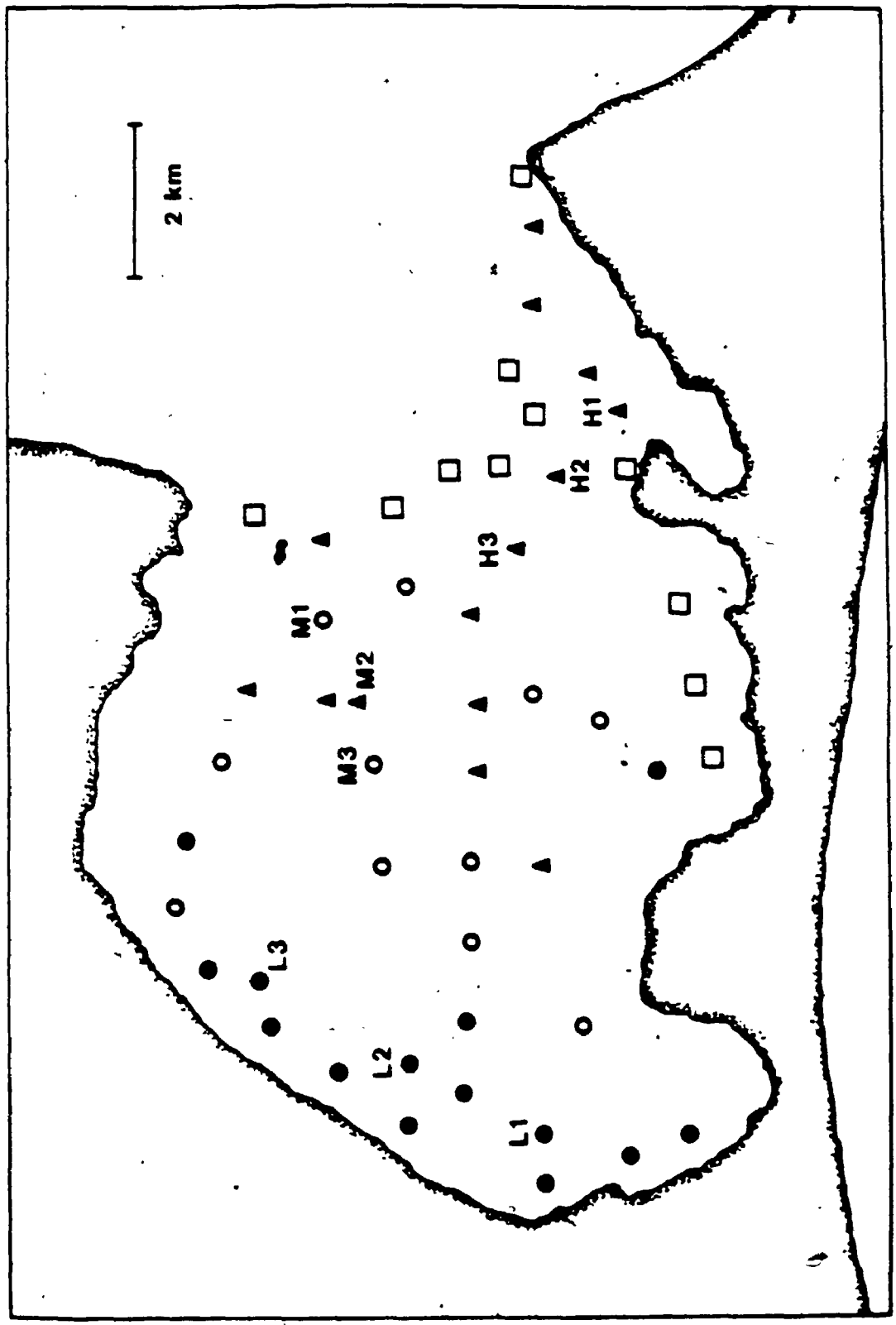

2 km
H1
H2
H3
M1
M2
M3
L1
L2
L3

Table 3. Frequency of occurrence, mean density, total variance in density (n = 41 sites * 3 samples/site = 123 samples), and the percentage of variation in density due to among (%A) and within (%W) site variance of each unionid species found in Inner Long Point Bay in June, 1984.

Species	Frequency (out of 41)	Density (no/m^2)
Anodonta grandis	13	0.16
Anodonta imbecilis	3	0.041
Elliptio dilatata	3	0.031
Fusconaia flava	2	0.021
Lampsilis radiata	23	0.58
Lampsilis ventricosa	2	0.021
Ligumia nasuta	10	0.12
Pleurobema coccineum	1	0.010
Proptera alata	3	0.031
Villosa iris	1	0.010

(continued)

Table 3. (continued)

Species	Total Variance (n=123)	%A	%W
Anodonta grandis	0.21	13	87
Anodonta imbecilis	0.078	0	100
Elliptio dilatata	0.039	0	100
Fusconaia flava	0.026	0	100
Lampsilis radiata	0.91	28	72
Lampsilis ventricosa	0.026	0	100
Ligumia nasuta	0.17	0	100
Pleurobema coccineum	0.013	0	100
Proptera alata	0.039	0	100
Villosa iris	0.013	0	100

no L. radiata were found.

CVA showed that there was a highly significant difference in shell morphology among L. radiata from the three exposure areas (Table 4). Only the first canonical axis was important in describing differences among the groups. The distribution of scores on the first axis, together with the standardized and structure coefficients (Table 4), indicated that as exposure increased, shell thickness (i.e. weight/length ratio) increased. This is more clearly illustrated with a bivariate 95% confidence ellipse of weight and length (log-transformed) for each group (Fig. 10). All three groups had about the same (geometric) mean length (59mm), but the average shell weights at this length were 21.1g (high exposure), 18.5g (medium), and 17.1g (low).

Analysis of covariance of consecutive ring measurements (Walford plot technique) showed that the estimated time to reach asymptotic size was the same in the three groups (i.e. slopes of Walford plots were the same) so I compared the absolute growth rates (i.e. using the intercepts) (Table 5; Fig. 11). Mussels from the high exposure area had significantly ($p<0.01$) faster growth rates than those from medium or low exposure areas. Growth rates (i.e. Walford intercepts) of mussels from the low and medium exposures were statistically indistinguishable ($p>0.5$). The equations and plots of the von Bertalanffy growth curves derived from the Walford plot analysis (as described in McCuaig and Green 1983) showed how the high exposure mussels were faster

Table 4. Canonical variates analysis of variation in shell morphology (log-transformed moephometric variables) among mussels from the high, medium, and low exposure areas. Standardized coefficients are eigenvectors multiplied by the "pooled within" standard deviation of each variable. Structure coefficients are simple correlations between the CV scores and the original variables.

	CV 1		CV 2	
	Standardized	Structure	Standardized	Structure
Weight	2.40	0.46	-0.69	0.27
Length	-1.92	-0.04	-0.29	0.40
Height	0.26	0.20	1.75	0.77
Width	-0.49	0.19	-0.24	0.20
Eigenvalue	0.34		0.004	
Canonical Correlation	0.50		0.06	
Significance	F=7.5 df=8,374 p<0.0001		F=0.2 df=3,188 p=0.87	
Class Means				
High	0.67		0.03	
Medium	-0.16		-0.07	
Low	-0.84		0.07	

Figure 10. 95% confidence ellipses for weight and length (log-transformed) of L. radiata from high (H), medium (M), and low (L) exposure areas in IB.

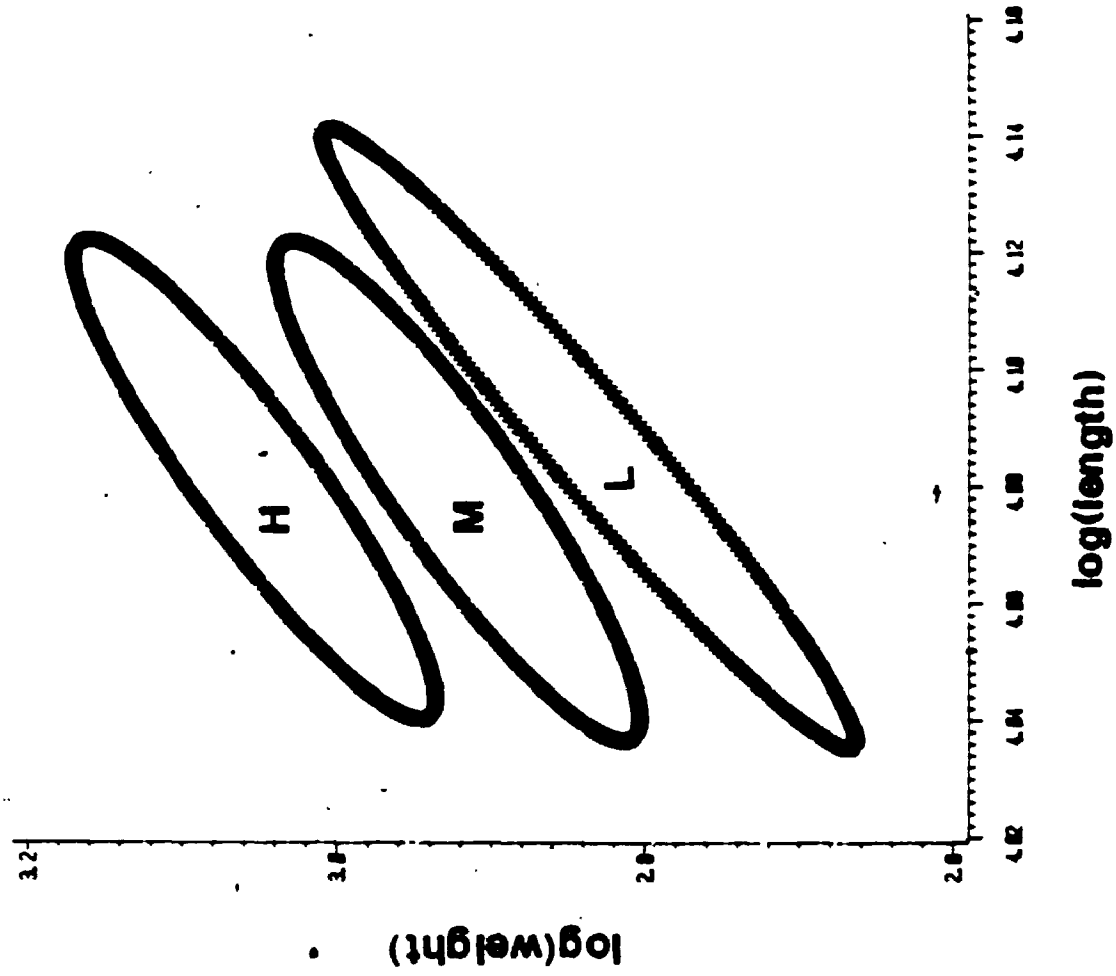

H
M
L
log(weight)
log(length)

Table 5. Analysis of covariance of consecutive growth rings using the Walford Plot method. Resulting Walford plot regression equations are also given, along with the derived von Bertalanffy growth curve equations.

Dependent variable = Length of 2nd Ring (L_2)

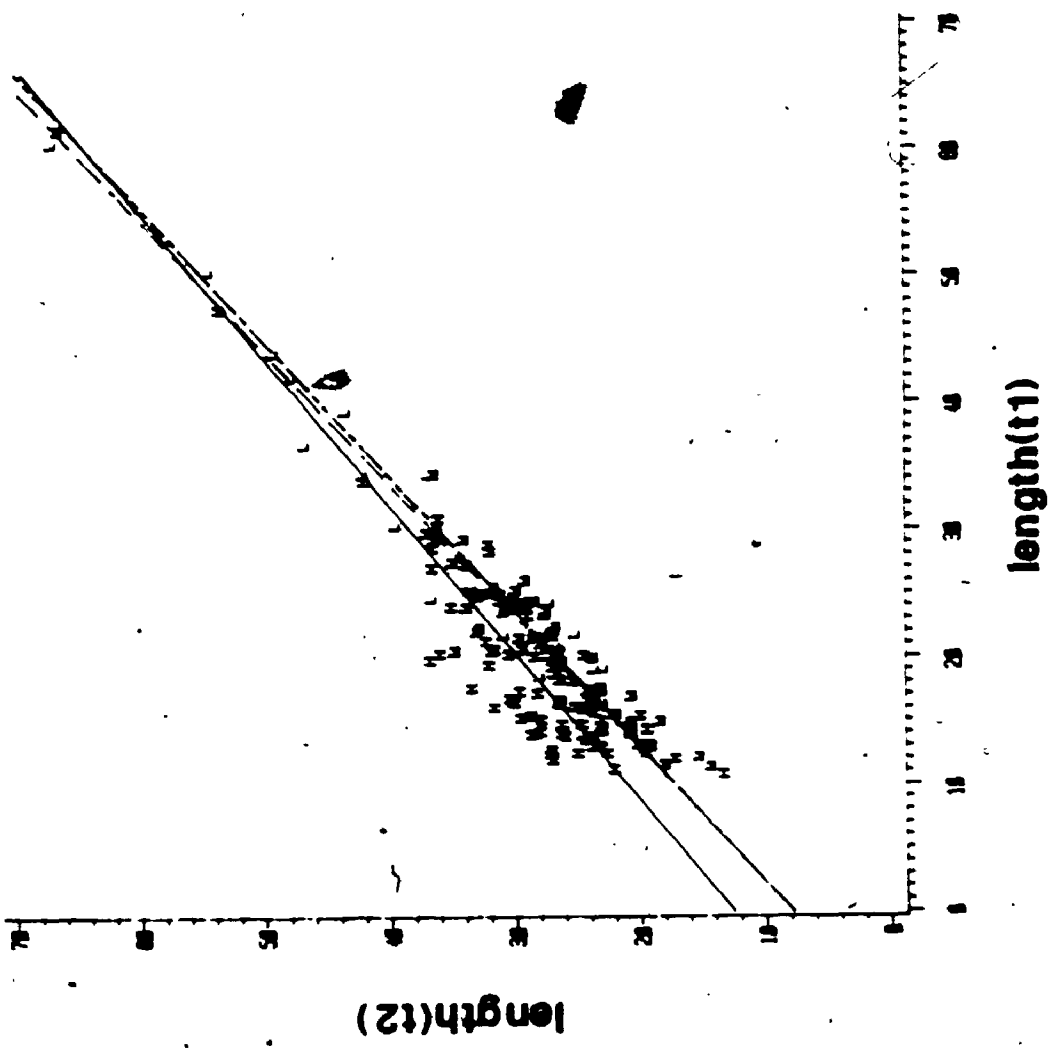
Covariate = Length of 1st Ring (L_1)

Grouping Variable = Exposure (high, medium, or low)

Source	df	SS	MS	F	p
L_1	1	10177.2	10177.2	1315.5	<0.0001
Exposure	2	323.2	161.6	20.9	<0.0001
L_1 * Exposure	2	14.5	7.2	0.9	0.39
Error	152	1175.0	7.7		

Exposure	Walford Regression Line	von Bertalanffy Eqn
High	$L_2 = 11.42 + 0.92\ L_1$	$L_t = 142.8\ (1 - e^{-.08t})$
Medium	$L_2 = 8.18 + 0.92\ L_1$	$L_t = 102.2\ (1 - e^{-.08t})$
Low	$L_2 = 8.65 + 0.92\ L_1$	$L_t = 108.1\ (1 - e^{-.08t})$

Figure 11. Walford plot of consecutive annual ring measurements made on high (H), medium (M), and low (L) exposure L. radiata from IB.


length(t2)
length(t1)

growing than the other exposure groups (Table 5; Fig. 12). In an independent comparison of growth, length of the first annual ring also varied significantly among the three groups ($F=13.55$; $df=2,150$; $p<0.001$), and correlated with the gradient in exposure (high=13.6mm; medium=12.6mm; low=11.2mm).

3.3 Allozyme Variation

Analysis of PGI (Table 6) showed no deviation from Hardy-Weinberg equilibrium in any of the three exposure groups. There was also no difference in genotype frequencies among the exposure groups, and after pooling animals from the three groups there was still no evidence of any deviation from Hardy-Weinberg equilibrium.

A comparison of my results with Hebert's data showed no difference in genotype frequency between the IB mussels and other populations in the Lower Great Lakes waterway (i.e. Lake St. Clair, Detroit River). But the mussels from Balsam Lake (on the Trent-Severn waterway) showed fixation on the slow PGM allele, causing significant differences between its genotype frequencies (all SS) and those of the Great Lakes populations (Fig. 13).

PGM (Table 7) showed a somewhat different pattern than PGI. Again, none of the exposure groups in IB deviated from Hardy-Weinberg equilibrium. There was heterogeneity in genotype frequencies, however, due to a lower frequency of heterozygotes (and thus a lower frequency of the F allele) in the low exposure group.

Figure 12. von Bertalanffy growth curves for L. radiata from high (———), medium (– – – –), and low (— — —) exposure areas in IB.

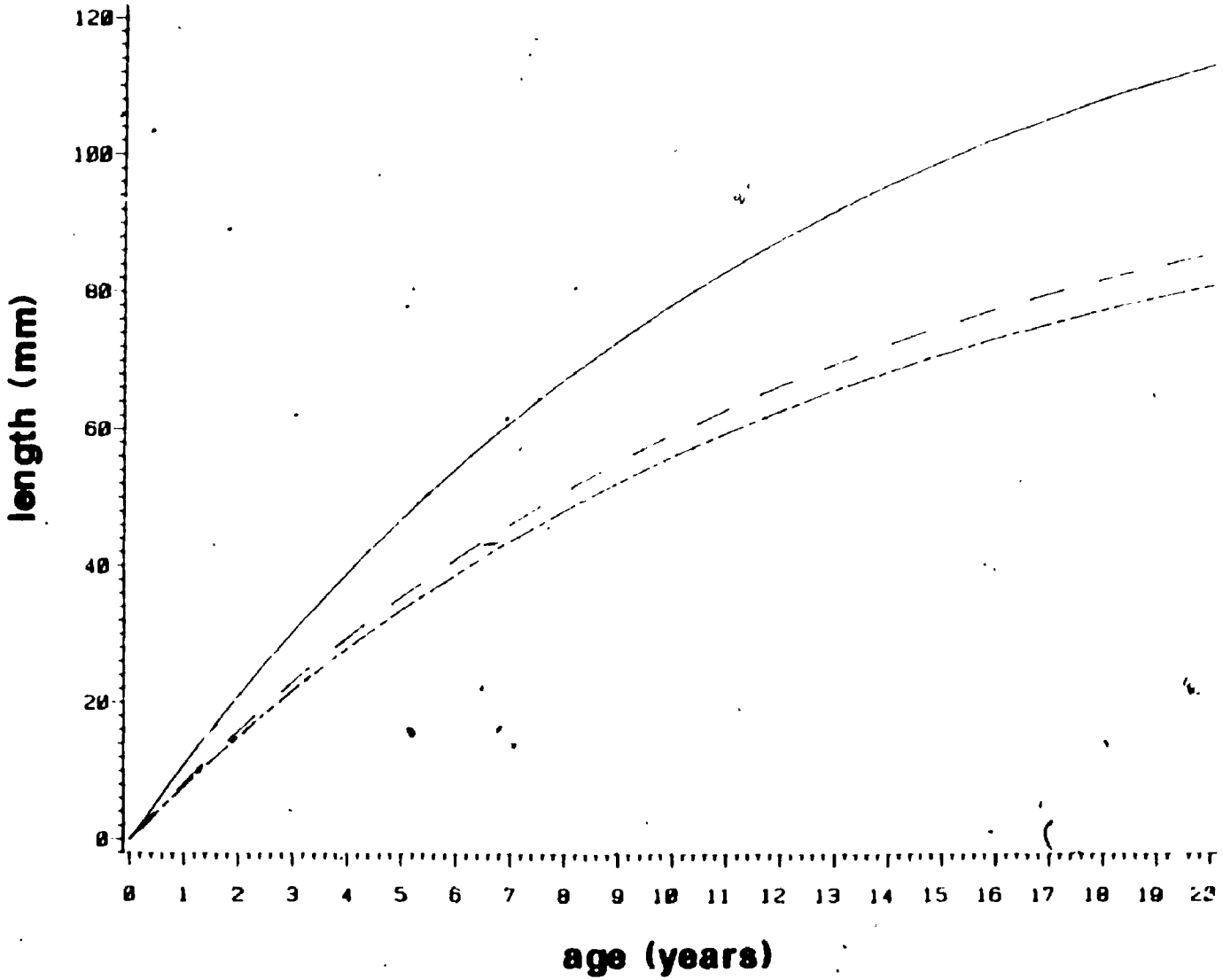

length (mm)
120
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
age (years)

Table 6. Variation in PGI of L. radiata from the September, 1984 sample in Inner Long Point Bay and from unpublished data collected by P.D.N. Hebert's (U. of Windsor) laboratory in 1984. X^2_{HW} indicates test statistic for Hardy-Weinberg equilibrium. For all of the H-W tests, the critical X^2 value is 3.84 (df = 1; p = 0.05).

i) IB Data

Exposure	n	FF	SF	SS	X^2_{HW}	p_S
High	99	0	21	78	1.4	0.89
Medium	78	1	22	55	0.6	0.85
Low	51	1	14	36	0.1	0.84
Total	228	2	57	169	1.5	0.87

Independence X^2 = 3.2 (df = 4; p > 0.5)

ii) Hebert Data

Site	n	FF	SF	SS	X^2_{HW}	p_S
Lake St. Clair	271	8	69	194	0.40	0.84
Detroit River	22	1	9	12	0.18	0.75
Balsam L.	49	0	0	49	0.00	1.00

Independence (IB + Lake St. Clair + Detroit R. + Balsam L.) X^2 = 25.2 (df = 6; p < 0.001). Independence (all except Balsam L.) X^2 = 6.2 (df = 4; p = 0.18).

Figure 13. Geographic variation in proportion of most common PGI (top number) and PGM (bottom number) allele from four L. radiata populations in southern Ontario.

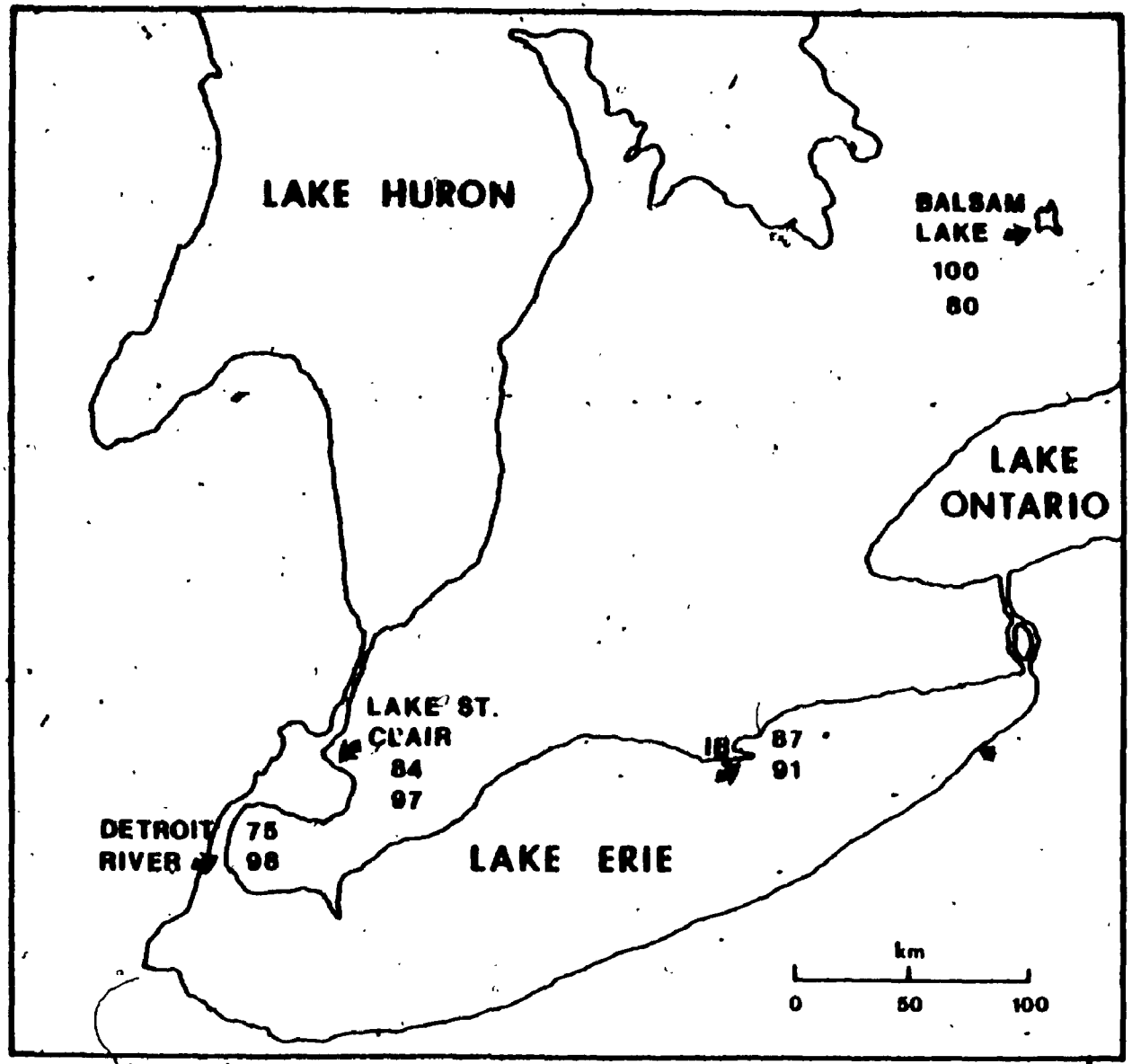

LAKE HURON
BALSAM LAKE
100
80
LAKE ONTARIO
LAKE ST. CLAIR
84
97
IBC
87
91
DETROIT RIVER
75
98
LAKE ERIE
km
0
50
100

Table 7. Variation in PGM of L. radiata from the September, 1984 sample in Inner Long Point Bay and from unpublished data collected by P.D.N. Hebert's (U. of Windsor) laboratory in 1984. X^2_{HW} indicates test statistic for Hardy-Weinberg equilibrium. For all of the H-W tests, the critical X^2 value is 3.84 (df = 1; p = 0.05).

i) IB Data

Exposure	n	FF	SF	SS	X^2_{HW}	p_S
High	98	0	16	82	0.8	0.92
Medium	82	1	20	61	0.2	0.86
Low	50	0	2	48	1.0	0.96
Total	230	1	38	191	0.4	0.91

Independence X^2 = 11.4 (df = 4; p = 0.02)

ii) Hebert Data

Site	n	FF	SF	SS	X^2_{HW}	p_S
Lake St. Clair	259	0	17	242	0.4	0.97
Detroit River	22	0	1	21	0.01	0.98
Balsam L.	46	.7	14	25	3.5	0.80

Independence (Lake St. Clair + Detroit R. + Balsam L.)
X^2 = 73.1 (df = 4; p < 0.001).

Hebert's data also showed heterogeneity in genotype frequencies, again due to a substantial difference in allele frequencies in the Balsam Lake population (Fig. 13). IB mussels from low exposure areas were closer in allele frequencies to the Lake St. Clair and Detroit River populations than they were to high or medium exposure IB mussels.

There was no evidence of linkage between PGM and PGI in the high, medium, or low exposure areas of IB (Table 8).

3.4 Heritability of Glochidial Size and Shape

Nested analyses of variance, followed by the estimation of variance components, indicated that over 90% of the variation in both the total length (TL; Table 9) and hinge length (HL; Table 10) of glochidia was due to within-family variation. For TL, 9.35% of the variation was due to among-family (i.e. additive genetic variance, maternal effects, dominance deviations) differences, which after doubling (since full sibs share an average of half of their genes), gave a heritability estimate of 18.7% (95% confidence intervals are 9% and 43%). The same calculation for hinge length of the glochidia (Table 10) gave a heritability estimate of 10.6% (with 95% confidence intervals of 4% and 33%).

The analysis of the covariance between TL and HL of the glochidia, followed by the estimation of the components of covariance (Table 11), showed a total phenotypic correlation between the two characters of - 0.23 ($p<0.001$) : longer

Table 8. Evaluating linkage between PGI and PGM in the high, medium, and low exposure areas of IB.

i) High Exposure

		PGM	
		SF	SS
PGI	SF	3	18
	SS	13	64

(Independence X^2 = 0.081; df=1; p=0.78)

ii) Medium Exposure

		PGM		
		FF	SF	SS
PGI	FF	0	1	0
	SF	1	6	14
	SS	0	12	43

(Independence X^2 = 6.3; df=4; p=0.18)

iii) Low Exposure

		PGM	
		SF	SS
PGI	SF	1	13
	SS	1	35

(Independence X^2 = 0.5; df=1; p=0.48)

Table 9. Analysis of variance of glochidia total length. Mean total length (n = 749) was 247.3um, coefficient of variation was 9.2% (SS = sum of squares; MS = mean square; EMS = expected mean square).

Source	df	SS	MS	EMS
Among Families	14	40454.06	2889.6	$49.9s^2_{LA} + s^2_{LW}$
Within Families	734	344499.0	469.3	s^2_{LW}

$s^2_{LW} = 469.3$ $\quad s^2_{LA} = (2889.6 - 469.3) / 49.9 = 48.5$

$s^2_{LT} = s^2_{LW} + s^2_{LA} = 469.3 + 48.5 = 517.8$

$h^2_L = 2\, s^2_{LA} / s^2_{LT} = 0.187 = 18.7\%$

Table 10. Analysis of variance of glochidia hinge length. Mean hinge length (n = 749) was 112.0um, coefficient of variation was 10.4% (SS = sum of squares; MS = mean square; EMS = expected mean square).

Source	df	SS	MS	EMS
Among Families	14	6857.9	489.8	$49.9s^2_{HA} + s^2_{HW}$
Within Families	734	94109.5	128.2	s^2_{HW}

$s^2_{HW} = 128.2$ $\quad s^2_{HA} = (489.8 - 128.2) / 49.9 = 7.24$

$s^2_{HT} = s^2_{HW} + s^2_{HA} = 128.2 + 7.24 = 135.4$

$h^2_H = 2 s^2_{HA} / s^2_{HT} = 0.053 = 10.6\%$

Table 11. Analysis of covariance of glochidia total and hinge length (SP = sum of products; MP = mean products; EMP = expected mean products).

Source	df	SP	MP	EMP
Among Families	14	6168.8	440.6	49.9 cov_A + cov_W
Within Families	734	-50935.2	-69.39	cov_W

cov_W = -69.39 cov_A = (440.6 - 69.39) / 49.9 = 10.2

$cov_T = cov_W + cov_A$ = -69.39 + 10.2 = -59.2

$$r_{PHENO} = \frac{cov_T}{(s^2_{LT} + s^2_{HT})^{1/2}} = \frac{-59.2}{(517.8 + 135.4)^{1/2}} = -0.23$$

$$r_{"ENV"} = \frac{cov_W}{(s^2_{LW} + s^2_{HW})^{1/2}} = \frac{-69.4}{(469.3 + 128.2)^{1/2}} = -0.28$$

$$r_{"GEN"} = \frac{cov_A}{(s^2_{LA} + s^2_{HA})^{1/2}} = \frac{10.2}{(48.5 + 7.24)^{1/2}} = 0.54$$

glochidia have shorter hinges. The "genetic" (includes maternal effects) correlation, however, is positive ($r = 0.54$; $p<0.05$), indicating that differences among families tend to be differences in overall size rather than shape differences. In summary, families of glochidia vary from those which are short in both hinge and total length to those which are long on both of these dimensions. Within a given family, glochidia with longer shells tend to have shorter hinges.

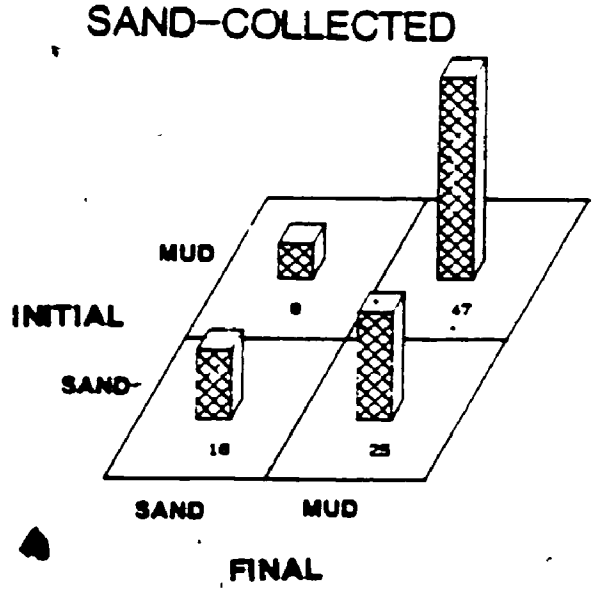
3.5 Habitat Preference Experiment

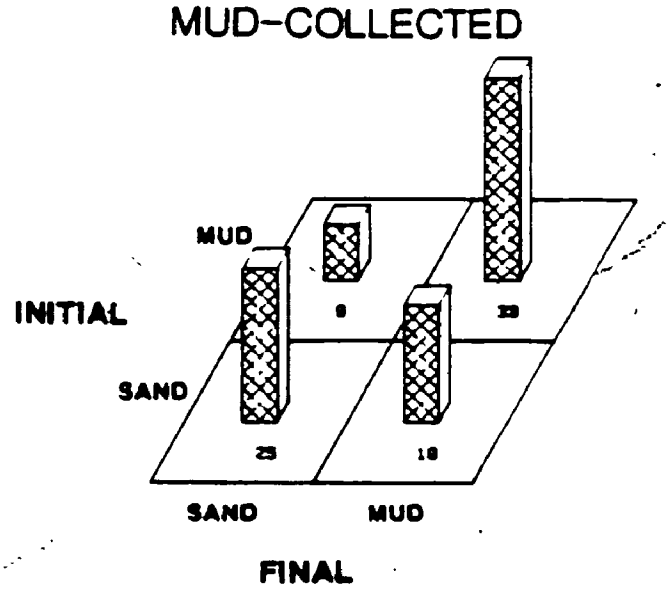
Of the 200 mussels initially planted in the ponds (June 12, 1985), 182 were recovered at the conclusion of the experiment (October 9, 1985). Of these, 159 (146 alive) were found within three days of terminating the experiment and draining the ponds. Twenty-three dead mussels were recovered from the dry ponds the following spring. Two thirds of the mussels recovered (124/182) were found in the mud. The loglinear model analysis (Table 12) showed that this preference for mud was somewhat influenced by the source of the mussels (i.e. where they were collected from the IB), and more strongly influenced by their initial substrate (i.e. where they were first planted in the ponds). There was a tendency for those initially placed in mud to stay there, but those initially placed in sand had about a 50/50 chance of switching to mud (Fig. 14). More of the mussels that were collected in exposed, sandier areas of IB tended to stay in mud or move to mud from sand relative to those collected from

Table 12. Log-linear model analysis showing the significance of "Source" (area collected in IB), "Initial Substrate" (substrate in which the mussel was initially planted in the ponds), and the interaction between these factors in predicting the final substrate of the mussels.

Source	df	X^2	p
"Source"	1	2.9	0.08
"Initial Substrate"	1	17.4	<0.001
Interaction	1	0.13	0.72

Figure 14. Initial placement and final substrate choice of L. radiata from high exposure, sandy areas (top) and low exposure, muddy areas (bottom) in IB.


SAND–COLLECTED
MUD
INITIAL
SAND
0
47
18
25
SAND
MUD
FINAL


MUD–COLLECTED
MUD
INITIAL
SAND
0
39
25
18
SAND
MUD
FINAL

the protected, muddy areas of the bay. "Sand-collected" mussels preferred mud 3:1, while "mud-collected" individuals were relatively ambiguous (1.4:1).

CVA of the two "source" groups (Table 13) showed a similar result to that in the intensive sample of L. radiata (Section 3.2). Mussels collected from exposed, sandy areas had thicker shells than those collected in protected, muddy areas. In addition to this, the "sand- collected" mussels were larger than the "mud-collected" group. This was not observed in data obtained from the intensive sample of L. radiata (Section 3.2).

3.6 Burrowing Experiment

PCA of the mussels used in the burrowing experiment (Table 14) revealed a more or less isometric, first principal component axis (MORPH1) of size which accounted for over 95% of the variation in shell morphology. All the linear measurements have coefficients of the same sign and magnitude, while weight has a coefficient which is about three times the size of those for the linear measurements. In a given shaped object, weight increases proportionally to the cube of length if density doesn't change. Given that the variables in this analysis were log-transformed, if shell thickness doesn't change with size (isometry), one would expect the coefficient for weight to be three times that for the linear measurements.

The second axis (MORPH2) summarized a trend of increasing length and height in relation to shell weight, or

Table 13. Canonical variates analysis of variation in shell morphology (log-transformed morphometric variables) between mussels collected from high ("sand-collected") and low ("mud-collected") exposure areas for the substrate preference experiment. Standardized coefficients are eigenvectors multiplied by the "pooled within" standard deviation of each variable. Structure coefficients are simple correlations between the CV scores and the original variables.

	CV 1
	Standardized Structure
Weight	1.78 / 0.99
Length	-0.78 / 0.88
Height	0.54 / 0.92
Width	-0.03 / 0.94
Eigenvalue	1.39
Canonical Correlation	0.76
Significance	F=47.7 df=4,137 p<0.0001
Class Means	
Sand-collected	1.03
Mud-collected	-1.03

Table 14. PCA of shell measurements from mussels used in the burrowing behaviour experiment. Variables were all log-transformed prior to analysis.

	MORPH1	MORPH2	MORPH3	MORPH4
Eigenvectors				
Weight	0.88	-0.36	-0.28	-0.10
Length	0.26	0.88	-0.18	-0.35
Height	0.24	0.30	0.02	0.92
Width	0.30	0.06	0.94	-0.12
Eigenvalues	0.17	0.005	0.002	0.001
%Accounted For	95.3	2.7	1.2	0.8
Cumulative %	95.3	98.0	99.2	100

variation in shell thickness at a given size. The third PC (MORPH3) was an axis of increasing width in relation to weight, or variation in shell obesity at a given size. The fourth axis (MORPH4) was a trend of increasing height in relation to length.

The coefficients from the logistic regression (Table 15) can be interpreted as in a multiple regression, where the dependent variable is 'log(p/1-p)', where 'p' is the probability of burrowing. Positive coefficients in the model indicate factors which increase the predicted probability of burrowing. Substrate was dummy coded (Mud = 1; Sand = - 1). Two, two-way interactions were particularly significant relative to the other predictors in the model. The coefficients for these interactions showed the following :

i) MORPH2 * Substrate. Mussels with relatively thick shells were less likely to burrow in sand, but more likely to burrow in mud.

ii) MORPH3 * Substrate. Relatively obese mussels were less likely to burrow in sand, but more likely to burrow in mud.

Table 15. Logistic regression predicting burrowing probability from substrate and shell morphology.

Source	df	parameter estimate	X^2	p
Intercept	1	-0.50	4.6	0.03
Experiment	5		16.3	0.006
Substrate(S)	1	-0.31	1.95	0.16
MORPH1(M1)	1	0.16	0.69	0.41
MORPH2(M2)	1	0.14	0.17	0.68
MORPH3(M3)	1	-0.02	0.00	0.95
MORPH4(M4)	1	0.60	5.42	0.02
S*M1	1	-0.06	0.08	0.77
S*M2	1	-1.21	11.34	<0.001
S*M3	1	0.74	7.16	0.008
S*M4	1	0.32	1.43	0.23
M1*M2	1	-0.47	2.50	0.11
M1*M3	1	-0.34	2.15	0.14
M1*M4	1	-0.24	1.04	0.31
M2*M3	1	0.20	0.40	0.53
M2*M4	1	0.50	2.40	0.12
M3*M4	1	-0.02	0.00	0.96
S*M1*M2	1	0.71	4.69	0.03
S*M1*M3	1	0.44	3.31	0.07
S*M1*M4	1	-0.12	0.21	0.65
S*M2*M3	1	0.20	0.27	0.60
S*M2*M4	1	0.42	0.81	0.37

(cont.)

(Table 15 continued)

Source	df	parameter estimate	X^2	p
S*M3*M4	1	-0.37	0.83	0.36
S*M1*M2*M3	1	0.15	0.14	0.71
S*M1*M2*M4	1	-0.14	0.12	0.73
S*M2*M3*M4	1	-0.72	1.31	0.25
S*M1*M2*M3*M4	1	0.55	0.48	0.49

4. DISCUSSION

4.1 The Exposure Gradient

To justify interpretation of the observed variation in sediment characteristics in IB as an "exposure" gradient, it is necessary to consider the source and dynamics of fine particulate matter in the bay. The major sources of silty material are the primary inflows (Big Creek and Dedrick Creek; Fig. 2), with some addition from decomposing macrophyte and plankton debris.

The accumulation and resuspension of this fine material are controlled by water movement at the sediment-water interface (i.e. the benthic habitat). The direction, speed, and ultimate effect of this water movement are primarily influenced by three factors, i) the location and flow rate of the inflows, ii) prevailing winds, and iii) surface seiches :

i) Both Big Creek and Dedrick Creek are in the southwest corner of the bay (Fig. 2). Their mean daily discharge is equal to about 1% of the bay's volume (Leach 1981), although periodic storms cause much higher inflow rates and sediment loads.

ii) In IB the prevailing winds are from the southwest (Kohli and Farooqui 1980). The bay is shallow enough (maximum depth of my sites was 3.6m) for wind-induced shallow waves (Wetzel 1975) to cause turbulence in the benthic habitat, which would resuspend and transport fine sediments.

iii) After prolonged winds, Lake Erie is subject to surface seiches which have amplitudes as high as 2m (Wetzel 1975). The prevailing winds (as noted above) would tend to cause seiches on a southwest/northeast axis in the lake. These seiches would "flush" the part of IB not protected by Turkey Point (the northeastern "gate" of IB).

Taking these three factors into account, the distribution of exposure areas in IB (Fig. 9) can be explained. The mucky area along the western coast is caused by the accumulation of silt from the two inflows and decaying organic material. It is not subject to significant resuspension by southwesterly winds and it is protected by Turkey Point from the flushing action of surface seiches. As one moves eastward, the distance from the southwestern shore, and thus the impact of southwesterly winds, increases. Thus one finds moderately and then extremely sandy sediments.

The area of extremely sandy sediments along the southern shore of IB is probably caused by the flushing action of surface seiches, since this area lies directly on the major "seiche axis". It is also subject to occasional storms from the northeast.

In summary, I feel that the exposure gradient which I have quantified in this study reflects more than just sediment variation. It is a measure of water movement or turbulence in the benthic habitat. The effects of this environmental variation on the habitat of the mussels are directly analagous to changes in the benthic habitat as one moves downstream in a lotic system. In a river, the speed of

current flow generally decreases as one moves downstream (Ortmann 1920). Thus, fast-flowing upstream sites generally have coarser substrates than slow-flowing downstream areas.

4.2 Shell Morphology and Growth Rate of L. radiata

Ortmann (1920), in compiling evidence for what later became known as "Ortmann's Law" (mussels from downstream, quieter areas in a river are more obese than those from upstream, fast-flowing areas), noted that many unionid species (including L. radiata) do not show this correlation. He hypothesized that the relationship would only be observed in mussels from the more primitive taxonomic groups. Although he did not define "primitive" in this context, he mentioned that the genera Fusconaia, Amblema, Quadrula, and Pleurobema "belong to the most primitive types of North American Naiades" (Ortmann 1920).

My results for L. radiata agreed with those of Ortmann (no difference in obesity with differences in exposure), but demonstrated another relationship (increased shell thickness with higher exposure). A correlation between exposure and morphology may exist in many species previously thought to be "unresponsive" to their habitat. It could be obscured by just looking at ratios of morphological measurements, which may distort or not show the relationship of interest (Atchley et al. 1976; Atchley 1978; Atchley and Anderson 1978). The precise nature of the relationship between exposure and shell morphology will vary among species depending on allometric relationships among the various morphometric

variables. This would be true even if the effect of exposure was identical for each species (e.g. increased growth rate). It is clear that a multivariate consideration of shell morphology is necessary to properly characterize the relationship between shell form and habitat. Jolicoeur's (1963) generalization of the allometry equation to multivariate analysis is particularly useful in this regard.

In contrast to Ortmann's (1920) and my results, Clarke (1973) found that L. radiata tend to be more obese in larger lakes. He thought that increased obesity would increase the stability of mussel shells in the more exposed habitats of large lakes. This is contrary to the usual "adaptive hypothesis" of greater obesity in low exposure areas to prevent sinking into soft sediments (Section 1.1). Since Clarke (1973) did not collect any environmental data at the particular sites of mussel collection, and didn't actually quantify a relationship between shell form and habitat, his conclusions about the variation in shell morphology of L. radiata in lakes must be regarded as tentative.

Hinch et al. (1986) observed differences in shell morphology and growth rate between L. radiata collected from sand and mud in IB. Although the growth rate comparison gave the same result as mine (discussed below), they found smaller and more obese shells in the mud habitat (in agreement with Ortmann's Law). Since their comparison of high and low exposure areas is based on only one site in each area, I would argue that the relationship I observed better describes the general situation in IB.

In contrast to Hinch et al.'s (1986) and my results, both Brown et al (1938) and Green (1972) reported finding smaller, "stunted", L. radiata in more exposed lentic habitats. Brown et al (1938) sampled L. radiata in an extremely exposed area of western Lake Erie off Pelee Island. They found, after comparison with specimens collected in less exposed areas near to this site, that the mussels from the highly exposed site were much smaller. Their results, combined with mine and those of Hinch et al. (1986), indicate that the positive relationship between exposure and shell growth rate has a limited range, beyond which the mussel is unable to function and grow efficiently. I tried to test this hypothesized relationship by sampling L. radiata in Outer Long Point Bay along the northern coast of Long Point. In the highly exposed, sandy, "washboard bottom" habitats found in this area, there were no mussels present. Brown et al. (1938) noted that at the Pelee Island site mussels were cast up along the shore during days of only mild westerly winds, and many dead mussels (with bodies still intact) were found in the shore drift.

Green's (1972) evidence of "stunting" in high exposure habitats is somewhat more equivocal than Brown et al.'s (1938). He collected L. radiata only from areas beyond the littoral zone, thus reducing the variation in exposure among sampling sites. He also collected mussels from several lakes, thereby including among-lake variation in his dataset. Green's (1972) canonical correlation of environmental (water chemistry and sediment variables) and shell morphometric

variables showed no relationship between the shell morphology of L. radiata and the sediment characteristics of its habitat. Nevertheless, he interpreted the main canonical axis of environmental variables as one of "exposure" because it roughly correlated with maximum fetch in the lakes sampled.

In summary, within a given lake L. radiata (this study; Hinch et al. 1986) and other species (Hinch and Bailey 1987) have faster-growing shells in more exposed habitats. In very extreme conditions, stunting can occur (Brown et al. 1938). Other factors such as water chemistry and lake morphology may be more important in explaining among lake variation in shell morphology and growth (as in Green 1972).

The positive relationship between growth rate and exposure, which I measured using the ANCOVA of Walford regression lines, was similar to that found by Hinch et al. (1986) with L. radiata from a sandy and a muddy site in IB. The small differences between Hinch et al.'s (1986) and my estimates of the coefficients of the Walford plot regression lines (Table 16) are probably due to sampling error, differences in the range of ring measurements used to compute the regression lines (Hinch et al. [1986] generally used larger pairs of rings), and the different set of sites used in each study.

Other Walford plot analyses of growth have been done on this and other populations of L. radiata (McCuaig and Green 1983; Day 1984; Magnin and Stanczykowska 1971; see Table 16). Differences in the method of collecting and analysing

Table 16. Other estimates of Walford plot coefficients for L. radiata.

Location	Walford Plot Equation
Lake Champlain (Day 1984)	$L_{t+1} = 12.5 + 0.85\ L_t$
Lac St. Louis (Magnin and Stanczykowska 1971)	$L_{t+1} = 22.5 + 0.74\ L_t$
Lac des Deux Montagnes (Magnin and Stanczykowska 1971)	$L_{t+1} = 16.2 + 0.77\ L_t$
Inner Long Point Bay (LRB) (McCuaig and Green 1983)	$L_{t+1} = 16.0 + 0.75\ L_t$
Inner Long Point Bay (other sites) (McCuaig and Green 1983)	$L_{t+1} = 12.7 + 0.81\ L_t$
Inner Long Point Bay (high exposure) (Hinch et al. 1986)	$L_{t+1} = 12.68 + 0.89\ L_t$
Inner Long Point Bay (low exp.) (Hinch et al. 1986)	$L_{t+1} = 7.45 + 0.89\ L_t$
Inner Long Point Bay (high exp.) (this study)	$L_{t+1} = 11.42 + 0.92\ L_t$
Inner Long Point Bay (medium exp.) (this study)	$L_{t+1} = 8.18 + 0.92\ L_t$
Inner Long Point Bay (low exp.) (this study)	$L_{t+1} = 8.65 + 0.92\ L_t$

the data make direct comparison of the estimated coefficients difficult. In fact, although the use of Walford plots as a method of comparing growth rates within the range of the annual ring data collected is sound, one should view the derived von Bertalanffy growth curves with caution. Small differences in the Walford plot coefficients can have a major impact on the von Bertalanffy growth curve, which is derived from the Walford coefficients.

Shells from high exposure habitats were thicker but not longer than those from lower exposure areas, and yet both of the annual ring analyses (Walford plot; length at first ring) showed faster growth in length in the high exposure mussels. This implies one of two things : either the growth curve of each exposure group changes as age increases beyond the range of annual ring data collected, or there is a higher mortality rate in the high exposure mussels. The first scenario suggests the possibility of different growth strategies : quick, early growth in the high exposure mussels followed by a virtual cessation of growth in length; slow, steady growth of mussels living in low exposure areas which continues beyond the age at which high exposure mussels have stopped growing. This seems unlikely, since a reciprocal transplant experiment over one growing season (Hinch et al. 1986) showed faster growth in high exposure mussels, even though all animals used in the experiment were close to asymptotic size. The second scenario, higher mortality in high exposure mussels, also suggests the possibility of different strategies for reproduction and

growth, but no data were collected to adequately test such an idea.

4.3 Allozyme Variation in L. radiata

Allozyme variation is valuable in examining population structure. The observed variation in allozyme phenotypes is almost always heritable, although both ontogenetic (Morgan et al. 1978) and environmentally induced (Oxford 1975) variation has been observed. Some authors (reviewed in Nei and Graur 1984) feel that allozyme variation is primarily due to genetic drift. This would enable differences in allele frequencies of allozymes to act as a sort of "biological clock", which measures the time since two populations have become reproductively isolated (reviewed in Thorpe 1982). Other workers, particularly those interested in marine invertebrates (e.g. Murdock et al. 1975; Singh and Zouros 1978; Koehn and Shumway 1982; Gartner-Kepkay et al. 1980) have collected either observational or experimental data supporting the hypothesis that some allozyme variation is maintained by selection.

Although the relative importance of the various causes of genetic divergence is difficult to determine, the existence of divergence is relatively easy to test by examining contingency tables of gene frequency data (but see Allendorf and Phelps 1981). The data examined in this study showed a clear difference between Lower Great Lakes populations of L. radiata and a population in Balsam Lake (east of Lake Simcoe) in the allele frequencies of both gene

loci examined. This can probably be explained by reproductive isolation between the Balsam Lake and Great Lakes populations since the retreat of the Wisconsin ice sheet about ten thousand years ago. At that time the Trent-Severn watershed (including Balsam Lake) and the Lower Great Lakes watershed became separated enough to greatly reduce fish transport of glochidia between these geographic areas (Clarke and Berg 1959). Although these historical processes probably played a major role in the observed genetic divergence between the Balsam Lake and Great lakes populations, selection could have augmented (or reduced) this divergence. In contrast to the observed geographic heterogeneity of gene frequencies in the present study, Kat and Davis (1984) found little variation in allelic frequencies of L. radiata radiata from three populations in eastern Canada. However, they included monomorphic loci in their analysis of genetic similarity.

Within IB, there was significant heterogeneity in PGM among the exposure areas. To illustrate how low the gene flow between the exposure areas in IB would have to be to generate this amount of genetic divergence, I calculated Wright's (1943) fixation coefficient :

$$F_{st} = \frac{s}{\bar{p}\,(1 - \bar{p})}$$

where s = variance among sub-populations in frequency of allele 'a'

$\bar{p}$ = mean frequency of allele 'a' across all sub-populations

For PGM's variation among the three exposure areas in IB the value was 0.0319, while for PGI (which did not show significant heterogeneity in IB) Fst was 0.0058. The value for two populations with exactly the same gene frequencies (i.e. no divergence) would be zero. For comparison, if L. radiata from IB and Balsam Lake are considered two populations, Fst is 0.139 for PGI and 0.049 for PGM. The value calculated for PGM among exposure groups within IB is low, indicating a small amount of genetic divergence among the three exposure areas. As another measure of reproductive isolation, I calculated the number of individuals straying from their sub-populations given the observed divergence in allelic frequencies (using Wright 1975):

$$Nm = .1/4 \left(1/Fst - 1 \right)$$

where N = effective population size

m = proportion of individuals straying from sub-populations

This formula assumes that migration and drift without selection determine allele frequencies (Allendorf and Phelps 1981). 'Nm' was equal to about 8 individuals for PGM and about 43 individuals straying per generation for PGI. Recalling that freshwater mussels can disperse as parasites of fish, and that the density of L. radiata in IB was about half a million mussels per km (Section 3.1), the supposed sub-populations in IB represent a large number of animals. Thus, whether 8 or 43 mussels are straying from their sub-populations to breed, the wanderers represent an extremely small percentage of the effective population size. However, it takes only one straying individual per breeding season to render a set of sub-populations into a panmictic unit (Spieth 1974; but see Allendorf and Phelps 1981). By comparison, the value of 'Nm' when one considers the reproductive isolation between IB and Balsam Lake is about 2 individuals for PGI and 5 individuals for PGM.

In summary, examination of the variation in two polymorphic enzymes revealed some heterogeneity in gene frequencies among different exposure areas in IB. The magnitude of the heterogeneity, when considered relative to genetic divergence between the Great Lakes and Balsam Lake

mussels was insufficient to support an hypothesis of reproductive isolation among the exposure areas.

4.4 Heritability of Glochidia Shell Morphology

Compared to other estimates (Table 17), heritabilities calculated for glochidia total length (19%) and hinge length (10%) appear low. They are also low relative to those calculated for larval size of various marine bivalves (reviewed in Newkirk 1980), which often exceed 50% when estimates are based on full-sib correlations in laboratory rearing conditions. Since maternal effects and dominance deviations may have positively biased my heritabilty estimates, I conclude that the heritability of these traits is low.

According to Fisher's Fundamental Theorem of Natural Selection (Fisher 1958), traits showing low heritability are highly correlated with fitness. Although the generality and utility of this theorem have been debated (e.g. Stearns 1980; Etges 1982; Stearns 1982), it seems plausible that selection has pushed glochidia shell size to some general optimum, thus resulting in low heritability. Low heritability is not consistent with the hypothesis that sub-populations of L. radiata in the different exposure areas have had glochidial sizes pushed to different optimal sizes. Indeed, such disruptive selection would maintain high additive genetic variance in the IB population as a whole.

One assumption in my computation of heritabilty was that the glochidia sampled from a given female were full sibs. It

Table 17. Examples of other estimates of heritability (referred to in Ayala [1982] and Falconer [1981]).

Litter size in mice	15%
Egg production in Drosophila	20%
Egg production in poultry	10-20%
Plant height in corn	70%
Stature in humans	65%

was assumed that sperm from one male would enter the incurrent siphon of the female and fertilize all of her ova. Since this reproductive pattern has not been verified by observation, the effect of multiple paternity on my heritability estimates should be considered. The proportion of genes which two glochidia share, on average (given a large number of glochidia and an equal number of offspring sired by each male) is :

$$p = (f + 1) / 4f$$

where 'f' is the number of males inseminating the female. The equation illustrates that as the glochidia approach half sib status, where there is a different father for each glochidium, they share one quarter of their genes; when only one father is responsible for all the glochidia, they share one half of their genes. Heritability is '1/p' times the estimate of the among-family component of variance in the ANOVA's of total length and hinge length of glochidia. Thus, my estimates would be one half of what they should be if the glochidia are half sibs. Although it is unlikely that glochidia are all half sibs, it is quite possible that two or three males fertilize one female. This would mean that my estimtes are two thirds (3 males) or three quarters (2 males) of their true values. Unequal division of paternity, which seems quite likely, would increase the accuracy of my estimates.

There is a more important question about these heritabilty estimates : what does the heritability of glochidia shell dimensions tell one about additive genetic

variation in the shell form of juvenile and adult mussels? Although there is no shell growth from the pre- parasitic, mature glochidia to the post-parasitic juvenile mussels, data from this study show that L. radiata grows from approximately 250u as a post-parasitic juvenile in early summer to about 12mm by formation of the first winter's annual growth line (a 50-fold increase in length). Although there was significant, exposure-related variation in length of this first annual ring, this variation could be due to genetic variance in physiological mechanisms (e.g. filtering apparatus) which develop during or after parasitism of the fish. Thus, I could have missed an increase in additive genetic variance with respect to shell dimensions by measuring only pre-parasitic glochidia.

Some evidence indicates that, ceteris paribus, size of glochidia may be a good indication of adult growth rate. Haley and Newkirk (1977) found that early settling, faster-growing oyster spat became faster-growing adult oysters. With respect to freshwater mussels, B. G. Isom (unpublished data) has compiled extensive growth data on several species which he has cultured in vitro from glochidia to juveniles (Isom and Hudson 1982; Hudson and Isom 1984). He reports that large glochidia grow faster as juveniles than small glochidia in a homogeneous laboratory environment. Given this evidence, the genetic variance of shell dimensions estimated for glochidia would be relevant to that in at least the juvenile year of growth. Uncontrolled conditions outside of the laboratory aquarium would only increase environmental

variance, causing a reduction in heritability.

Also, in order for the heritability of shell form to be markedly higher by the first winter of life, any genetic variation introduced by variation in post-parasitic anatomical, physiological, or behavioural development must "outrun" variation introduced by the environment in these later life stages. I have no data to compare the dynamics of genetic and environmental variance in shell dimensions. However, environmentally induced variation in morphology arising during the parasitization process, the establishment of the juvenile mussel, and its growth in the benthic habitat probably keeps pace with genetic variation due to differences in anatomy, physiology, and behaviour. Thus, I would conclude that the heritability of at least juvenile shell dimensions is also low.

The genetic and environmental correlations between the total length and hinge length of the glochidia had opposite signs (genetic : positive correlation; environmental : negative correlation). This shows that genetic and environmental sources of variation affect the characters through different physiological mechanisms (Falconer 1981). Since the genetic correlation also includes maternal effects, the positive correlation could be indicative of the habitat or general health of the mother. The negative environmental correlation shows that shell allocation to width or length is just an environmentally induced phenomenon, at least in glochidia.

4.5 Habitat Preference or "Stuck in the Mud"?

Although many authors (Harman 1971; Cvancara 1972; Tevesz and McCall 1979; Kat 1982) have speculated about the nature of (or the lack of) substrate preference in freshwater mussels, Kat (1981) pointed out that frequency of occurrence does not indicate preference. Gale (1971), in a laboratory experiment, showed strong preference by a fingernail clam for muddy substrates, although its distribution in the field did not reflect such a preference. Fretwell and Lucas (1970) and Rosenzweig (1981) showed how both the fitness cost of expressing a preference and the density-dependent quality of a preferred habitat can reduce its relative quality until it is no longer "cost effective" for an organism to occupy a "preferred" habitat.

I showed a preference for the mud substrate by L. radiata in the ponds. The preference for the mud substrate can be partially explained by comparing the particle size distributions of the pond substrates (Fig. 5) with two examples of IB substrates (Fig. 7). The particle size distribution of the mud used in the ponds resembles that of the lake mud and sand. The sand used in the ponds was quite different - coarser and less variable than the sandy substrate from the lake.

There is another explanation for the observed distribution of mussels when they were recovered from the ponds. If the mud substrate significantly slowed down the movement of the mussels as they were randomly moving through the pond, the observed pattern of "substrate preference"

would emerge. Given the fact that these mussels must move through the substrates they occupy, it is impossible to distinguish an observation of "habitat preference" from one of mussels "stuck in the mud".

This problem is also relevant when considering the different response of the "sand-collected" and "mud-collected" mussels. Mussels which were collected from the sand in IB showed a stronger preference for the mud in the ponds than mud-collected mussels. This was not due to a lack of movement in the mud-collected group: 33% switched substrates during the study compared to 34% of the sand-collected mussels. The CVA of shell morphology showed the sand-collected individuals were larger and heavier-shelled. This could mean that substrate choice is more important for larger, thicker-shelled L. radiata. If so, the ability to express substrate preference may bring together mussels of similar shell morphology and impose a breeding structure on the IB population. Alternatively, the larger-shelled, sand-collected mussels may have just been more prone to getting "stuck in the mud".

What influence could habitat preference have on the structure of the population in IB? Even if there are preferred substrates, the fitness cost of getting to them may be too high. Little is known about the potential for movement in unionids. I have observed marked mussels which have moved (or have been moved by currents?) about 20m in three months. Given the large area of each exposure level in IB, it is likely that substrate preference could act only as

a fine tuning mechanism of reproductive isolation near the borders of sub-populations.

4.6 Shell Morphology as a Burrowing Adaptation

Both Stanley (1970), for bivalves in general, and Wilson and Clarke (1914) for unionids, proposed that less obese shells are better adapted to burrowing in hard substrata. The interaction between substrate and MORPH3 (shell obesity), in the burrowing experiment is consistent with this hypothesis. The coefficient for this term showed that in the sandy substrate, narrow mussels were more apt to burrow than obese individuals. The opposite was true in the muddy substrate.

The other important interaction, between substrate and MORPH2 (shell thickness), indicated a lower probability of burrowing by heavy-shelled individuals in the sandy substrate. It has been argued (e.g. Stanley 1970; Wilson and Clarke 1914) that heavy shells are well-adapted to more exposed habitats because they prevent dislodgement of the shell. If this is true for L. radiata, the thicker shell may be increasing the stability of the animal in exposed habitats at a cost of some burrowing efficiency. Stanley (1970) proposes, in agreement with my results, that thinner shells enable bivalves to burrow faster.

Neither of the important interactions in the burrowing experiment would generate correct predictions about patterns of morphological variation in the natural population. The M3*Substrate interaction would lead to a prediction of

reduced obesity in the more exposed, sandier areas of IB. I found no evidence of such a relationship (but see Hinch et al. 1986). The M2*Substrate interaction would lead to a prediction of thinner shells in the more exposed areas. I found the opposite trend (Section 3.2). Three reasons may be proposed for the failure of the experimental results to predict natural variation; i) burrowing rate alone is a poor fitness surrogate (e.g. stability of the shell may also be important), ii) there is more to the natural habitat variation than variation in substrate characteristics (e.g. water turbulence differs in high and low exposure areas), and iii) the natural variation in shell morphology may represent a non-adaptive pattern (e.g. variation due to varying food supply).

4.7 Conclusions - A Proposed Mechanism for the Correlation

I will now summarize what I observed in each component of the study following my initial observation of the correlation between the habitat of L. radiata and its shell morphology and growth rate (i.e. mussels in high exposure areas have faster growing, thicker shells than those from low exposure areas). I will indicate what I have concluded from each component, and what results I would have expected for an alternative conclusion to be drawn :

i) Allozyme variation. Little divergence in allele frequencies was observed, especially when compared to that between geographically isolated populations in Balsam Lake and the Lower Great Lakes. Reproductive isolation among the

exposure areas in the bay, necessary for either past or present disruptive selection with respect to shell morphology, would have been indicated by greater divergence.

ii) Heritability of glochidia shell size. Low heritability of total length and hinge length was found, especially considering that the estimates included maternal effects. Disruptive selection of shell growth rate across IB would maintain additive genetic variance in the IB population as a whole, causing high heritabilities.

iii) Substrate Preference Experiment. Substrate preference was displayed, and appeared to be at least partially dependent on shell morphology. This may affect the breeding structure of the population, at least near the borders of different habitat types. I expected the same type and degree of preference, regardless of morphology, to conclude that substrate preference has no effect on the breeding structure.

iv) Burrowing Experiment. Shell morphology affected burrowing ability differently in muddy and sandy substrates. This showed that there were different optimal morphologies for burrowing in the different substrates. I expected no difference between the substrates in the effect of shell morphology on burrowing (i.e. no interactions between morphological variables and substrate in predicting the probability of burrowing) if there is no difference in "optimal burrowing morphology" in the two substrates. Since the observed differences between substrates in morphological effects on burrowing rate did not lead to correct predictions

about morphological variation in IB, I concluded that either my definition of burrowing rate was a poor fitness surrogate, or my laboratory habitats did not mimic important, natural variation in the environment, or the variation in shell form found in IB is a non-adaptive response to environmental variation.

The above evidence strongly suggests that the proximal cause of variation in the shell morphology and growth rate of L. radiata in IB is phenotypic plasticity. The heritability estimates and allozyme data did not support an hypothesis of divergent sub-populations in the different exposure areas. The question of whether or not the observed relationship between phenotype and environment represents differential adaptation to different habitats remains unanswered. The burrowing experiment showed differential adaptation of shell form to different habitats, but predictions made from the experiment concerning morphological variation did not hold in the natural population. A more elaborate study of processes other than burrowing rate which may be affected by shell morphology (e.g. stability, filtering rate), in conditions that better mimic the natural environmental variation (e.g sediment and turbulence variation), would lead to a stronger test of the "adaptation" hypothesis.

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