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Physiological And Growth Responses Of Macoma Balthica (mollusca:pelecypoda) To Environmental And Genetic Factors, With Emphasis On A Hudson Bay Population Near Churchill

Shirley Siew-lee Lim

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PHYSIOLOGICAL AND GROWTH RESPONSES OF *MACOMA BALTHICA* (MOLLUSCA, PELECYPODA) TO ENVIRONMENTAL AND GENETIC FACTORS, WITH EMPHASIS ON A HUDSON BAY POPULATION NEAR CHURCHILL

by

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ABSTRACT

Growth rate of most intertidal organisms generally declines in an upshore direction. However, *Macoma balthica* from 1.0 m above mean low water (MLW) grew faster than those from MLW in Churchill (Hudson Bay). This anomaly was the focus of this study. The first objective was to determine if this phenomenon is widespread or localised. Clams were collected from two tide levels from four sites along the St. Lawrence River and three sites at the Bay of Fundy. Results from the analyses of the growth ring data gathered showed that the "Churchill phenomenon" was unique and confined to sites along the coast of Hudson Bay east of Churchill to Cape Henrietta Marie on James Bay.

The second objective was to examine the effects of biotic and environmental factors on the growth rate and physiological responses of the Churchill clams. At ebb tide *M. balthica* makes crawling tracks on the intertidal sand flats. The parasite load (i.e. number of trematode metacercariae) and growth rate of crawling and buried *Macoma* from the two tide levels were compared. High tide *Macoma* were more heavily parasitized and grew faster than low tide ones and crawlers (more parasitized) grew faster than the buried (less parasitized) clams. Parasitic castration resulting in enhanced growth could be an explanation for these observations.

Transplant experiments were conducted for two field seasons. Results showed that the *Macoma* transplanted from MLW to 1.0 m above MLW grew at a rate similar to that of the high tide "residents", indicating that the phenomenon is probably influenced by local environmental conditions at the two tide levels rather than by the genotypic differences. No

positive correlation was detected between growth rate and degree of heterozygosity (scored for the enzymes PGM, PGI, MDH and LAP) in the clams from either tide level.

The Q_{10} of *Macoma* from both tide levels was 1.5 which is indicative of a moderately temperature dependent metabolism. Oxygen consumption per gram dry weight was inversely related to the size of *Macoma* for both high and low tide levels. For clams of a given size and temperature, low tide *Macoma* consumed more oxygen than high tide clams. This implies that the high tide clams have greater metabolic efficiency: this energy "saved" by higher metabolic efficiencies in faster growing animals could be used to further enhance their growth.

Low tide clams were able to withstand longer periods of cold stress than high tide clams in the laboratory. This is contradictory to the general rule that species living higher up the shore are expected to have greater tolerance to freezing. Clams from the low tide level burrowed deeper into the substratum than high tide clams. Higher temperatures experienced near the surface of the substratum and at the high intertidal region could be associated with the faster growth rate of the high tide *Macoma*.

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To my parents

who introduced me

to the wonders of the sea-shore

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1. INTRODUCTION

1.1 Study animal

Macoma balthica (Linnaeus 1758) is a common, extensively studied species of bivalve mollusc belonging to the family Tellinidae. It is generally found in temperate, boreal and arctic low salinity waters of the North Atlantic and Pacific. The geographical distribution of *M. balthica* ranges from the North Sea, Baltic Sea (Vogel 1959, Segerstrăle 1960) Hudson Bay (Green 1973, Green et al. 1983) and Frobisher Bay (Lubinsky 1980) in the north to as far south as the Gironde estuary (Bachelet 1980), Chesapeake Bay (McErlean 1964) and off the coast of Georgia (Abbott 1974).

Aspects of the biology of *M. balthica* studied to date include: its growth (Segerstrăle 1960, Lammens 1967, Lavoie et al. 1968, Gilbert 1973, Green 1973, de Wilde 1975, Bachelet 1980, McGreer 1983, Nichols and Thompson 1982, Green et al. 1983, Hummel 1985, Wenne and Klusek 1985, Beukema and Desprez 1986, Vincent et al. 1987), reproduction (Lammens 1967, McGreer 1983, Wenne 1985), parasite infection (Swennen 1969, Swennen and Ching 1974, Pekkarinen 1984a, 1984b, 1987a, 1987b, 1987c, Wenne 1985), burrowing behaviour (Hulscher 1973, Reading and McGrorty 1978), predation by shore-birds (Hulscher 1982, Evans 1984, Prokosh 1984, Wolff and Smit 1984), biochemistry (Pekkarinen 1983, Wenne and Styczynska-Jurewicz 1987) and population genetics (Green et al. 1983, Meehan 1985, Singh and Green 1986).

M. balthica is dioecious with synchronous spawning and external fertilisation. The larval stage is distinct and planktonic. Its maximum length ranges from 25 mm to 38 mm. Annual shell growth rings in

Macoma are clear in many populations: on Hudson Bay (Green 1973, Green et al. 1983), along the south shore of the St. Lawrence River (Lim, unpubl. data), Dutch Wadden Sea (Lammens 1967) and Gdánsk Bay, South Baltic (Wenne and Klusek 1985).

1.2 Growth rate of *M. balthica*

The growth rate of many marine organisms varies with tide level. Numerous studies have shown that growth rate generally declines in an upshore direction in most intertidal molluscs (Vermeij 1980). For example, in the limpet, *Patella vulgata*, growth rate was much higher in the mid-tide level than in the high intertidal (Lewis and Bowman 1975). Similarly, growth rate of the mussel, *Mytilus edulis*, was observed to be much lower at high shore levels than in lower shore habitats (Newcombe 1935, Seed 1976).

A Norwegian fjord intertidal population of *M. balthica* showed the same trend, with larger individuals (implying higher growth rates) at lower levels on the shore (Strömgren et al. 1973). Beukema et al. (1977) observed that the *Macoma* at the high intertidal area in the Dutch Wadden Sea grew slower than those found lower down the shore. At Baie des Roses on the southern shore of the St. Lawrence River, greater shell growth was also observed in the lower intertidal *Macoma* than the higher intertidal ones (Vincent et al. 1987).

However, Green (1973) and Green et al. (1983) found the reverse to be true for a population of *M. balthica* in Hudson Bay near Churchill. *Macoma* living at a tidal level of 1.1 m above mean low water (MLW) grew faster than those at the MLW level.

Macoma collected by the Canadian Wildlife Service (C.W.S.) from other Hudson Bay and James Bay sites, south-east of Churchill (Appendix I

(i)), were examined by the author. High tide *Macoma* were found to grow significantly faster than low tide ones at Severn Partridge Island (Appendix I(ii)(a), (iii)(a) and (iv)). At Hook Point South, a faster rate of growth was also observed for the high tide *Macoma* although it was not statistically significant (Appendix I (ii)(b), (iii)(b) and (iv)). There was no significant difference between the growth rates of the high and low tide *Macoma* at Swan River South. At North Point D, there was a significant difference between the growth rates of the high and low tide *Macoma*. The growth of young high tide clams was faster than low tide ones but as clams grew older (i.e. larger than 7mm), low tide *Macoma* grew faster than high tide ones. These results indicated that the "Churchill phenomenon" was present in Hudson Bay, east of Churchill to Cape Henrietta Marie and not present in James Bay.

The only other location where faster growth of higher than lower tidal *M. balthica* was ever reported is the Gironde estuary in France. Bachelet (1980) attributed this differential growth rate between clams from 2 m above MLW and those from MLW to the hydrodynamics that govern sedimentation in Le Verdon, a small protected bay in the estuary. The clams at 2 m above MLW were in a region of the intertidal that was relatively stable where water conditions were calm, whereas the MLW clams were constantly under stress in shifting sediments caused by strong water disturbance. These low tide *Macoma* did not survive beyond two years: as a result, the population at MLW consisted exclusively of young individuals which were less than 5 mm in length. Higher up the shore, a wider size range and greater longevity of *Macoma* were recorded.

1.3 Factors affecting growth rate

1.3.1 Parasite load

Parasite load is a biotic factor that could affect *Macoma* growth rate. "Gigantism" (or enhanced growth) caused by the presence of parasites in molluscan hosts, has been well studied and reviewed by many researchers e.g. Rothschild and Rothschild 1939, Cheng 1971, Baudoin 1975, Joose and van Elk 1986 etc. *M. balthica* is the intermediate host to at least three gymnophallid trematodes: *Gymnophallus gibberosus* Loos-Frank, *Parvatrema affinis* (Jameson and Nicoll) and *Lacunovermis macomae* (Lebour) (Loos-Frank 1971). Trematode daughter sporocysts of all three species are found mainly in the gonad of the clam causing parasitic castration (Pekkarinen 1987a, b, c). Various stages of tissue degradation were observed around the daughter sporocysts of *Lacunovermis macomae* in *M. balthica* (Pekkarinen 1987b). *Parvatrema affinis* causes partial or total disappearance of the gonads and can also induce shell deformations in *M. balthica* (Wenne and Klusek 1985). Hydrolytic enzymes may be secreted by trematode daughter sporocysts to break down the exogenous carbohydrates in the host which could then be absorbed through the parasite's body wall (Thomas and Pascoe 1973, Popiel and James 1976). Enzyme activities in host tissues can be altered by infections of daughter-sporocysts (Moore and Halton 1973, Marshall et al. 1974).

The definitive hosts of these digenean trematodes are shorebirds. In Hudson Bay, *Limosa haemastica,* Hudsonian Godwits, forage extensively on *Macoma* on the upper levels of the

flats prior to their southward migration (R.D. Elliot, C.W.S. pers. comm.). Other species such as *Pluvialis squatarola*, the Black-bellied Plover which migrate through Hudson Bay during autumn, probably feed on *Macoma* as they do in other areas where this clam is abundant (P.W. Hicklin, C.W.S. pers. comm.).

Swennen (1969) and Hulscher (1973) reported that 100% of the clams that made crawling tracks at various sites along the Dutch coast and Wadden Sea were heavily infected with parasites while those normally buried in sand had a much lower infection rate. Swennen (1969) postulated that this track-making behaviour could be due to a shortage of oxygen caused by the high density of parasites present in the clams. In Hudson Bay, *M. balthica* is observed to make conspicuous U-shaped furrows or tracks on the sand flats at ebb tide.

1.3.2 Abiotic environmental factors

Examples of abiotic environmental factors that could influence growth rate are food availability and temperature. *M. balthica* has been described by Brafield and Newell (1961) and Newell (1965) as a "facultative filter-feeder cum surface film deposit-feeder". Micro-organisms, which are more abundant in fine sediments because of the greater particle surface area (Dale 1974) are used by *Macoma* when it deposit-feeds. The sand on the Churchill beach is finer at MLW than at 1.1 m above MLW (Green et al. 1983). The *Macoma* at the higher tidal level would, arguably, have less time to filter-feed, and when deposit-feeding, less food would be available because of the coarser sediments present there.

Vincent et al. (1989) found that there was a strong negative effect of clam density on *Macoma* shell growth in the St. Lawrence estuary. This density effect on growth was more significant as tidal level increases, and was attributed to food limitation in the upper parts of the tidal flat. At Churchill, the density of *Macoma* was much higher in the upper regions of the shore than at the MLW level. Hence, based on particle grain size and density of clams, it is difficult to attribute the higher growth rate at the 1.1 m level to food availability.

Green (1973) proposed that temperature is the factor causing the higher *Macoma* growth rate at the higher tidal level. Since the 1.1 m level is exposed to air about 37% of the time whereas the MLW level is only exposed at below average low tides, high summer air temperatures were suggested to play a major role. It is well known that marine organisms are able to adjust the rate of a variety of physiological as well as behavioural processes to seasonal, latitudinal or local environmental conditions such as temperature fluctuations. The metabolic processes of *M. balthica*, a poikilotherm, are obviously going to be affected by temperature. Cold tolerance of *M. balthica* from the St. Lawrence River has been studied by Bourget (1983) who found that small clams were less cold tolerant than larger ones. The higher an intertidal species live on the shore, the greater is the expected tolerance to freezing (Murphy 1979).

1.3.3 Genetics

Growth is a result of the conversion of energy to somatic tissue. The regulation of the enzymes involved in energy conversion is undoubtedly under genetic influence. There is increasing evidence in marine bivalves that individuals with high levels of heterozygosity have physiological characteristics which differ from those of more homozygous individuals. In fact, a positive correlation between degree of heterozygosity and growth rate has been found; the more heterozygous an individual is, the faster the growth. This correlation has been shown in many marine bivalves e.g. *Crassostrea virginica* (Zouros et al. 1980), *Mytilus edulis* (Koehn and Gaffney 1984) and *Mulinia lateralis* (Garton et al. 1984, Koehn et al. 1988).

Many different measures of growth were used in the studies mentioned above, such as direct increment in length over a known period of time, oxygen consumption, ammonia excretion, algal suspension feeding rate etc. Koehn and Shumway (1982) found that the more heterozygous *Crassostrea virginica* consumed less oxygen than the less heterozygous ones under both ambient (10^0 C, 28‰) and stressful (30^0 C, 14‰) conditions. They concluded that heterozygosity was associated with higher metabolic efficiency as a result of a decreased cost of enzyme biosynthesis in heterozygotes, thereby enabling them to grow faster. A similar inverse correlation between individual heterozygosity and rate of oxygen consumption was established in *Mytilus edulis* (Diehl et al. 1985). Green et al. (1983) showed that degree of heterozygosity as well as growth rate was greater at 1.1 m than at MLW in the Churchill *M. balthica*

population. Also, within the 1.1 m population, faster growing individuals had greater genetic heterozygosity and lower tolerance to high temperature. Thus, there is already some evidence that genetic factors are involved with the phenomenon at Churchill.

1.4 Thesis structure and objectives

This study was divided into two main parts. The first addressed the question of whether the faster growth rate of *M. balthica* at the higher tidal level is widespread or localised. Clams were collected from four locations along the southern shore of the St. Lawrence River and three sites at the Bay of Fundy. These sites were chosen based on their accessibility and the presence of *M. balthica*. The growth rates of the clams collected from two tidal levels, whenever possible, were compared for each site.

The second part of the study concentrated on the two sub-populations of clams from Churchill where the phenomenon occurs. There are three sections in this part. The first dealt with the effect of parasite load on the growth rate of *M. balthica* from Churchill. The percentage of parasitized clams at MLW and 1 m above MLW were determined and compared. The number of parasites per clam was ascertained and related to size, track-making behaviour and growth rate of the clams from the two tidal levels.

The second examined the influence of environmental and genetic effects on the growth of *Macoma*. This was approached by conducting transplant experiments in Hudson Bay. To the extent that the growth rate of the *Macoma* transplanted to the 1.1 m above MLW increases, and the growth rate of those transplanted to the MLW level decreases, the

suggestion is that the phenomenon is influenced by local environmental conditions at the two tide levels rather than by genotypic differences.

The last section consisted of three laboratory experiments comparing the response of high and low tide *Macoma* to temperature stress. Oxygen consumption at various sub-lethal temperatures and the tolerance of clams to different lengths of freezing treatment were the physiological responses studied. These response variables were analysed in conjunction with tidal level (that is, the source of the clams), clam length or size (wherever applicable), metacercaria load and genetic composition. Finally, the behaviour of *Macoma balthica* at three sub-lethal water temperatures was investigated in order to study their response to different environmental temperatures as well as modification of clam behaviour by parasites. This behaviour, quantified by the depth to which the clam burrowed was related to which tidal level the clams came from, clam length, metacercaria load and temperature of the water under investigation.

2. MATERIALS & METHODS

2.1 Study Areas

2.1.1 St. Lawrence River

Sampling was carried out at two tidal levels at four sites along the south shore of the St. Lawrence River (Fig. 1). The four sites were: Cacouna-est, St. Fabien-sur-mer, Capucins and Baie des Capucins; hereafter referred to as CE, SF, C and BC respectively. The tidal levels at which sampling was done at each site are tabulated in Table 1(a). All of the sampling sites were mud-flats except for SF which was a mixed rock and mud beach. The surface water salinity ranged from 11.5 to 23‰ among the four sites during the period of sampling. Mean tidal range is about 2.62 m.

2.1.2 Bay of Fundy

As *M. balthica* was only found in the high intertidal region at Indian Point (IP), St. Andrews, the tidal level difference between the high and low tide collection sites was small (Table 1b). Clams were only found in the upper region of the mud flat at Dorchester Cape (DC). The locations of IP and DC are shown in Fig. 2. IP is a mixed sand, boulder and muddy beach whereas DC is an extensive mud-flat extending about 2 km from the shore. The mean tidal range at IP and DC are 5.6 and 11 m respectively. The salinity of the seawater ranges from 26 to 31 ‰ (Trites and Garrett 1983).

Fig. 3 shows the location of the sampling site at the Selma Bar mud-flats (SB) in the Minas Basin, Nova Scotia. The tidal levels of the two collection sites on the mud-flat are given in Table 1(b). The

Figure 1. **Map of St. Lawrence River region showing location of four sampling sites viz. Cacouna-est, St. Fabien-sur-mer, Capucins and Baie des Capucins.**

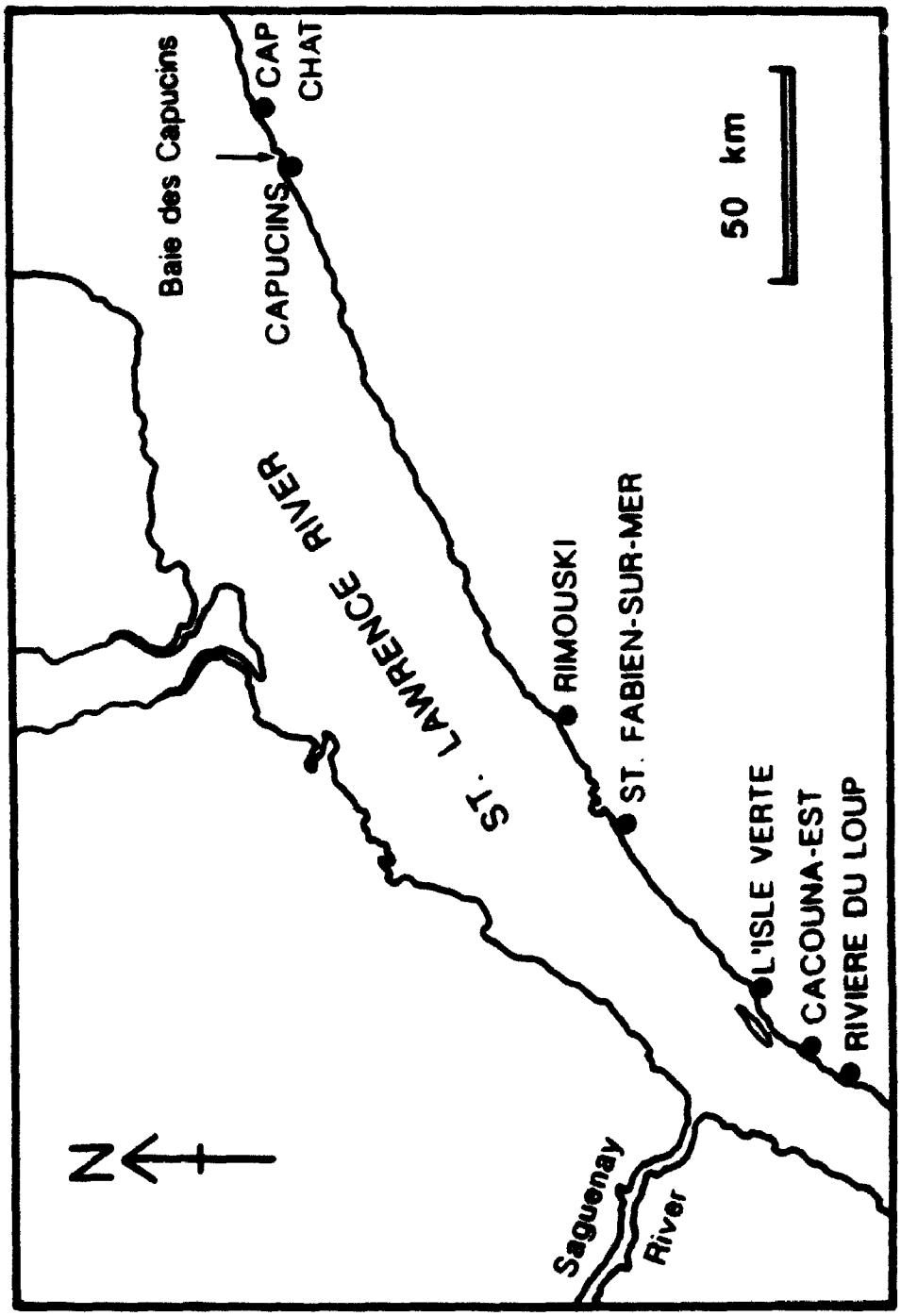

Baie des Capucins
CAP CHAT
CAPUCINS
ST. LAWRENCE RIVER
RIMOUSKI
ST. FABIEN-SUR-MER
L'ISLE VERTE
CACOUNA-EST
RIVIERE DU LOUP
50 km
N
Saguenay River

Table 1. Tide levels at which sampling was carried out : (a) four sites along the southern shore of the St. Lawrence River; (b) three sites in the Bay of Fundy; (c) one site in Hudson Bay. MLW = Mean low water.

Sampling Site	High tide level (m above MLW)	Low tide level (m above MLW)
(a) St. Lawrence River		
Cacouna-est	1.0	0.2
St. Fabien-sur-mer	1.2	0.1
Capucins	1.0	0.0
Baie des Capucins	1.0	0.0
(b) Bay of Fundy		
Indian Point	4.0	3.5
Dorchester Cape	10.0	-
Selma Bar	5.0	2.0
(c) Hudson Bay		
Churchill	1.0	0.0

Figure 2. Map of the Bay of Fundy showing location of two sampling sites viz. Indian Point and Dorchester Cape.

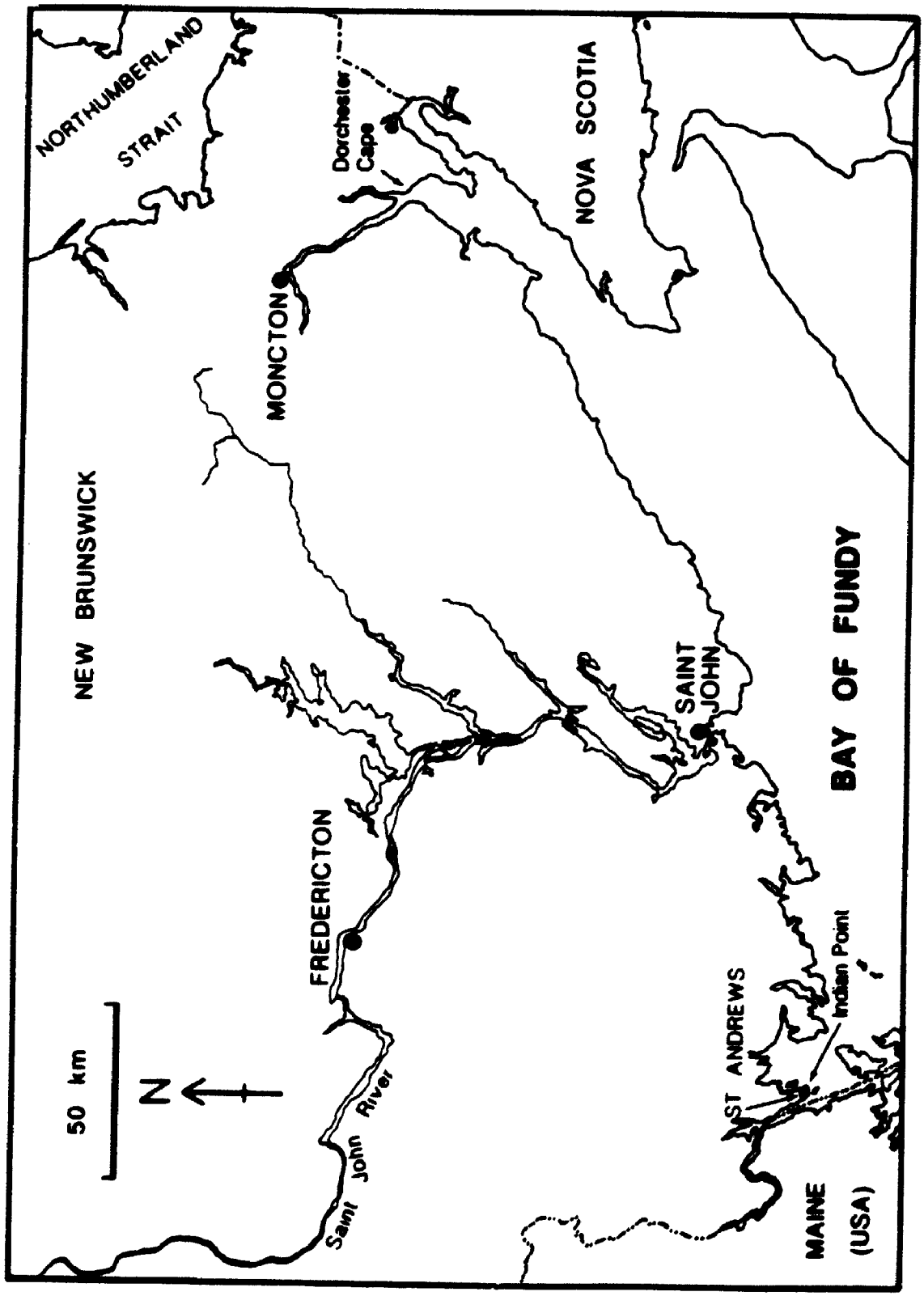
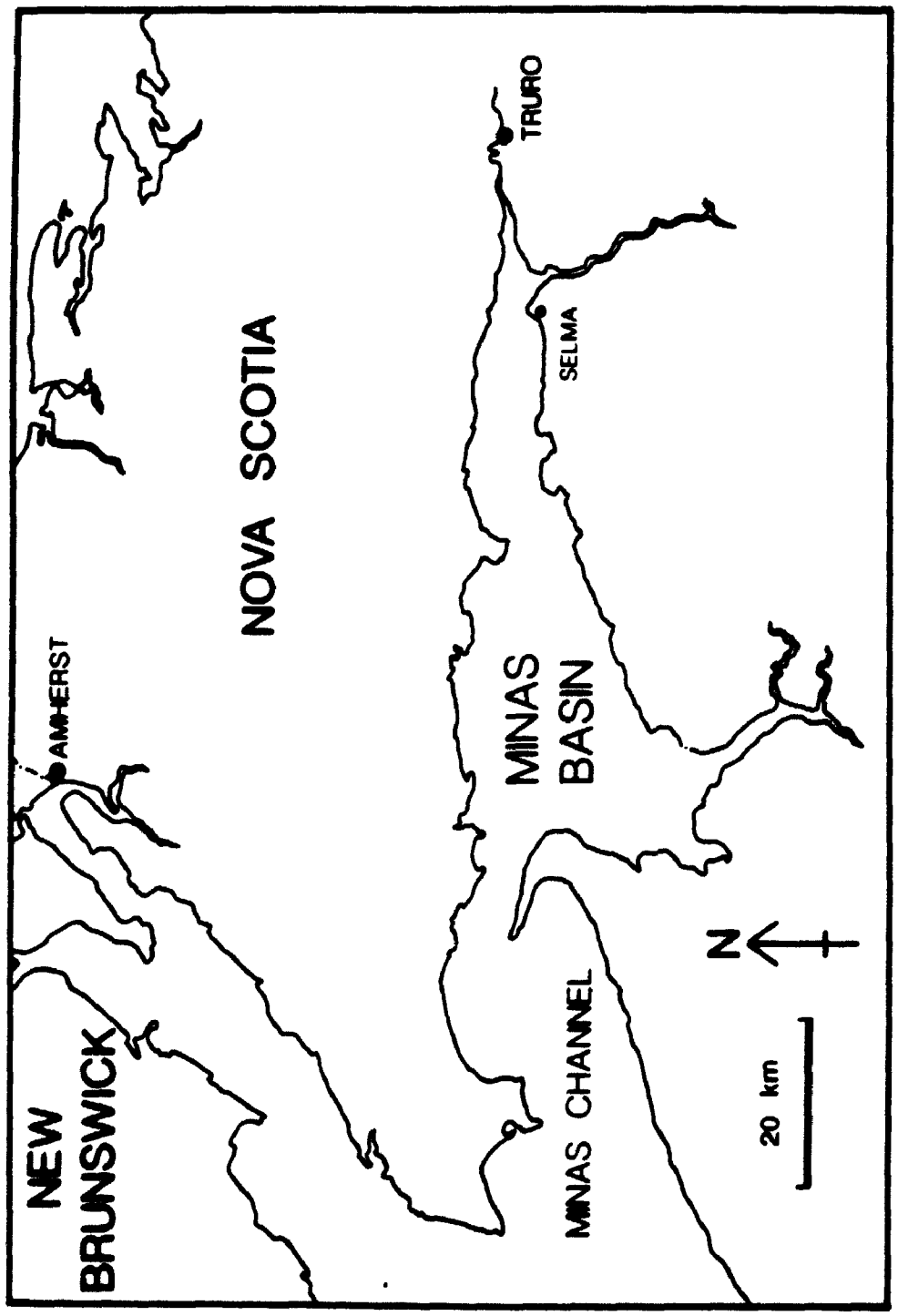

NORTHUMBERLAND
STRAIT
Dorchester
Cape
NOVA SCOTIA
MONCTON
NEW BRUNSWICK
SAINT
JOHN
BAY OF FUNDY
FREDERICTON
ST ANDREWS
Indian Point
MAINE
(USA)
Saint John River
50 km
N

Figure 3. Map of Minas Basin (Bay of Fundy) showing location of sampling site at Selma.


NEW
BRUNSWICK
AMHERST
NOVA SCOTIA
TRURO
SELMA
MINAS
BASIN
MINAS CHANNEL
N
20 km

mean tidal range in the Minas Basin is 11.5 m and the salinity ranged from 26 to 30‰ during the sampling period.

2.1.3 Hudson Bay

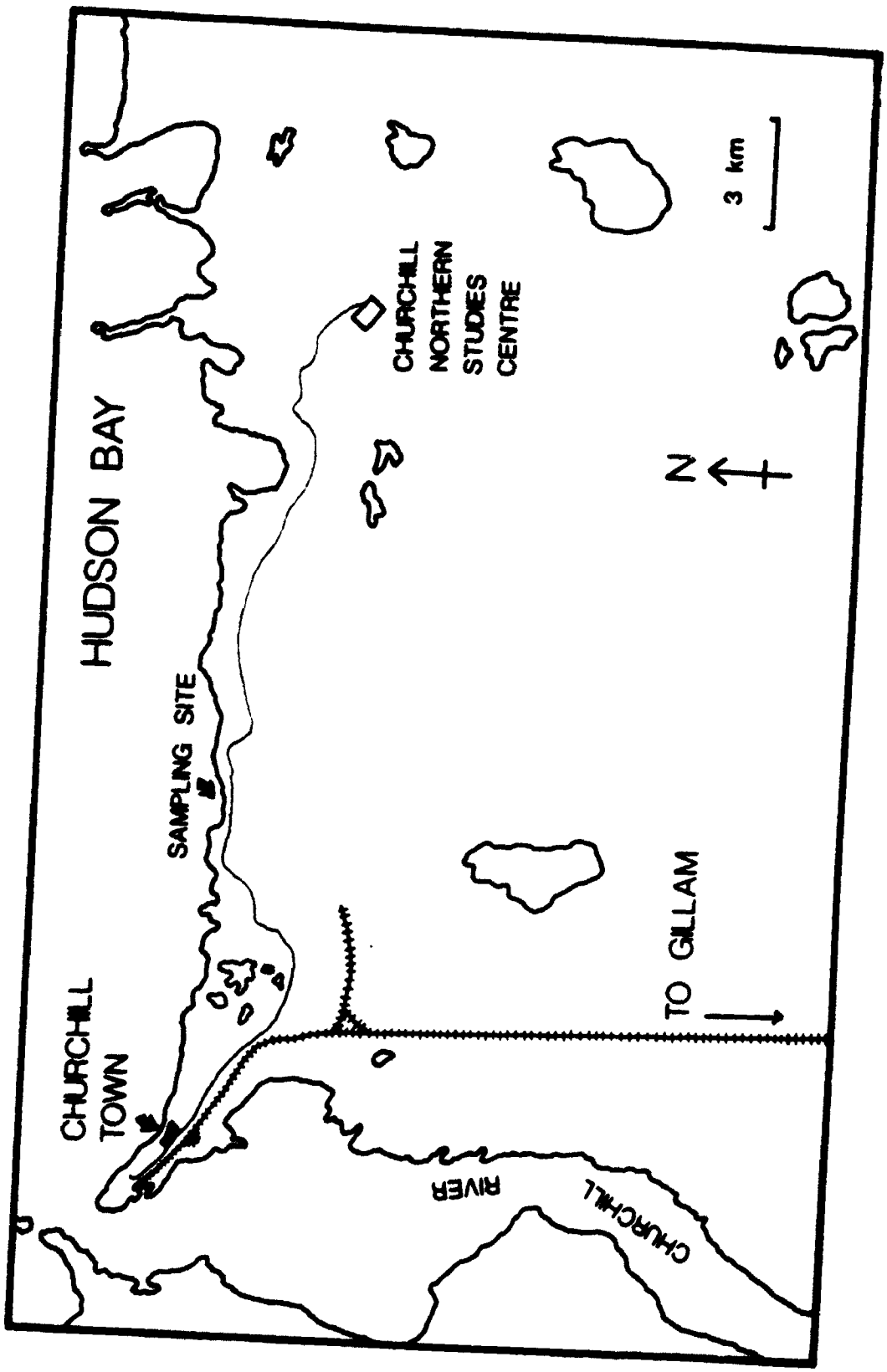
Sampling at two tidal levels was carried out on a mixed sand and boulder beach 7 km east of Churchill, Manitoba on Hudson Bay (Fig. 4). Table 1(c) gives the tidal levels of the sampling sites. The two sub-populations of *Macoma* are separated by a 200 m expanse of rock reef and tidal pool system. It is unlikely that vertical migration of adult clams occurred between the two tidal levels. The salinity of the nearshore water during the summer months is about 18‰. The mean tidal range is approximately 3.35 m.

2.2 General sampling method

At all three sampling areas, clams were collected by shovelling and sieving the substrate (mesh size 1 mm) at the respective tide levels given in Table 1. *M. balthica* from the St. Lawrence and Bay of Fundy areas were preserved in 70% ethanol immediately after collection for growth rate comparisons.

Clams collected from Hudson Bay for physiological and behavioural experiments were bagged together with wet *Fucus* and transported back to London, Ontario where they were kept alive in aerated artificial seawater of salinity 18‰ at $4 \pm 1^{\circ}$ C. Mortality rate of clams in the holding tanks was negligible (<1 %).

Figure 4. **Map of Hudson Bay showing location of sampling site near Churchill (Manitoba).**


HUDSON BAY
CHURCHILL NORTHERN STUDIES CENTRE
SAMPLING SITE
CHURCHILL TOWN
TO GILLAM
CHURCHILL RIVER
3 km
N

2.3 Geographical variation in growth rate with respect to tide levels

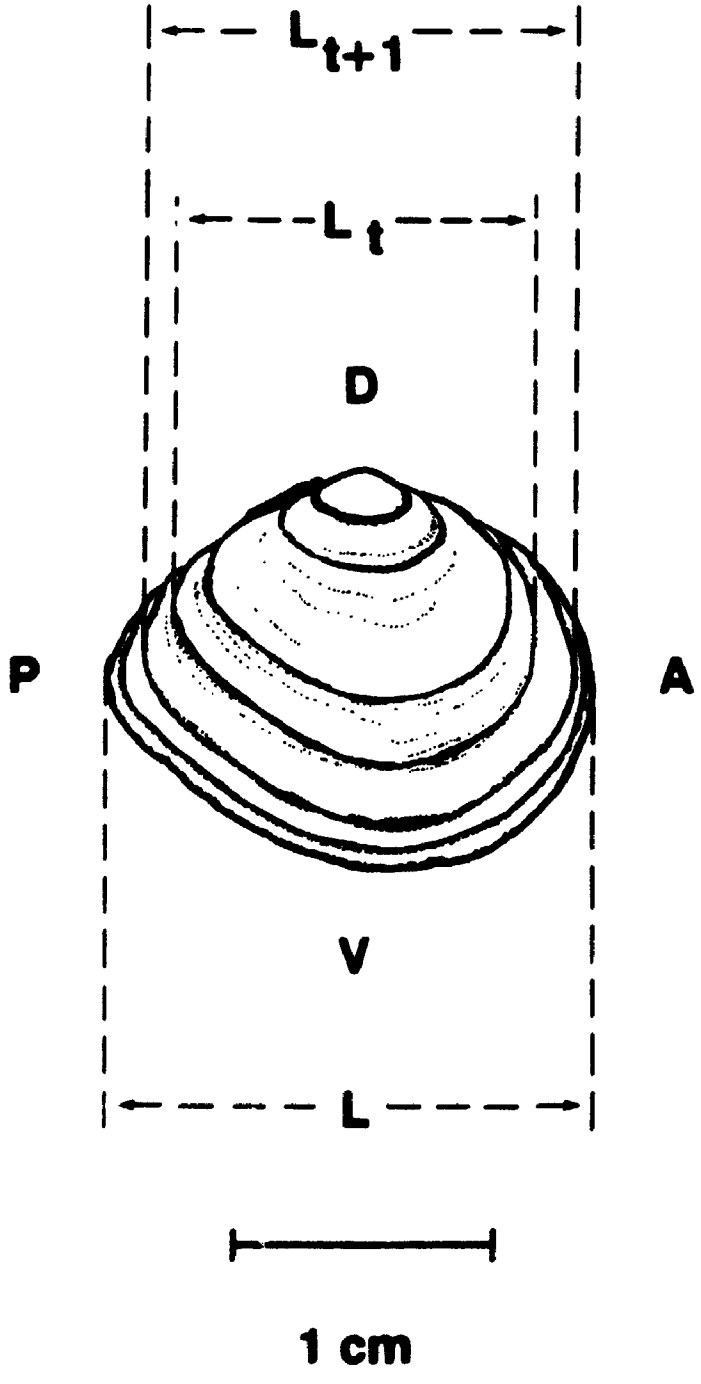
2.3.1 Growth rate

Lengths at two consecutive winter rings (L_t and L_{t+1} respectively) (Fig. 5) were measured on each clam under a dissecting microscope with a pair of Mitutoyo 500-115 Digimatic calipers, correct to 2 decimal places. Growth curves of the *Macoma* from the tide levels at all sampling sites mentioned in Sections 2.1.1 and 2.1.2 were determined by the Walford Plot method of Ford (1933) and Walford (1946) as used by Green (1973) and Green et al. (1983).

An analysis of covariance (ANCOVA) using SAS PROC GLM (SAS Institute Inc. 1985) was applied to the Walford Plot relationship $L_{t+1} = \alpha + \beta L_t$ where L_t and L_{t+1} are the lengths at two consecutive winter rings on a clam. Hypotheses about differences between tidal levels in the parameters of regression lines were tested. $L_{t+1} = \alpha + \beta_1(L_t) + \beta_2(\text{Tide level}) + \beta_3(\text{Tide level}*L_t) + \varepsilon$ is the linear form of a one way ANCOVA with tide level as the class variable. If the null hypothesis $H_1 : \beta_3 = 0$ is rejected, then the linear regressions of L_{t+1} on L_t have different slopes at the two tide levels. If H_1 is not rejected then β_3 is set to zero and $H_2 : \beta_2 = 0$ tested. Rejection of H_2 would indicate parallel regression lines with different intercepts. β_1 is the ratio of a given year's growth to that of the previous year and α represents the first year's growth.

When there was no significant difference in the slopes of the lines being compared, a power analysis determined δ, the minimum detectable vertical difference between the lines. α was set at 0.05 and β at 0.25 for this calculation. The equation for this calculation was :

Figure 5. Diagram of *M. balthica* showing orientation and L_t and L_{t+1}. A=Anterior; P=Posterior; D=Dorsal; V=Ventral; L=Maximum length of clam; Lt and Lt+1 =Length of clam at consecutive years.


L_{t+1}
L_t
D
P
A
V
L
1 cm

$\delta = (t_{\alpha,2,df} + t_{\beta,1,df})$ EMS $(1/n_1 + 1/n_2)$ where EMS is the error mean square for the common slope model, and n_1 and n_2 are the sample sizes of the two groups of clams being compared (see Zar 1984).

2.3.2 Parasite-load

Daughter sporocysts of all three species of trematodes mentioned earlier (see Section 1.3.1) occupy principally the gonadal region of the clam (Pekkarinen 1987a, b, c). The number of sporocysts can vary between 50 to over 3,000 per clam depending on the size of the clam (Swennen and Ching 1974) and can be difficult to enumerate. Since *M. balthica* is both the primary as well as secondary intermediate host, the number of metacercariae was used as a measure of parasite load.

Most metacercariae found between mantle and shell were encysted on the inner surface of the shell. There may be one or more metacercaria(e) per cyst. When the soft tissue is removed from the shell, metacercariae may be lost from their cysts. However, cyst walls and slight depressions on the internal surface of the shell as well as the metacercariae in the extra pallial space are detectable under a dissecting microscope, when stained with Tryphan Red. As the metacercariae of many gymnophallid species are very much alike (Pekkarinen 1987b) and difficult to identify (M. Pekkarinen pers. comm.), no attempt was made to distinguish among the metacercariae of the three known parasites of *Macoma*.

The number of parasites and imprints of them on the right and left valves were counted and used as an index of "metacercaria load". There was no significant difference between left and right valves

($p<0.05$, paired t-test); the total number on both valves was used to compare the parasite load of high and low tide clams at each sampling site.

2.4 Parasite-load of Churchill *M. balthica*

Buried clams were collected by shovelling and sieving the substrate (mesh size 1 mm) at MLW and 1.0 m above MLW levels. Crawling clams were sampled by digging at ends of tracks. Clams were preserved in 70% ethanol. The parasite-load of the clams was determined as described in Section 2.2.

The maximum length and lengths at two consecutive winter rings of each clam were measured using vernier calipers. A log-linear model analysis by SAS PROC CATMOD (SAS Institute Inc. 1985) was used to test for difference in the incidence of infection (i.e. percentage of clams parasitized) of crawlers and burrowers at both tide levels.

The model was : Parasitism = tide level | Behaviour. Parasitized clams were coded with 1 and uninfected ones with 0. The two independent variables, tide level and behaviour, are both dichotomous: high vs low tide and crawler vs burrower respectively. If the 'tide level*behaviour' interaction term is not significant, it can be inferred that degree of parasitism is related to behaviour in a way that does not differ with tidal level, and the term can be dropped from the model. The analysis will be run again to test for the significance of the main effects. A plot of the raw data showed that larger clams generally harbour more metacercariae than smaller ones. Hence, clam length was used as a covariate in this analysis.

The number of metacercariae per clam + 1 (because of the presence of zeros) was log-transformed; the class variables, tide level (MLW and 1.0 m above MLW) and behaviour (crawling vs buried clams) were crossed in a 2*2 factorial design. The factorial ANCOVA model is :

$\text{Ln(total+1)} = \alpha + \beta_1(\text{length}) + \beta_2(\text{tide level}) + \beta_3(\text{behaviour}) + \beta_4(\text{length*tide level}) + \beta_5(\text{length*behaviour}) + \beta_6(\text{tide level*behaviour}) + \beta_7(\text{length*tide level*behaviour}) + \varepsilon.$

If the null hypothesis $H_0 : \beta_7 = 0$ is accepted, then the relationship between parasite load and behaviour varies with length of clams in the same way at both tide levels. β_7 can then be set to zero and the $H_0 : \beta_6 = 0$ tested. Acceptance of this null hypothesis means that degree of parasitism is related to behaviour in a way that does not differ with tidal levels. Similarly : if β_5 is found to be significantly different from zero, then clam length influences whether crawlers have more parasites than burrowers and if β_4 is significantly different from zero, then the influence of clam length on parasite load changes with tide level.

The remaining hypotheses of whether parasite load differs between crawlers/burrowers, source of the clams (i.e. high or low tide) and lengths of the clams can be tested by examining whether β_3, β_2 and β_1 differ significantly from zero respectively.

Growth curves and ages were determined by the Walford Plot method of Ford (1933) and Walford (1946) as used by Green (1973) and Green et al. (1983). A factorial ANCOVA (crossed in the same way as mentioned above) was applied to the Walford Plot relationship $L_{t+1} = \alpha + \beta L_t$, where L_t and L_{t+1} are the lengths at two consecutive winter rings on a clam.

2.5 Transplant experiments at Churchill, Hudson Bay

2.5.1 1985 Transplant

In early July 1985, *M. balthica* from both MLW (low tide) and 1.0 m above MLW (high tide) were collected using the method described earlier in Section 2.2. Five hundred active clams covering as wide a size range as possible from each tide level were marked with the following procedure.

A number was etched on the exterior of one shell valve using a diamond-tipped pencil; right valve for high tide *Macoma* and left for the low tide clams. Waterproof coloured super fine-tipped felt pen markers were used to go over the etched numbers. A layer of clear nail varnish was applied to further protect the number on the clam shell.

The length of each clam was measured with a pair of Mitutoyo 500-115 Digimatic calipers correct to 2 decimal places. Mortality due to handling, marking and measuring was negligible (< 0.5%). Clams which died before release were replaced.

A transplant area at the high tide level was selected and the 1,000 marked clams (500 from each tide level) were randomly scattered onto the substrate. The time of release was less than 2 weeks after ice-breakup. Predators such as shore birds were kept away until all the clams had burrowed into the substrate. This normally took about 15 minutes.

Another 300 high tide *Macoma* were marked, measured and released in an adjacent plot to the high tide transplant site and left to

overwinter. Growth data from these clams were used to verify that the growth observed in summer is equivalent to annual growth.

A reciprocal transplant at MLW was not possible in 1985 as this part of the shore was not exposed during the early part of the field season.

Marked *Macoma* were retrieved from the summer transplant area by the same sampling technique in early October 1985 just before the bay froze. Over-wintered marked clams were similarly recovered during early July the following year. All clams were frozen immediately at -20° C and transported on ice to London, Ontario.

The lengths of the clams were remeasured. The increase in length of the marked high and low tide clams in the high tide transplant area were compared using an ANCOVA (see Section 2.3.1) in order to determine whether the growth rate of the two tidal level clams differed. In order to verify that growth of the *Macoma* differed between tidal levels prior to transplant, lengths of consecutive winter rings were also measured and compared by ANCOVA (see Section 2.3.1).

The tissue of the clams was removed from the shells, placed individually into marked 1.5 ml polypropylene micro test tubes and frozen at -70° C. Celluiose acetate electrophoresis (Hebert and Beaton 1986) was used to establish allozyme frequencies of three enzymes, phosphoglucomutase (PGM), phosphoglucose isomerase (PGI) and malate dehydrogenase (MDH), all of which are polymorphic in *Macoma*. Horizontal starch gel electrophoresis (Shaw and Prasad 1970) was used to determine the allozyme frequency of leucine aminopeptidase (LAP).

One drop of distilled water was added to each sample. Homogenisation was carried out mechanically (in an ice bath) using a glass rod whose tip had been ground to fit the bottom of the micro test tube. The sample was then centrifuged at 10,000 rpm for 15 minutes and quick frozen at -70° C. The staining recipe and electrophoretic procedure for PGM, MDH and PGI followed that of Hebert and Beaton (1986) and that of LAP after Shaw and Prasad (1970).

The allelic frequencies, p (as a measure of departure from the Hardy-Weinberg equilibrium) and the heterozygote deficiency, D (defined as the deviation of observed from expected under the Hardy-Weinberg equilibrium, divided by the expected heterozygosity) were calculated for each enzyme locus. These genetic parameters for the two tide levels were compared. Hypotheses testing statistics involving genotype combinations were not attempted as sample sizes were often too small in each genotype combination. The criteria of whether a clam is homozygous or heterozygous at a certain locus and whether a certain allele is present or absent was used. The hypotheses tested followed those mentioned in Section 2.3.1.

2.5.2 1986 Reciprocal Transplant

In early July 1986, 2,000 *Macoma* from each tide level (MLW and 1.0 m above MLW) covering as wide a size range as possible, were marked and measured following the methods described in Section 2.5.1. The clams from each tide level were divided into 2 groups of 1,000 clams and assigned to either transplant area: an area

at the MLW level (low tide transplant site) was chosen. The same high tide transplant area was used (Section 2.5.1).

Another 1,000 *Macoma* from the high tide site were marked and measured. They were released and left to over-winter in the same long-term transplant area as the previous year.

Summer transplant clams were retrieved just prior to ice-up in mid-October 1986, frozen at -20° C and brought back to the laboratory in London, Ontario on ice. Recovery of long-term transplant *Macoma* was carried out the following summer in early July. Similar measurements, preparation for electrophoresis and parasite-load determination as mentioned in Section 2.5.1 were done.

Genetic parameters mentioned in Section 2.5.1 were similarly calculated for the 1986 data. Statistical methods similar to those outlined in Section 2.5.1 were used to analyse the data.

2.6 Temperature-related experiments

2.6.1 Oxygen consumption

Two hundred live *Macoma* from each tide level were selected from their respective holding tanks (maintained at 4° C) approximately 2 months after collection from the field. They were randomly assigned to one of the four temperature regimes under investigation i.e. 5, 10, 15 and 20° C after which they were numbered, colour-coded and measured. Care was taken to ensure that the size range of the 50 clams in each group within a tide level was similar.

Oxygen consumption of the clams was determined using a modified YSI Model 55 Oxygen monitor. Each clam was placed in 10

ml of 18 ‰ sea water which had been saturated with oxygen at the corresponding test temperature. A thin well-perforated plastic disc was inserted into the sample vial to support and separate the clam from the magnetic stirring bar.

The two colder test temperatures were achieved in a cold room with ambient temperature of 4° C with appropriate adjustments on the heat control of the oxygen monitor. The 15° C temperature was achieved by immersing the cooling block superconductor of the oxygen monitor in a beaker of crushed ice and ice-cold water at room temperature (20° C). The heat control was set to zero for both the 15 and 20° C test temperatures. All temperature regimes were closely monitored so that fluctuations were only ± 0.5° C of the temperature under investigation. The oxygen probe of the apparatus measured the change in percentage of oxygen content of the solution in which it was immersed.

The percentage of oxygen consumed by each clam in two 30-minute trial periods was averaged and converted to µl per ml using the oxygen solubility table in Parsons et al. (1984). Clams were frozen immediately after the second trial. Tissues were treated for electrophoretic work as described in Section 2.5.1. Parasite-load in each clam was determined as outlined in Section 2.3.2. Cellulose acetate electrophoresis, as mentioned in Section 2.5.1 was used to determine the genotype of three enzymes, namely PGM, MDH and PGI in the clams used for this study.

Since tissues were frozen for electrophoretic studies, dry weights of clams were estimated with the following method. Fifty *Macoma* from both tide levels were randomly selected and the soft

parts dried overnight to constant weight at 40° C. A regression with ln(length of clam) as the predictor variable and ln(dry weight) as the dependent variable was carried out for clams from both tide levels (Appendix II). The equations were : ln(dry weight) = -10.53 + 2.64 ln(length) and ln(dry weight) = -9.32 + 2.08 ln(length) for high and low tide respectively. The r^2 for the regression lines were 0.67 and 0.70 respectively.

SAS PROC GLM (SAS Institute Inc. 1985) was used to evaluate the influence of the parameters measured i.e. clam dry weight, total number of parasites per clam, temperature and tide level, on the rate of oxygen uptake by the clams. The first two variables were log-transformed; since there were zeros in the latter, a ln(total number + 1) transformation was used. The volume of oxygen consumed by each clam was also log-transformed.

A four-way factorial analysis was carried out. Interaction terms were examined in turn and discarded if found to be statistically insignificant. The four-way interaction term : 'ln(dry weight)*temperature*ln(total number of parasites+1)*tide level' was examined first. If this term was found to be non-significant, the oxygen uptake per gram tissue dry weight would be some function of temperature that was independent of (i) which tide level was the source of the clams and (ii) of the parasite load in each clam. The next higher order interaction terms (i.e. the three-way interaction terms) would be examined in the same way and discarded if found to be insignificant, until only significant effects remained in the model.

The common slope model predicting oxygen consumption from the significant effects was derived using SAS PROC REG (SAS

Institute Inc. 1985). Since "tide" is a categorical variable, a dummy variable was introduced to code for tide level. Metabolic rate curves were generated by substituting the corresponding dummy variable that coded for the tide level. The common Q_{10} for both tide levels was calculated from the regression equation.

Genetic parameters such as allele frequency, p as a measure of departure from the Hardy-Weinberg equilibrium and D, the heterozygote deficiency were similarly calculated as mentioned in Section 2.5.1 for the clams used in this experiment. The purpose was to determine if these parameters were similar to those of the clams retrieved in the transplant study.

In order to study the effect of heterosis on the oxygen uptake of the clams, a further factorial analysis incorporating 'numhet' (the number of heterozygous loci of the three enzymes studied for each clam) with the significant main effects of the previous model was carried out. Since 'tide level' was significant in the previous GLM analysis and there is a difference in the heterozygosity of loci in the clams of the two tide levels (see Green et al. 1983) the data for the clams of the two tide levels were analysed separately. An ANCOVA using SAS PROC GLM to test for difference of slopes in rate of oxygen uptake was used. As mentioned earlier, hypothesis testing involving genotype combinations was not attempted as sample sizes were often too small in each genotype combination. The criteria of whether a clam is homozygous or heterozygous at a certain locus and whether a certain allele is present or absent was used instead. Factorial analyses with systematic discarding of non-significant effects were carried out.

2.6.2 Freezing tolerance

Two hundred and twenty *Macoma* from each tide level were randomly selected from their respective holding tanks (maintained at 4° C, approximately 1 month after collection from the field). They were numbered and measured. The volume of each clam (by water displacement) was also recorded.

The clams were randomly allocated to 11 freezing durations from 1 to 6 hours in half hour intervals. Care was taken to ensure that the size range in each group of 20 clams was similar. Ten clams were placed in each 100 by 15 mm square Intergrid Petri Dish filled with substrate from Churchill to a depth of 1 cm. The petri dishes were frozen at -15 ± 1° C for the assigned duration after which the substrates were allowed to thaw for a few minutes at room temperature. Clams were removed and placed into dishes with sea water (18 ‰) at 15° C.

The number of clams alive and dead were counted. The criterion for death was when shells gaped, showing no signs of closing when prodded gently. The live clams were frozen immediately for electrophoresis work and the number of parasites per clam (both dead and alive) was determined (see Sections 2.5.1 and 2.3.2 respectively). Similar genetic parameters as mentioned in Section 2.6.1 were calculated to ascertain that the clams used in this experiment were similar to those retrieved in the transplant study.

A log-linear model of a 2-way contingency table was used to analyse the effect of source of the clams (i.e. whether from high or low tide level) and the duration of cold exposure on the number of

clams that survived. In order to satisfy the criteria that fewer than 20% of the cells in the contingency table should have expected values of less than 5 and no cell should have an expected value of less than 1, (Cochran 1954), frequencies for cells of duration of cold exposure more than 4 hours were pooled. This gave rise to a 2*8 contingency table and a SAS PROC CATMOD (SAS Institute Inc. 1985) was used to analyse the data.

Log-linear models were used to analyse the survival of high and low tide clams separately as the results of the 2*8 contingency test showed that the source of the clams affected their survival when subjected to different lengths of freezing treatment.

Duration of cold exposure was further pooled to form three categories namely, <2 hours, >2<4 hours and >4 hours to increase sample sizes in each cell. The two morphometric variables, length and volume, were log transformed and combined into one variable, "size" using a Principal Component Analysis by the SAS PROC PRINCOMP procedure (SAS Institute Inc. 1985). The first principal component accounted for 70.7% of the total variation and it represents the size of the clams. This "size" variable was divided into two categories as the log-linear procedure by SAS PROC CATMOD (SAS Institute Inc. 1985) requires variables to be either all continuous or all categorical in nature.

Parasite load was divided into two classes, either with or without parasites. There were four categories used in the number of heterozygous loci per clam : 0 to 3 representing clams totally homozygous for all three loci to those totally heterozygous for all three loci.

The saturated model of the log-linear analysis for each tide level is thus :

State = Duration class | Size class | Parasite total class | Number of heterozygous loci per clam; where live and dead clams were coded as 1 and 0 respectively for the "state" variable. Systematic discarding of non-significant interaction terms was carried out until only significant effects remained in the model.

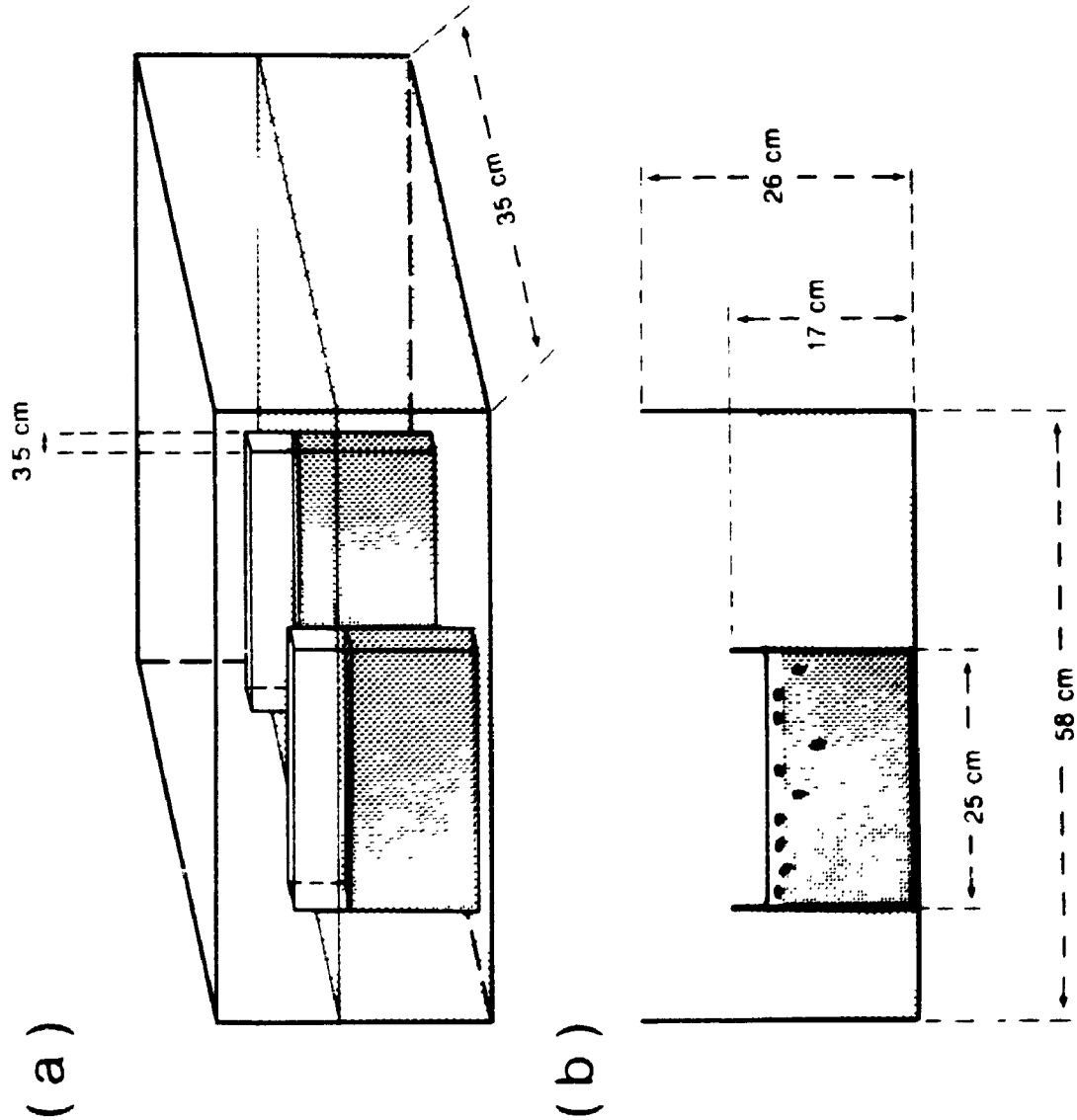
In order to test whether heterozygotes survive better than homozygotes, a similar analysis was done. Heterozygous clams were coded with a 1 and homozygotes with a 0 for the three enzyme loci studied. The model was similar to the one given above. Similar discarding of non-significant interaction terms was done.

2.6.3 Burrowing behaviour

Two hundred and twenty *Macoma* from each tide level were selected, numbered and measured. Burrowing behaviour was studied at 10, 20 and 30° C. The experimental set-up is shown in Fig. 6a. Ten clams were placed onto the surface of the substrate (from Churchill) in each trough in 10 mm depth of 18‰ sea water maintained at the test temperature. Temperature fluctuations were kept at ± 1° C. Eight mm of the sea water was pipetted out of the trough to simulate ebb tide. The test temperature regimes were applied to each clam randomly (i.e. no fixed order).

After half an hour, the depth which the clams burrowed into the substrate was measured to the nearest 0.5 cm (Fig. 6b). This duration was chosen because preliminary observations both in the field and in the laboratory have shown that clams usually burrow out

Figure 6. **Burrowing experiment (a) Experimental set-up; (b) Transverse** view.


(a)
35 cm
35 cm
(b)
26 cm
17 cm
25 cm
58 cm

of sight within fifteen minutes. The parasite-load of each clam was determined as in Section 2.3.2.

A repeated measures ANOVA done in the MINITAB statistical analysis package (MINITAB Inc. Release 6.1, 1988) was used to analyse the effect of temperature on burrowing depth. The model used was :

Depth = Tide level | Clams (Tide level) | Temperature - Temperature*Clams (Tide level); which uses 'Temperature*Clams (Tide level)' as the error term. Clams were nested within tide level as the same clams from each tide level were used at all three test temperatures.

Since both clam length and total number of metacercariae per clam are confounded with tide level (see Section 3.2), the effect of these two variables on burrowing depth was analysed for each tide level separately using ANCOVA. SAS PROC GLM (SAS Institute Inc. 1985) was used for the both analyses. The first model with clam length as the covariate was :

$$\text{Depth} = \alpha + \beta_1(\text{length}) + \beta_2(\text{temperature}) + \beta_3(\text{length*temperature}) + \varepsilon$$

where temperature is the class or group variable.

If the null hypothesis $H_1 : \beta_3 = 0$ is rejected, then the linear regressions of burrowing depth on length of clam have different slopes for the three test temperatures; in other words, the length of the clams influences the burrowing depth in a different way at the three temperatures. If H_1 is not rejected then β_3 is set to zero and $H_2 : \beta_2 = 0$ tested. Rejection of H_2 would mean that the regression lines are parallel but have different intercepts indicating that there is a

consistent difference in burrowing response across the size range of the clams at the three test temperatures.

The second model using number of metacercariae per clam as the covariate and temperature as the group variable was :

Depth = $\alpha + \beta_1$(number of parasites clam^{-1}) + β_2(temperature) + β_3(number of parasites clam^{-1}*temperature) + ε

Similarly, if the null hypothesis $H_1 : \beta_3 = 0$ is rejected, then the linear regressions of burrowing depth on the number of parasites per clam have different slopes for the three test temperatures; in other words, the degree of parasitism influences the burrowing depth in a different way at the three temperatures. If H_1 is not rejected then β_3 is set to zero and $H_2 : \beta_2 = 0$ tested. Rejection of H_2 would indicate parallel regression lines with different intercepts showing that the depth to which non-parasitized clams burrowed differed at the three test temperatures.

3. RESULTS

3.1 Geographical variation in growth rate with respect to tide levels

3.1.1 Growth rate

Fig. 7a, b, c and d show the Walford plots of *Macoma* sampled from CE, SF, C and BC (see Section 2.1.1 for full site names) and Fig. 8a, b, c and d show the von Bertalanffy growth curves generated from the Walford plots. Results of the ANCOVA for these four St. Lawrence sites are tabulated in Table 2. It can be seen that there is no significant difference between the growth rates of the high and low tide *M. balthica* at SF, C and BC. Intercepts also do not differ significantly which indicates that the first year's growth of the high and low tide clams are similar. δ, the minimum detectable vertical distance (given the sample sizes used) between the lines was calculated to be 0.21, 0.20 and 0.46 mm for SF, C and BC respectively. At CE, low tide *Macoma* grew significantly faster than the high tide clams. Since slopes differ, no test of intercepts nor power analysis was carried out.

The Walford plot for the clams collected from DC in the upper Bay of Fundy is given in Fig. 9. No comparison of growth rates of clams from different tidal levels was made at this site due to the absence of clams at lower tidal levels. The corresponding von Bertalanffy growth curve for this Walford plot and for all other subsequent Walford plots will not be presented graphically as it would essentially double the number of figures. The growth curves will only be presented when special mention is necessary.

Figure 7. Walford plots of *M. balthica* from four sites along the south shore of the St. Lawrence River (a) Cacouna-est; (b) St. Fabien-sur-mer; (c) Capucins; (d) Baie des Capucins. (- - - = High tide level; —— = Low tide level). Lines are drawn to cover the range of data actually used. The 95% confidence bands are shown.

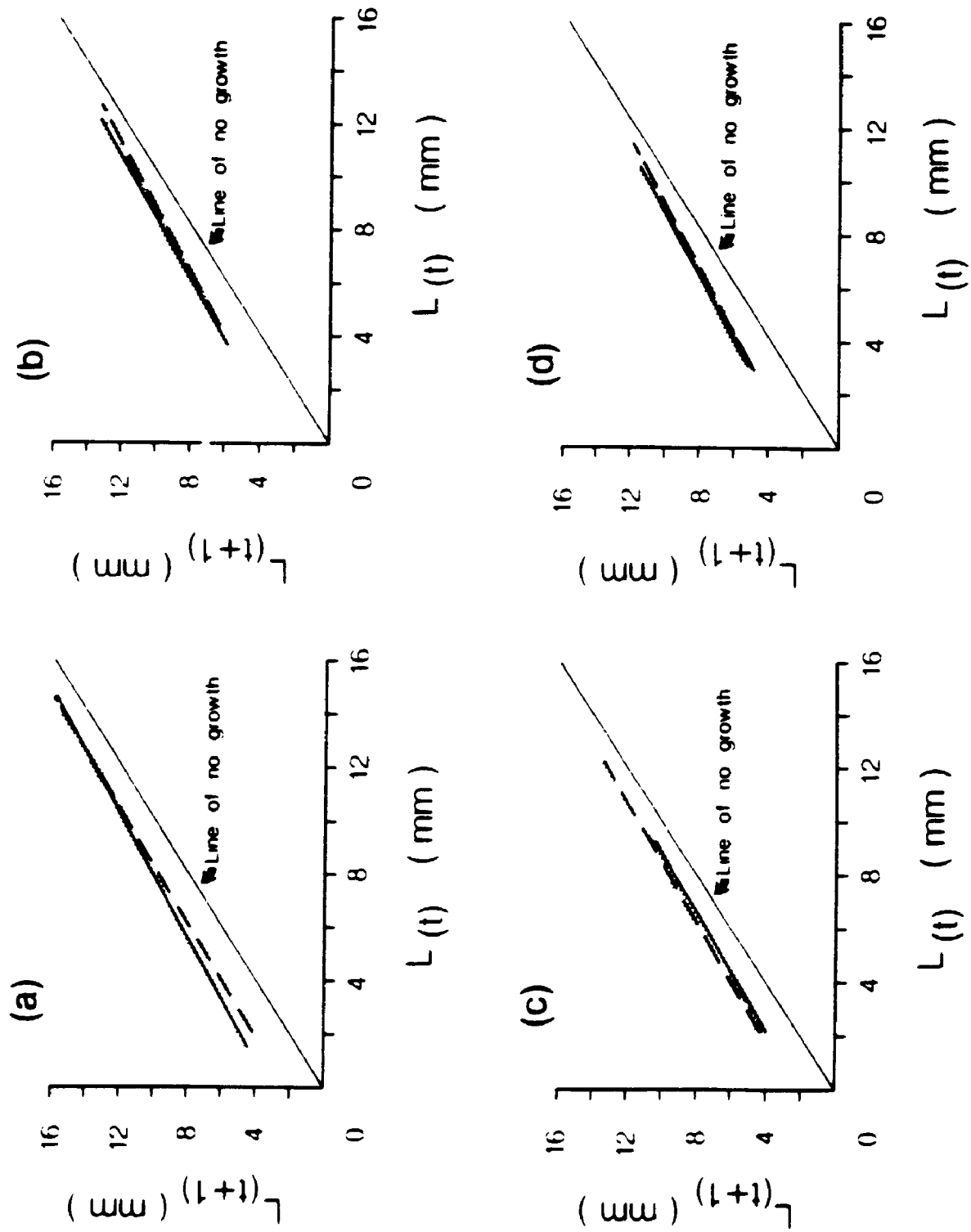
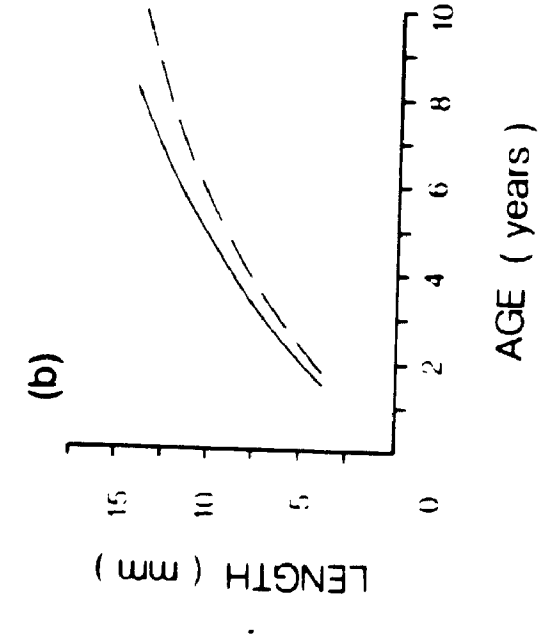
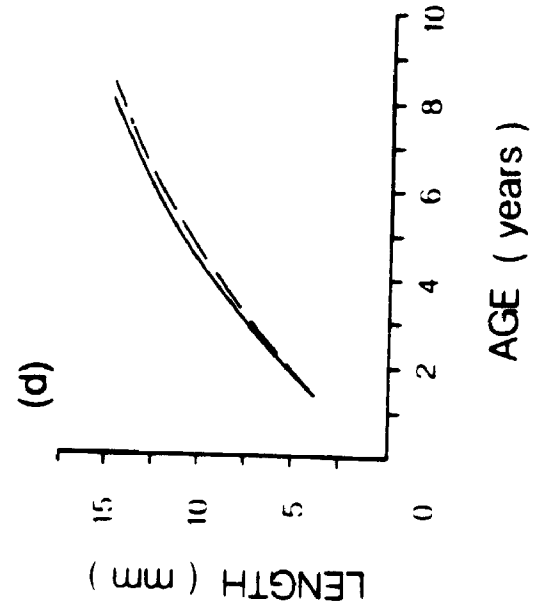
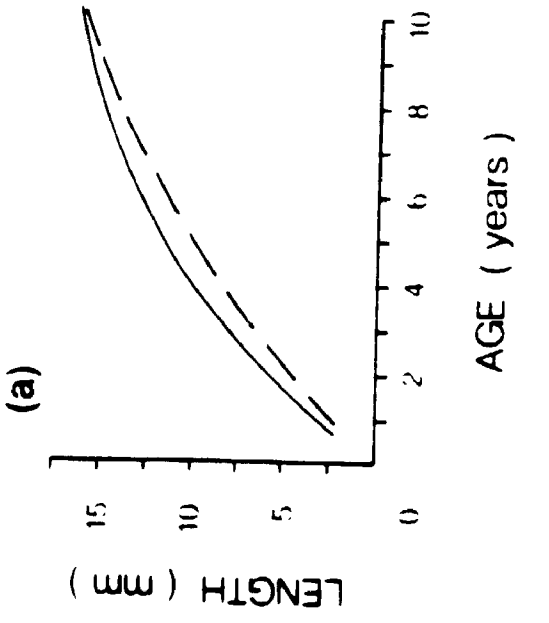

(a)
(b)
(c)
(d)
Line of no growth
L(t+1) (mm)
L(t) (mm)
0
4
8
12
16

Figure 8. von Bertalanffy growth curves corresponding to the Walford plots of Fig. 7 : (a) Cacouna-est; (b) St. Fabien-sur-mer; (c) Capucins; (d) Baie des Capucins. (- - - = High tide level; —— = Low tide level). Lines are drawn to cover the range of data actually used.


(b)
LENGTH (mm)
AGE (years)
0
5
10
15
2
4
6
8
10


(d)
LENGTH (mm)
AGE (years)
0
5
10
15
2
4
6
8
10


(a)
LENGTH (mm)
AGE (years)
0
5
10
15
2
4
6
8
10

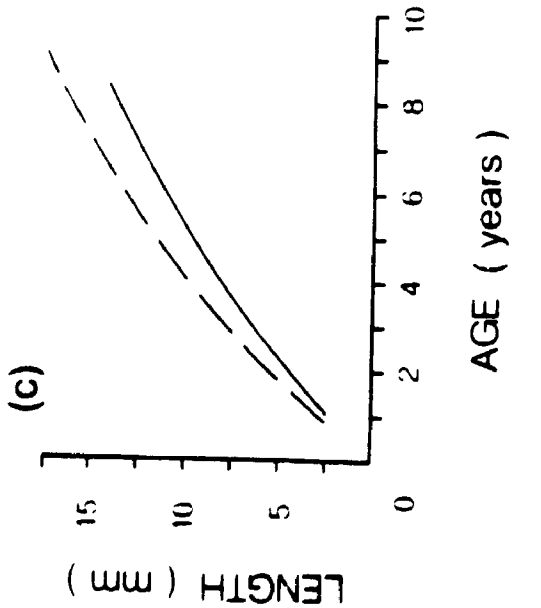
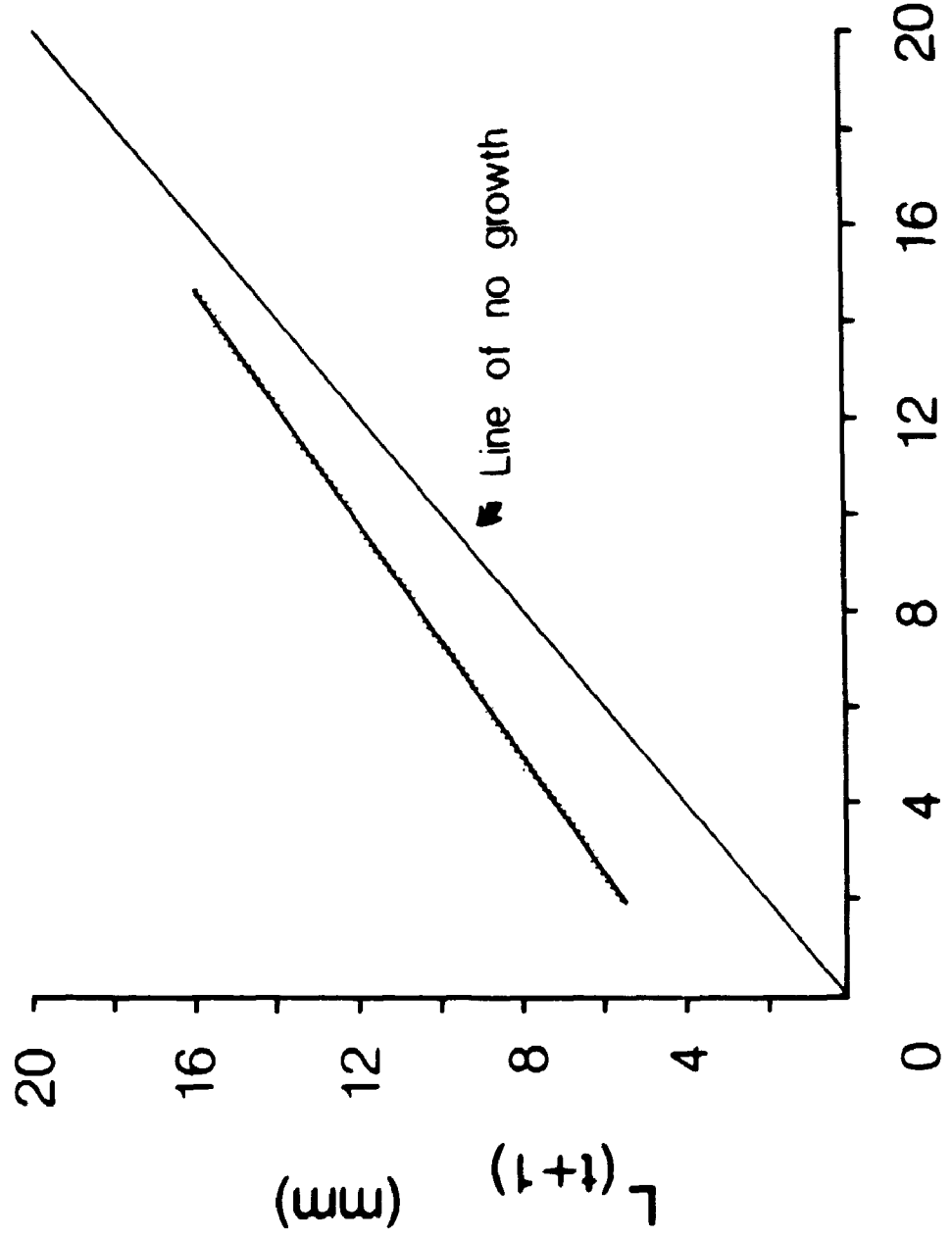

(c)
LENGTH (mm)
AGE (years)
0
5
10
15
2
4
6
8
10

Table 2. Tests of significance for differences in the Walford plot slopes and intercepts between high and low tide clams for: (a) sampling sites along the southern shore of the St. Lawrence River; (b) sampling sites at the Bay of Fundy.

Site	Slope	Intercept
(a) St. Lawrence River		
Cacouna-est	p=0.008 **	apparently different
St. Fabien-sur-mer	p=0.964 n.s.	p=0.360 n.s.
Capucins	p=0.621 n.s.	p=0.101 n.s.
Baie des Capucins	p=0.926 n.s.	p=0.953 n.s.
(b) Bay of Fundy		
Indian Point	p=0.129 n.s.	p=0.042 *
Selma Bar	p=0.406 n.s.	p=0.0001 **

Figure 9. Walford plot of *M. balthica* from the Bay of Fundy at Dorchester Cape. Line is drawn to cover the range of data actually used. The 95% confidence band is shown.


Line of no growth
20
16
12
8
4
0
4
8
12
16
20
L (t+1) (mm)
L (t) (mm)

Figs. 10 and 11 show the Walford plots for *M. balthica* collected from IP and SB respectively. ANCOVA results show that there was no significant difference between the slopes of the Walford plots of the high and low tide clams from both IP and SB (Table 2). Intercepts of the low tide clams were found to be significantly greater than those of the high tide clams for both locations ($p<0.05$ and $p<0.01$ for IP and SB respectively) (Table 2). It can be concluded that the first year's growth of the low tide clams is significantly greater than that of the high tide ones. δ was calculated to be 0.55 and 0.34 mm for IP and SB respectively.

3.1.2 Parasite-load

Table 3 shows the parasite-load of the clams from the St. Lawrence and Bay of Fundy study sites. There was no significant difference between the parasite loads of high and low tide clams from SF, C and BC ($p>0.05$ for all three sites). Low tide *M. balthica* from CE however, harboured a significantly higher number of metacercariae than those found in the high tide region ($p<0.01$).

At IP, there was no significant difference between the parasite loads of the high and low tide *Macoma* ($p>0.05$) (Table 3). No metacercariae were found in the DC and SB clams of the Bay of Fundy (Table 3).

3.2 Parasite-load of Churchill *Macoma balthica*

The percentages of parasitized crawlers and burrowers from the two tide levels as well as the results of the log-linear analysis of the data are given in Table 4(a, b and c). The 'tide level by behaviour' interaction term is

Figure 10. Walford plots of *M. balthica* from the Bay of Fundy at Indian Point. (- - - = High tide level; —— = Low tide level). Lines are drawn to cover the range of data actually used. The 95% confidence bands are shown.

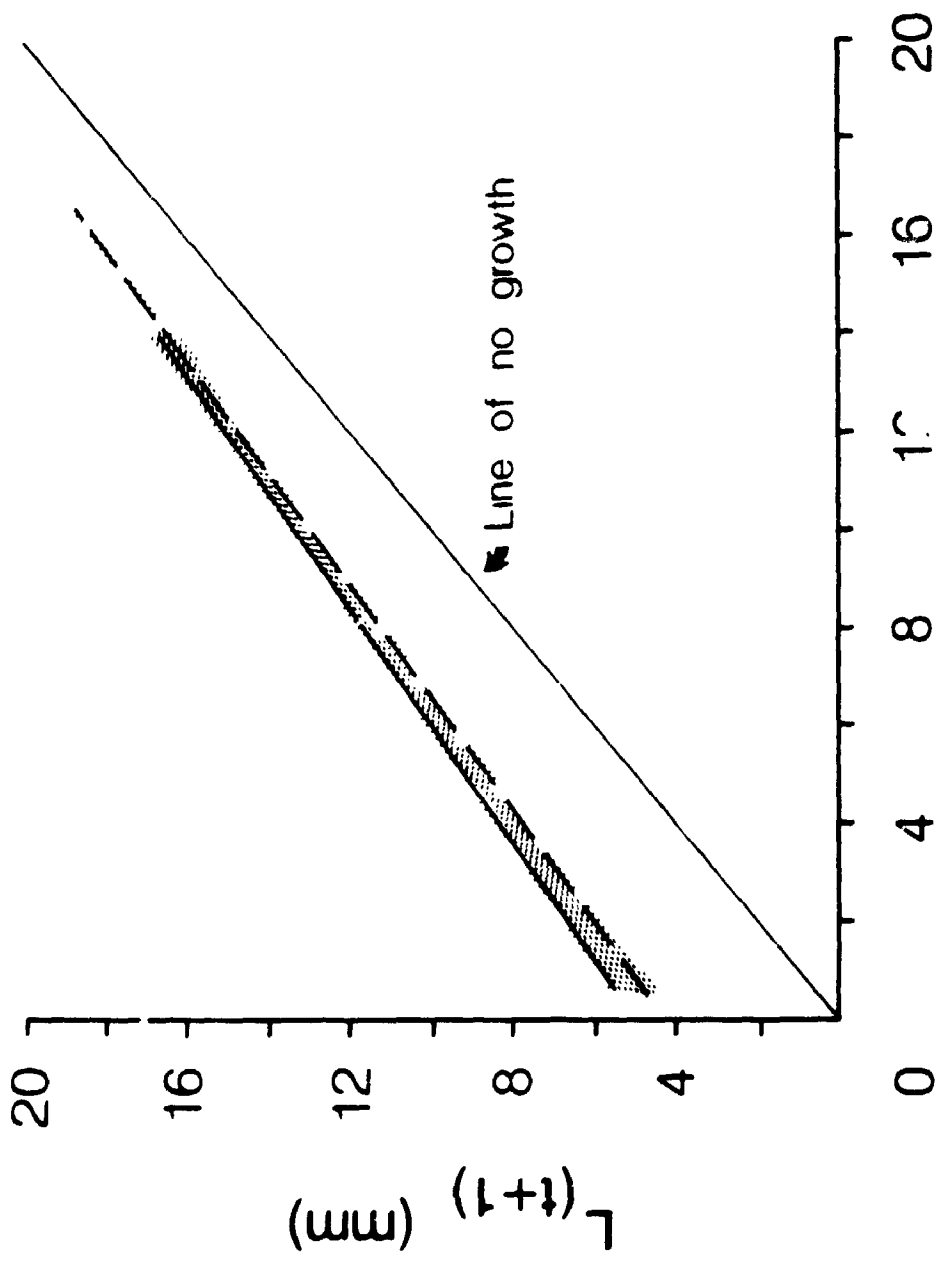

Line of no growth
$L_{(t+1)}$ (mm)
$L_{(t)}$ (mm)
0
4
8
12
16
20

Figure 11. Walford plots of *M. balthica* from the Bay of Fundy (Minas Basin) at Selma Bar. (- - - = High tide level; —— = Low tide level). Lines are drawn to cover the range of data actually used. The 95% confidence bands are shown.

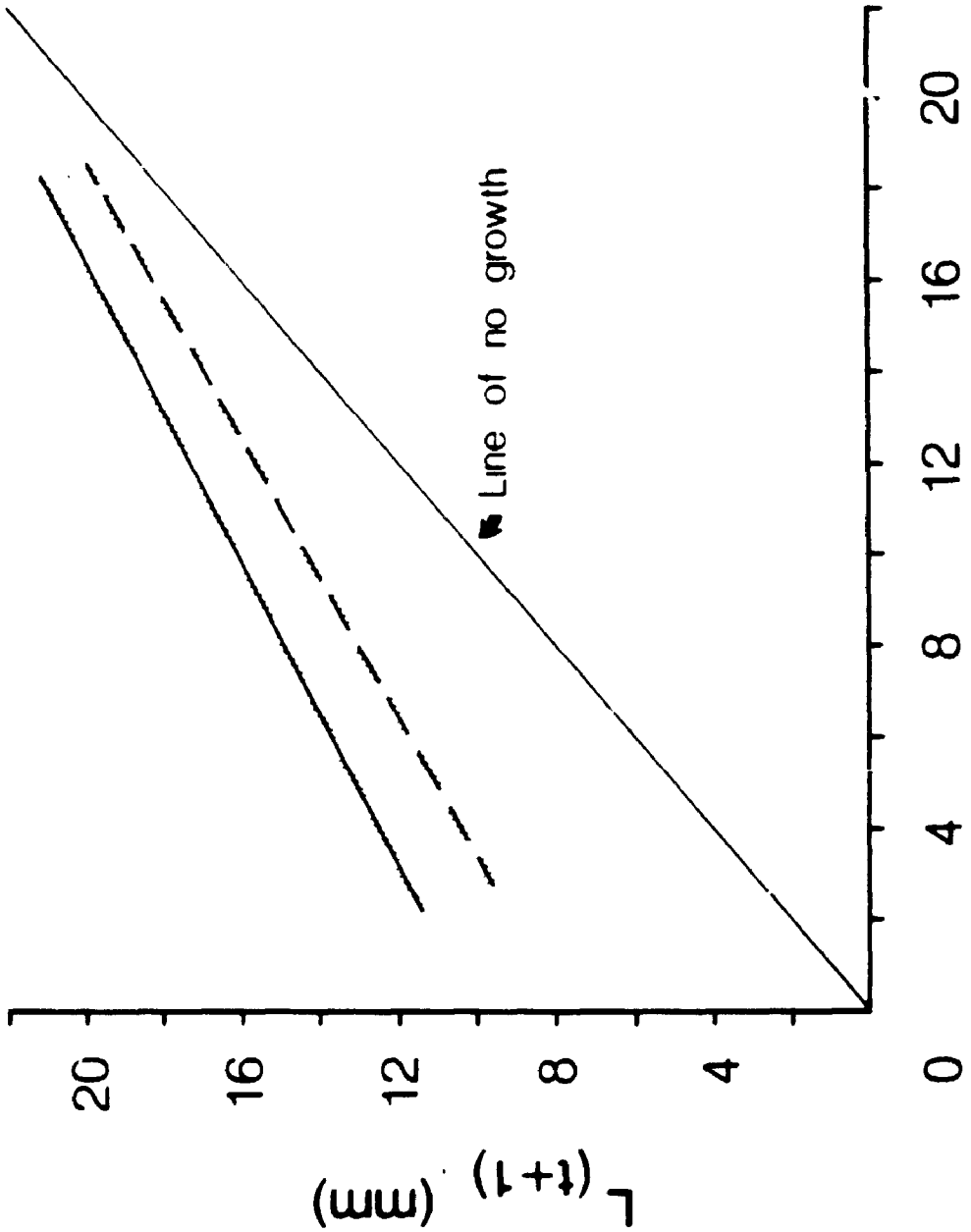

Line of no growth
0
4
8
12
16
20
$L_{(t)}$ (mm)
$L_{(t+1)}$ (mm)

Table 3. Least squares mean number of metacercariae per clam for *Macoma balthica* at the St. Lawrence River and Bay of Fundy sampling sites and ANOVA results.

Sampling site	Tide Level	Sample size	% parasitized	LS Mean # metacercariae per clam ± S.E.	Anova test
St. Lawrence River :					
Cacouna-est	High	102	64.7	1.56 ± 0.22	p=0.0001
	Low	101	84.2	3.09 ± 0.22	***
St. Fabien-sur-mer	High	145	45.5	1.20 ± 0.17	p=0.399
	Low	55	29.1	0.93 ± 0.27	n.s.
Capucins	High	91	3.3	0.03 ± 0.02	p=0.489
	Low	47	2.1	0.02 ± 0.02	n.s.
Baie des Capucins	High	55	0.0	0.0	p=0.489
	Low	41	4.9	0.15 ± 0.10	n.s.
Bay of Fundy :					
Indian Point	High	101	6.9	0.17 ± 0.06	p=0.25
	Low	58	3.4	0.05 ± 0.08	n.s.
Dorchester Cape	----	148	0.0	0.0	------
Selma Bar	High	152	0.0	0.0	------
	Low	101	0.0	0.0	------

Table 4. (a) Incidence of infection (percentage of clams parasitized) of *M. balthica* from Churchill, Manitoba; (b) log-linear analysis of incidence of infection data (saturated model); (c) log-linear analysis of same data - main effects only.

(a)

Tide level	Behaviour	n	# parasitized	% parasitized	# parasites Clam^{-1}
High	Burrower	203	181	89.2	0-39
High	Crawler	99	92	92.9	0-34
Low	Burrower	191	33	17.3	0-8
Low	Crawler	45	19	42.2	0-10

Source	df	χ^2	p
(b)			
Intercept	1	24.38	0.0001
Tide level	1	130.30	0.0001 **
Behaviour	1	8.31	0.0039 **
Tide level*Behaviour	1	2.03	0.1543 n.s.
(c)			
Intercept	1	26.87	0.0001
Tide level	1	185.63	0.0001 **
Behaviour	1	10.98	0.009 **

not significant ($p>0.05$). Both main effects, tide level and behaviour, were highly significant when the analysis was rerun without the interaction term ($p<0.01$ for both variables). A significantly larger number of clams were infected at the high tide level than at the low and more crawlers were parasitized than burrowers.

Fig. 12 shows the scatter plots of the total number of parasites and length of clams in the four groups of *Macoma*. It can be seen that parasite load generally increases with clam length. The regression lines of ln(total number of parasites+1) versus length for the four groups of *Macoma* are presented in Fig. 13. The results of the factorial ANCOVA showed that there is no 'tide level by behaviour by length' interaction ($p>0.05$). The 'tide level by behaviour' interaction term was also non-significant ($p>0.05$). Therefore, tide level and crawling behaviour are independent of one another.

Since the 'length by behaviour' interaction was non-significant ($p>0.05$), the number of parasites in crawlers vs burrowers is not influenced by clam length. Parasite load was found to be influenced by clam length in such a way that changes with tide level since the 'length by tide level' interaction was significant ($p<0.01$). The lower slope at the lower tide level indicates a slower gain of parasites with increasing length and age than that which occurs at the high tide level.

Growth of the four groups of clams are shown in the Walford plots in Fig. 14(a). The von Bertalanffy growth curves generated from these plots are presented in Fig. 14(b). Walford plot slopes differed between tide levels (i.e. the significance of the interaction term 'length by tide level', $p<0.01$) and also between crawlers and burrowers (i.e. the significance of the interaction term 'length by behaviour', $p<0.01$). Therefore, growth differed

Figure 12. The scatterplots of total number of trematode metacercariae harboured and length of *M. balthica* : (a) high tide (1.0 m above MLW) crawlers; (b) high tide burrowers; (c) low tide (MLW) crawlers; (d) low tide burrowers. Symbols represent number of clams - o : 1; ● : 2; x : 3 or more. Horizontal lines (i) and (ii) divide clams into three groups :- above (i) : > 10 parasites per clam; between (i) and (ii) : 1 - 10 parasites per clam; below (ii) : no parasites found.

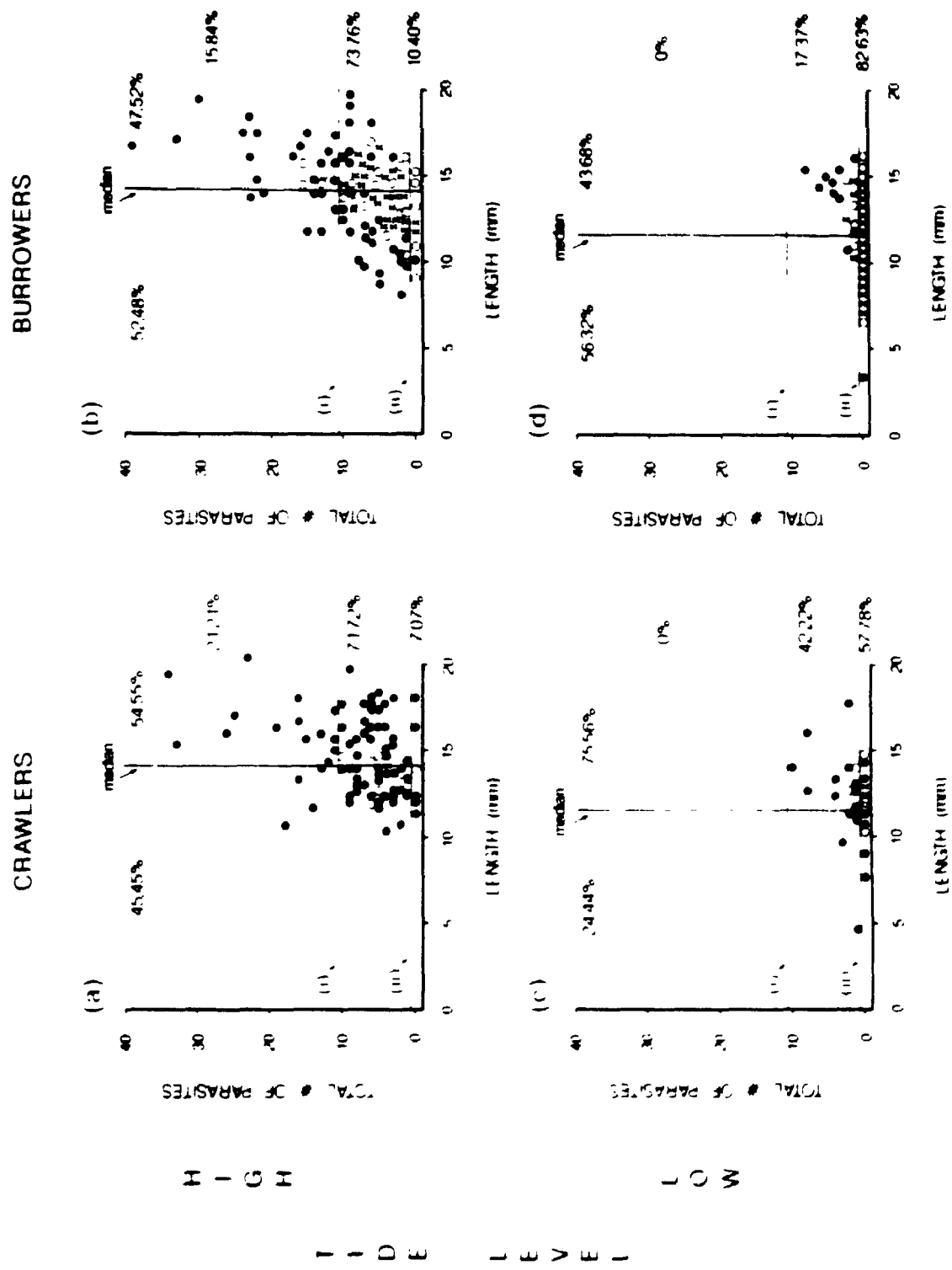

BURROWERS
CRAWLERS
(a)
(b)
(c)
(d)
median
47.52%
15.84%
52.48%
73.76%
10.40%
54.55%
45.45%
71.74%
70%
75.56%
24.44%
42.22%
57.78%
0%
56.32%
43.68%
17.37%
82.63%
TOTAL # OF PARASITES
LENGTH (mm)
HIGH
LOW
LEVEL
DENSITY

Figure 13. Least squares regression lines of ln(total number of trematode metacercariae + 1) against length of *M. balthica* of high and low tide crawlers and burrowers. The 95% confidence bands on the regressions are shown. H-C : high tide (1.0 m above MLW) crawlers; H-B : high tide burrowers; L-C : low tide (MLW) crawlers; L-B : low tide burrowers.

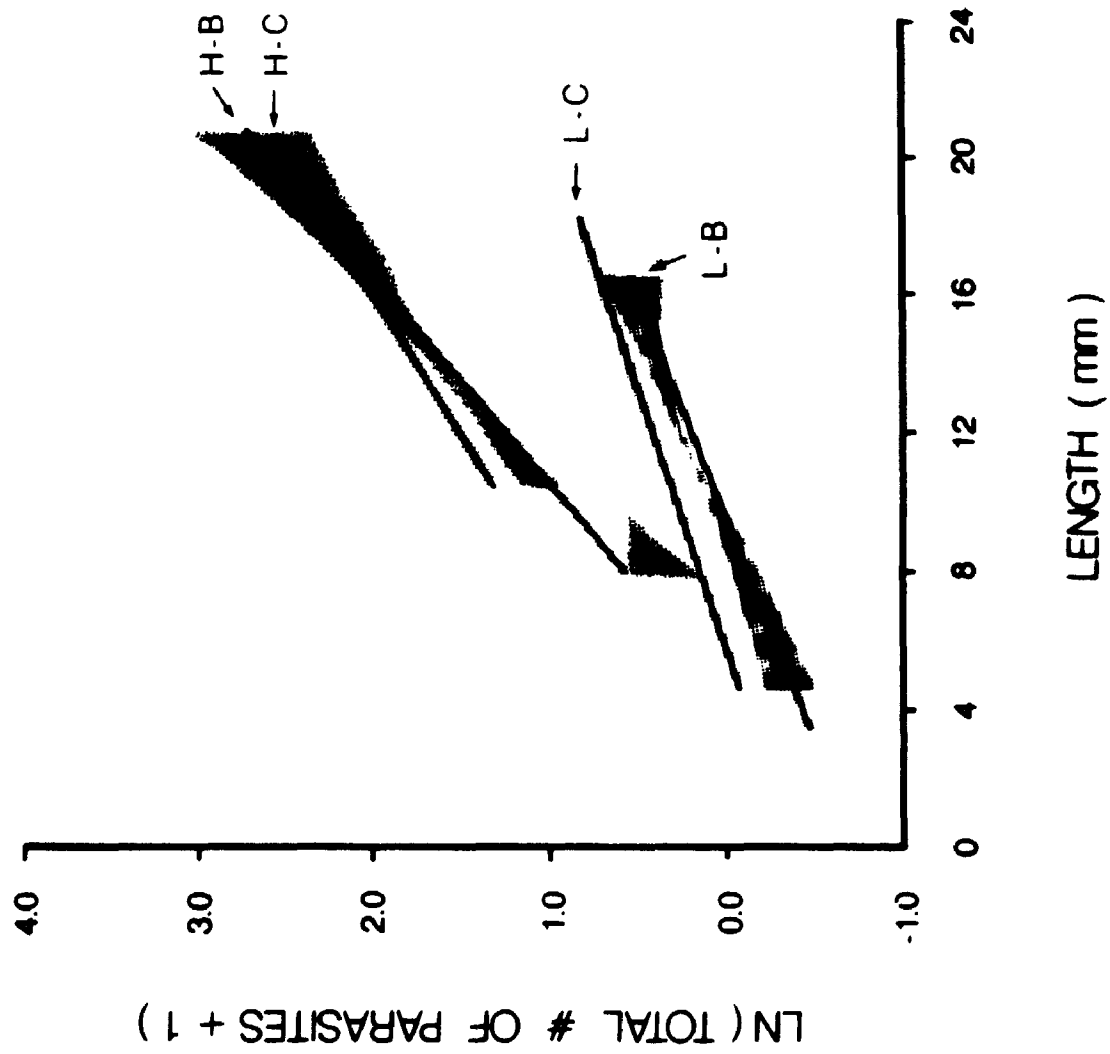
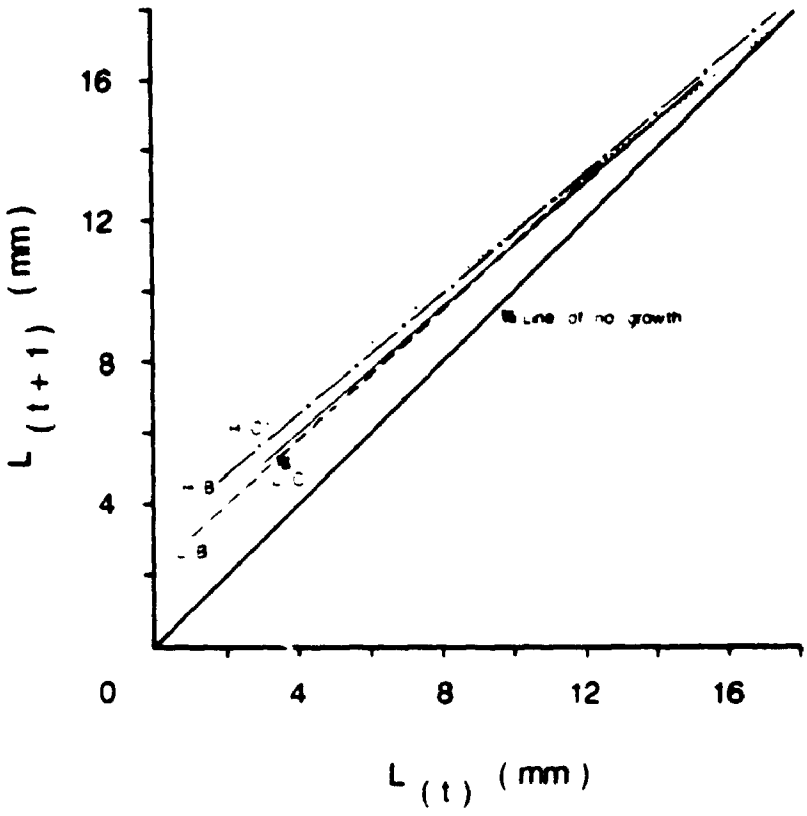
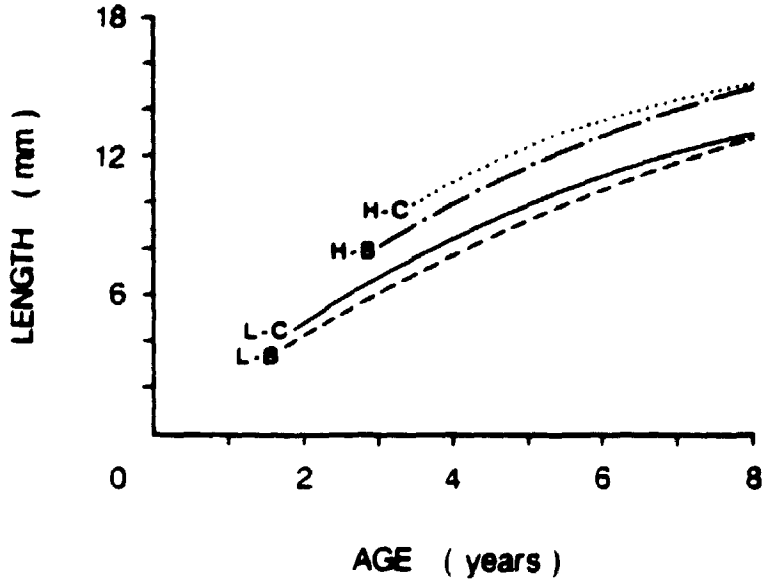

H-B
H-C
L-C
L-B
4.0
3.0
2.0
1.0
0.0
-1.0
0
4
8
12
16
20
24
LENGTH (mm)
LN (TOTAL # OF PARASITES + 1)

Figure 14. (A) Walford plot for high and low tide *M. balthica* crawlers and burrowers; (B) von Bertalanffy growth curves corresponding to the Walford plots. H-C : High tide crawlers; H-B : High tide burrowers; L-C : Low tide crawlers; L-B : Low tide burrowers.

(A)


16
12
8
4
0
4
8
12
16
L (t+1) (mm)
L (t) (mm)
Line of no growth
H-C
H-B
L-C
L-B

(B)


18
12
6
0
2
4
6
8
LENGTH (mm)
AGE (years)
H-C
H-B
L-C
L-B

between tide levels and between crawlers and burrowers. These significant slope differences are independent of each other because the 'tide level by behaviour by length' interaction was not significant ($p>0.05$). There is no 'tide level-by-crawling behaviour' interaction ($p>0.05$) which shows again that growth differences between tide levels are independent of the behaviour of the clams. Since slopes are different, no formal test of intercepts was done. However, it can be seen from Fig. 14(a) that high tide clams grow faster than low tide clams (independent of crawling behaviour), and that crawlers grow faster than burrowers (independent of tide level).

3.3 Transplant experiments at Churchill, Hudson Bay

3.3.1 1985 Transplant

Out of the 500 marked *Macoma* from each tide level left in the high tide transplant area, 127 (25.4%) and 68 (13.6%) of the high and low tide clams respectively were recovered. Results of the ANCOVA using the winter rings data showed that there was a significant difference between the growth rates of the high and low tide clams before the transplant experiment ($p<0.01$) (Table 5a) with the former growing faster than the latter (Fig. 15). This reconfirms that the phenomenon still exists amongst the two sub-populations of *Macoma* in Hudson Bay prior to the field experiment.

After one summer, however, no significant difference was found when the actual length increment of the low tide clams was similarly compared with that of the high tide clams ($p>0.05$) (Table 5b). The power of detection of a significant difference in the vertical distance between the two lines was 0.10 mm. Fig. 15 shows the

Table 5. ANCOVA results of *M. balthica* recovered after summer 1985 transplant (a) Before transplant experiment; (b) After transplant experiment; (c) After 1 year - between high tide clams only.

	Source of clams	p	
(a)	**Before transplant**		
	High tide level Low tide level	p=0.021	*
(b)	**After transplant**		
	High tide level Low tide level	p=0.327	n.s.
(c)	**After 1 year**		
	Between high tide clams only	p=0.540	n.s.

Figure 15. Walford plots of *M. balthica* before and after transplant (1985).

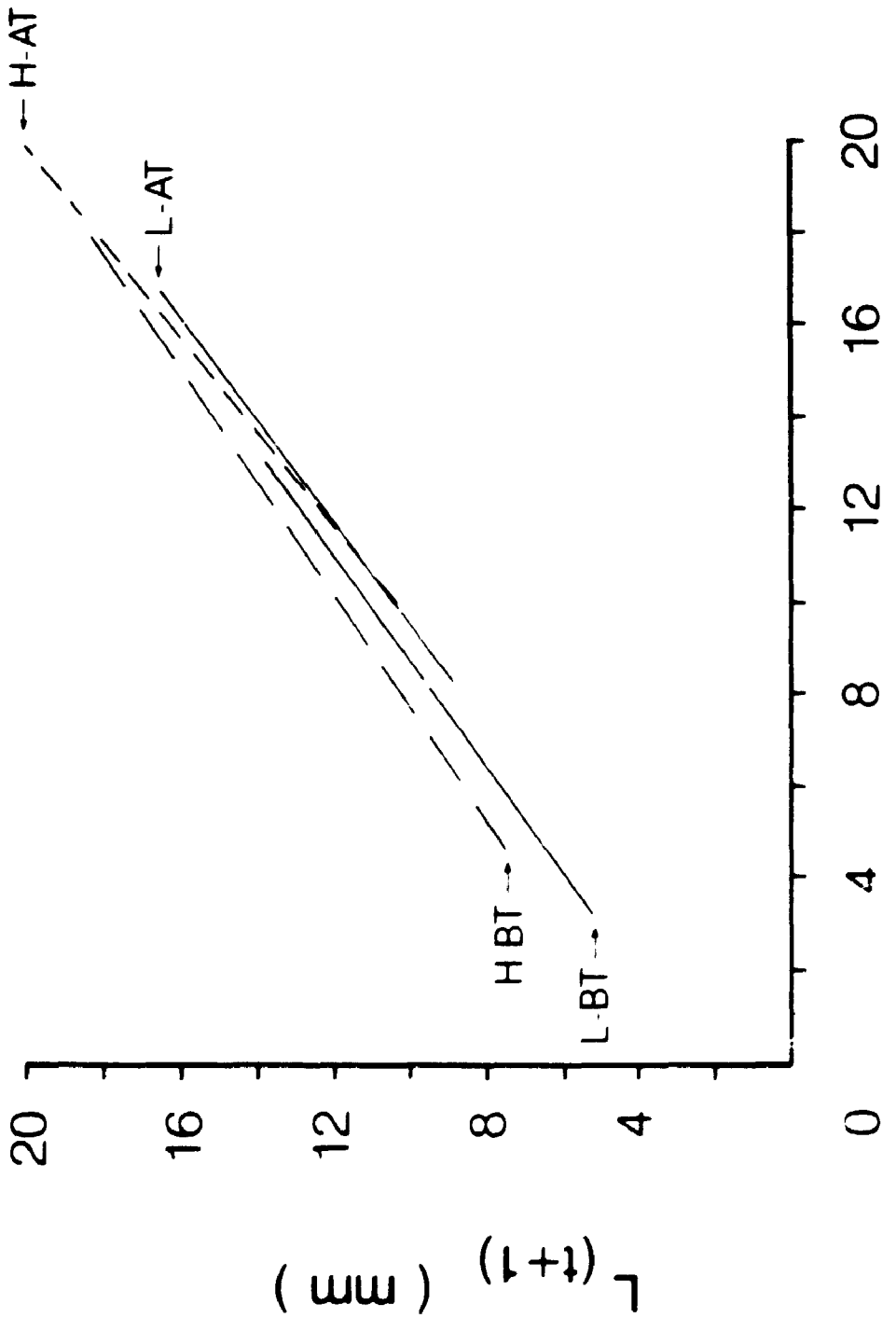
— — — — = H-BT (High tide level, before transplant)

— — — — = L-BT (Low tide level, before transplant)

— — — — = H-AT (High tide level, after transplant)

— — = L-AT (Low tide level, after transplant)

Lines are drawn to cover the range of data actually used.


H-AT
L-AT
H BT
L-BT
20
16
12
8
4
0
4
8
12
16
20
L (t+1) (mm)
L (t) (mm)

Walford plots for the clams before the transplant experiment and after one summer's growth.

When the growth that occurred in the high tide clams during one summer was compared with that which occurred during one year, no significant difference was detected ($p>0.05$) (Table 5c). This indicates that in 1985, the growing season did terminate after the retrieval of the summer transplant clams in mid-October when the bay froze. The growth measured in the summer is hence, equivalent to the annual growth. δ was calculated to be 0.08 mm. Fig. 16 shows the Walford plots for the *Macoma* left in the bay for one summer and one year.

It can be seen from Table 6 that the Hardy-Weinberg equilibrium exists for the PGM, MDH and LAP loci except for the PGI locus. The D values are all negative, indicating that there is an excess of homozygosity in the two tide level populations for the four polymorphic loci examined. Also, the low tide clams are generally more deficient in heterozygotes than the high tide ones (D values being more negative). These results agree with those reported by Green et al. (1983).

The results of the various ANCOVA tests for difference of slopes of the Walford plots of homozygotes versus heterozygotes for the four enzyme loci are summarised in Table 7. Only the homozygotes and heterozygotes at the LAP locus for the high tide clams differed in growth rates ($p<0.01$); all the rest were non-significant. The high tide homozygotes at the LAP locus grew faster than the heterozygotes (Table 7b and Fig. 17).

Figure 16. One year vs. one summer's growth - 1985 transplant (SUM = summer; YR = year). Lines are drawn to cover the range of data actually used. The 95% confidence bands are shown.

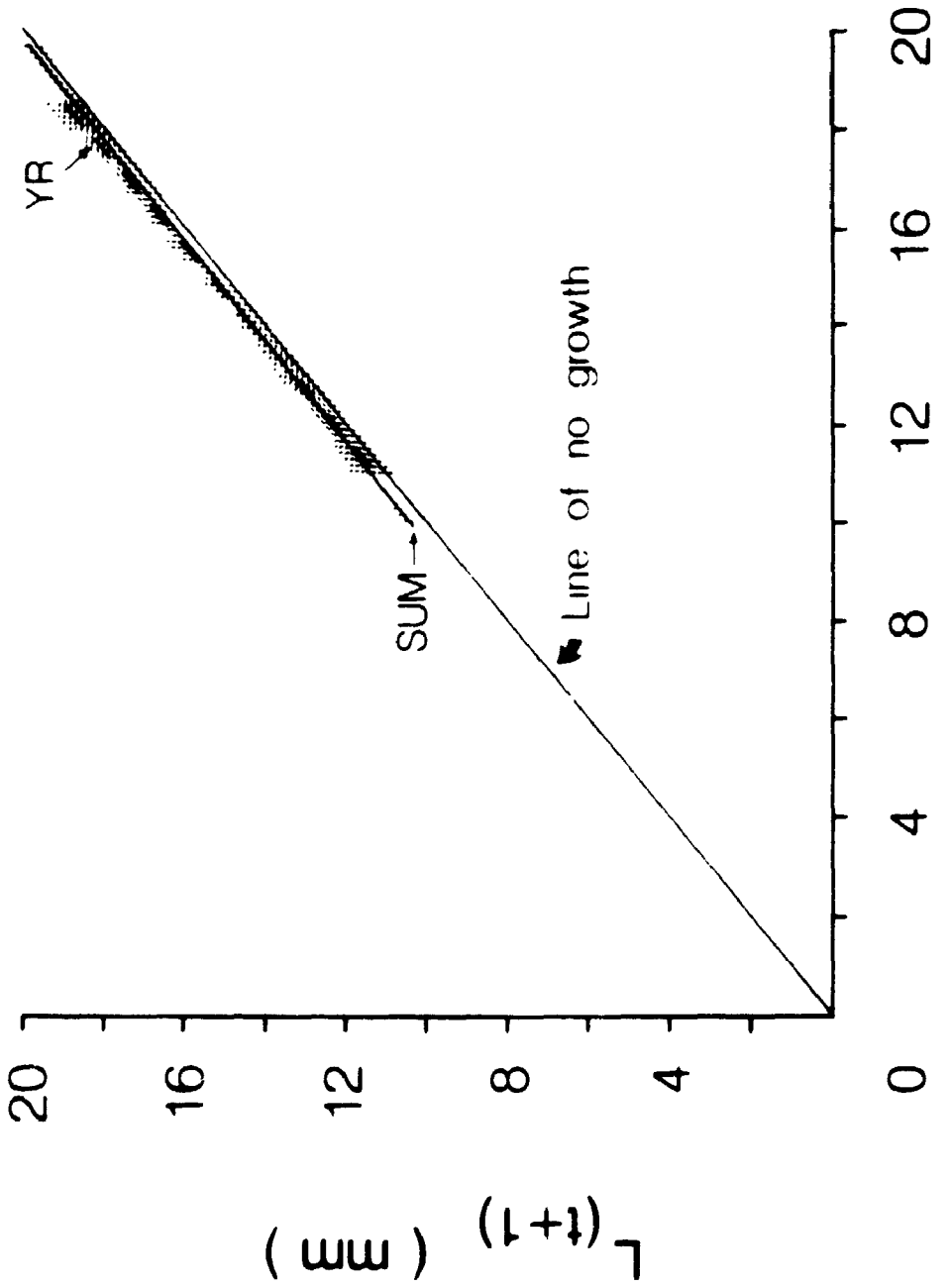

YR
SUM
Line of no growth
0
4
8
12
16
20
L (t) (mm)
L (t+1) (mm)

Table 6. Genetic parameters for four enzyme loci of *M. balthica* recovered in the 1985 summer transplant. ($n_{High\ tide} = 127$, $n_{Low\ tide} = 68$).
p (H.W.) is the chance probability for Hardy-Weinberg equilibrium;
D = (#Heterozygotes observed - #Heterozygotes expected)/(#Heterozygotes expected)

Locus	Allele	Tide level	
		High	Low
		(Allele frequency)	
PGM	1	0.56	0.60
	2	0.44	0.40
	p (H.W.)	>0.001	>0.05
	D	-0.281	-0.234
MDH	1	0.51	0.55
	2	0.49	0.45
	p (H.W.)	>0.025	>0.05
	D	-0.181	-0.198
LAP	1	0.50	0.52
	2	0.50	0.48
	p (H.W.)	>0.05	>0.025
	D	-0.070	-0.264
PGI	1	0.079	0.110
	2	0.531	0.508
	3	0.390	0.382
	p (H.W.)	<0.001	<0.001
	D	-0.226	-0.423

Table 7. (a) Comparison of slopes of the Walford plots (by ANCOVA) of *M. balthica* - homozygous versus heterozygous clams (summer 1985 transplant); (b) Walford plot equations of high tide homozygous and heterozygous clams for the LAP locus. LT and LT_1 are the length of the clam before and after the transplant experiment respectively.

(a)

Enzyme locus	High tide level		Low tide level	
	p		p	
PGM	0.7129	n.s.	0.4886	n.s.
MDH	0.1502	n.s.	0.7534	n.s.
LAP	0.0118	*	0.7942	n.s.
PGI	0.0946	n.s.	0.6950	n.s.

(b)

	n	Walford plot equation	r^2
Homozygotes	68	$LT_1 = 0.19 + 0.99\ LT$	0.99
Heterozygotes	59	$LT_1 = 1.13 + 0.93\ LT$	0.96

Figure 17. **Walford plots of *M. balthica* - 1985 transplant for clam homozygous and heterozygous at LAP locus.**
(- - - = Homozygous clams; —— = Heterozygous clams). Lines are drawn to cover the range of data actually used. The 95% confidence bands are shown.

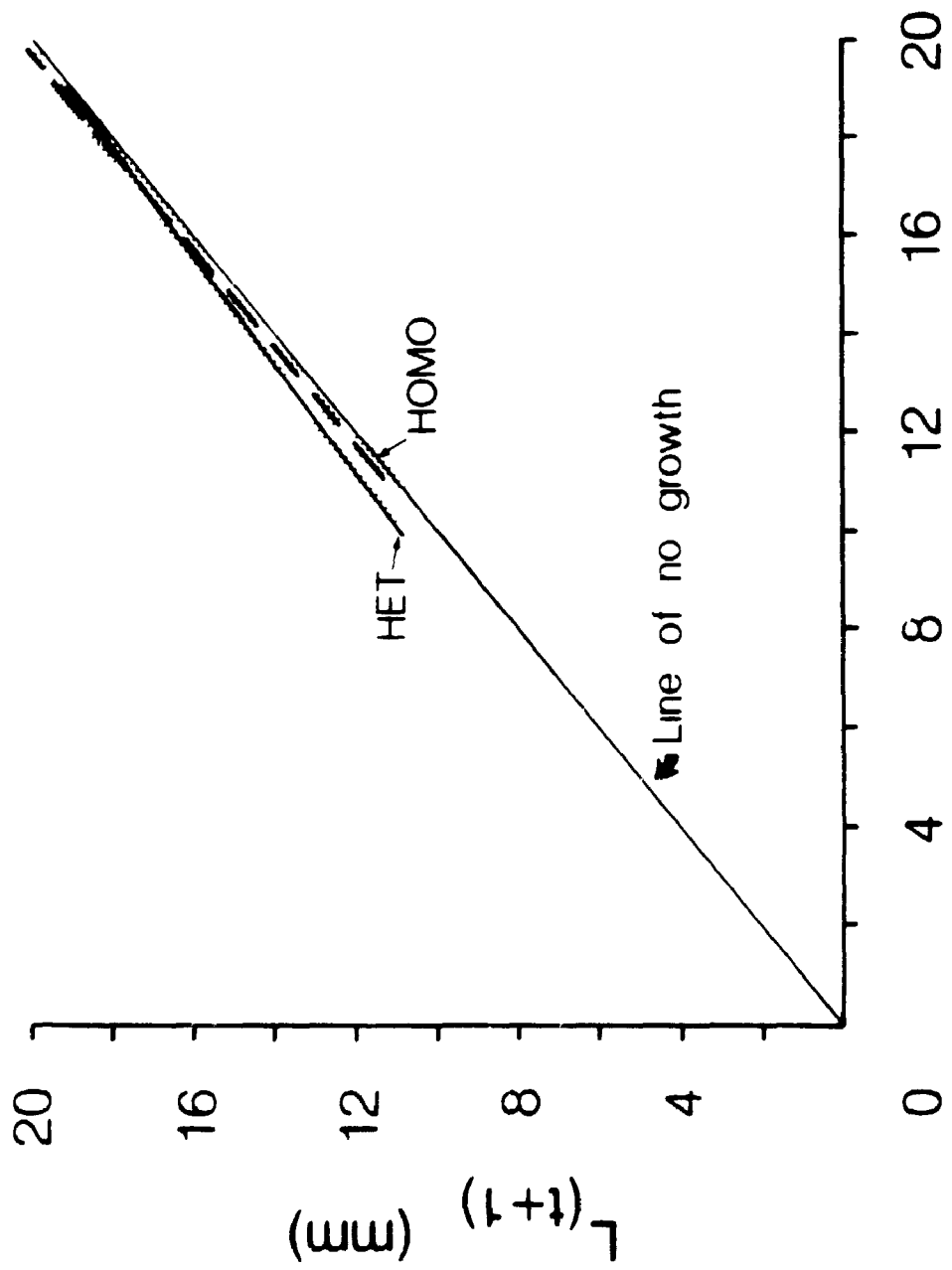

HET
HOMO
Line of no growth
0
4
8
12
16
20
$L_{(t+1)}$ (mm)
$L_{(t)}$ (mm)

Table 8 shows the results of the ANCOVA tests for difference of slopes of the Walford plots with presence or absence of alleles as the groups. Figs. 18, 19 and 20 show the Walford plots of the enzyme alleles that had significantly different slopes. It can be seen that for PGM the presence or absence of both alleles 1 and 2 affected the growth rates of the high tide clams; however, the direction of influence is opposite. When PGM-1 is absent, growth rate of the clams was greater than when it is present ($p<0.01$) (Fig. 18a). However, the reverse is true for PGM-2 (Fig. 18b). In the low tide clams, growth was not appreciably affected whether allele 1 or 2 of PGM is present.

The presence of allele 1 of MDH enhanced the growth of the high tide clams significantly ($p<0.01$) (Fig. 19) whereas that of allele 2 had no effect. Once again the presence of MDH-1 or MDH-2 had no influence on the growth of low tide *Macoma*. Both LAP-1, LAP-2 and PGI-1 had no significant influence on the growth rates of either high or low tide clams. Presence of PGI-2 enhanced the growth of high tide clams but not that of low tide ones (Fig. 20a). Growth was faster for clams without PGI-3 in the high tide region (Fig. 20b). Low tide *Macoma* growth was not significantly different whether PGI-3 was present or not.

3.3.2 1986 Reciprocal Transplant

Of the 2000 clams left in the high tide site, only 218 (21.8%) and 82 (8.2%) of the high and low tide *Macoma* were recovered respectively. None of the clams released at the low tide transplant area was recovered during October 1986 as the study area was

Table 8. Comparison of slopes of the Walford plots (by ANCOVA) of *M. balthica* with and without the alleles of four enzyme loci (summer 1985 transplant). W.P. (±allele) is the equation for the Walford plot with and without the allele specified respectively. LT and LT_1 are the length of the clam before and after the transplant experiment respectively.

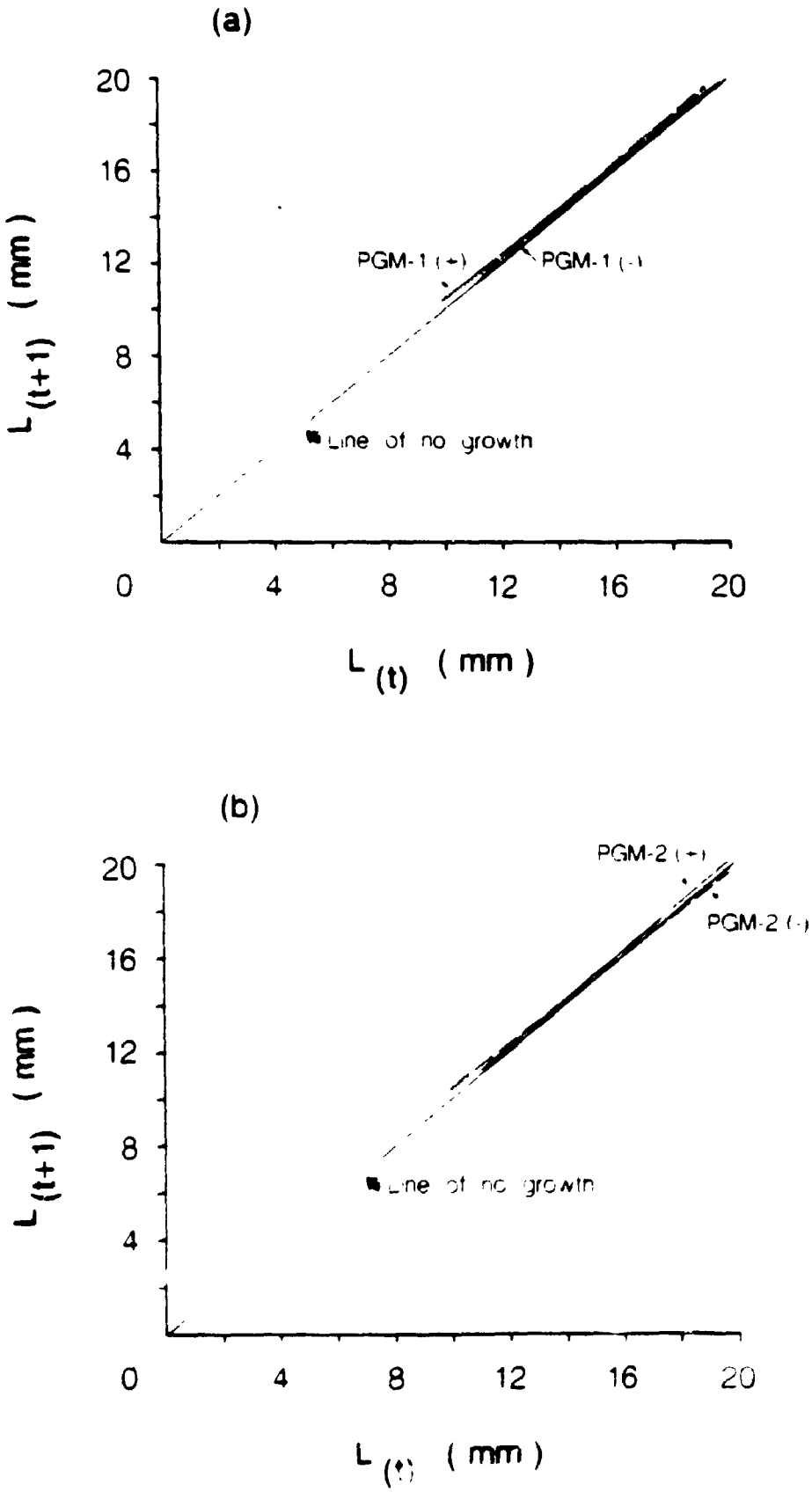
Tide level	Effect tested	Allele 1	Allele 2
(a) PGM			
High	Slope difference	p=0.009**	p=0.007**
	W.P. (- allele)	LT_1=-0.22 + 1.03LT	LT_1=1.16 + 0.94LT
	n	34	48
	r^2	0.996	0.964
	W.P. (+ allele)	LT_1=0.86 + 0.96LT	LT_1=0.13 + 1.00LT
	n	93	79
	r^2	0.978	0.995
Low	Slope difference	p=0.126 n.s.	p=0.435 n.s.
	W.P. (-allele)	LT_1=1.12 + 0.93LT	LT_1=0.53 + 0.97LT
	n	15	28
	r^2	0.984	0.988
	W.P. (+ allele)	LT_1=0.55 + 0.97LT	LT_1=0.84 + 0.95LT
	n	53	40
	r^2	0.990	0.986
(b) MDH			
High	Slope difference	p=0.023**	p=0.255 n.s.
	W.P. (- allele)	LT_1=1.08 + 0.95LT	LT_1=0.18 + 0.99LT
	n	36	39
	r^2	0.967	0.996
	W.P. (+ allele)	LT_1=017 + 0.99LT	LT_1=0.69 + 0.97LT
	n	91	88
	r^2	0.994	0.976
Low	Slope difference	p=0.888 n.s.	p=0.953 n.s.
	W.P. (-allele)	LT_1=0.71 + 0.96LT	LT_1=0.74 + 0.96LT
	n	17	24
	r^2	0.981	0.991
	W.P. (+ allele)	LT_1=0.75 + 0.95LT	LT_1=0.74 + 0.95LT
	n	51	44
	r^2	0.989	0.953

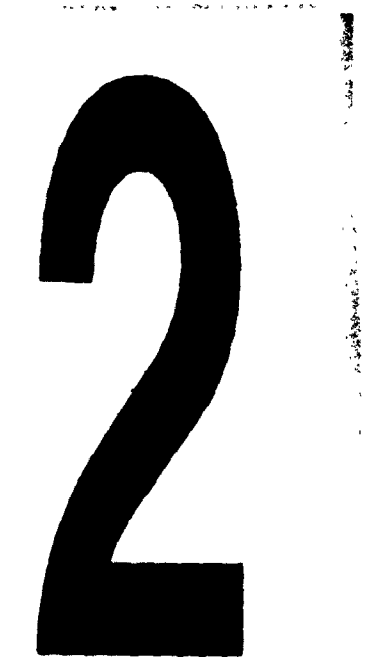
Table 8. (cont'd)

Tide level	Effect tested	Allele 1	Allele 2
(c) LAP			
High	Slope difference	p=0.363 n.s.	p=0.084 n.s.
	W.P. (- allele)	LT_1=0.31 + 0.99LT	LT_1=0.06 + 1.00LT
	n	34	34
	r^2	0.995	0.996
	W.P. (+ allele)	LT_1=0.67 + 0.96LT	LT_1=0.76 + 0.96LT
	n	93	93
	r^2	0.976	0.977
Low	Slope difference	p=0.809 n.s.	p=0.726 n.s.
	W.P. (-allele)	LT_1=0.90 + 0.95LT	LT_1=0.83 + 0.95LT
	n	20	33
	r^2	0.984	0.988
	W.P. (+ allele)	LT_1=0.73 + 0.95LT	LT_1=0.68 + 0.96LT
	n	48	45
	r^2	0.991	0.982

Tide level	Effect tested	Allele 1	Allele 2	Allele 3
(d) PGI				
High	Slope difference	p=0.919 n.s.	p=0.0001**	p=0.054*
	W.P. (-allele)	LT_1=0.59 + 0.97LT	LT_1=1.82 + 0.90LT	LT_1=0.17 + 0.99LT
	n	112	32	55
	r^2	0.980	0.950	0.997
	W.P. (+allele)	LT_1=0.59 + 0.96LT	LT_1=0.11 + 1.00LT	LT_1=0.98 + 0.95LT
	n	15	95	72
	r^2	0.997	0.996	0.971
Low	Slope difference	p=0.196 n.s.	p=0.756 n.s.	p=0.773 n.s.
	W.P. (-allele)	LT_1=0.59 + 0.96LT	LT_1=0.84 + 0.95LT	LT_1=0.80 + 0.95LT
	n	57	22	34
	r^2	0.994	0.972	0.983
	W.P. (+allele)	LT_1=1.26 + 0.92LT	LT_1=0.65 + 0.96LT	LT_1=0.67 + 0.96LT
	n	11	46	34
	r^2	0.962	0.992	0.992

Figure 18. Walford plots of *M. balthica* - 1985 transplant for (a) PGM-1; (b) PGM-2 [(+) = clams with PGM allele and (-) = clams without the PGM allele]. Lines are drawn to cover the range of data actually used. The 95% confidence bands are shown.


(a)
20
16
12
8
4
0
4
8
12
16
20
PGM-1 (+)
PGM-1 (-)
Line of no growth
L (t+1) (mm)
L (t) (mm)
(b)
20
16
12
8
4
0
4
8
12
16
20
PGM-2 (+)
PGM-2 (-)
Line of no growth
L (t+1) (mm)
L (t) (mm)



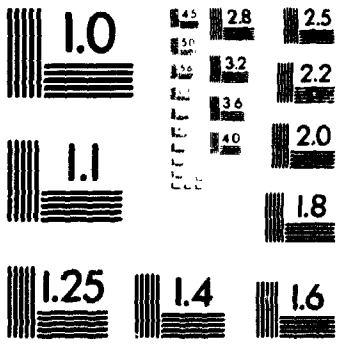

1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
5.6
6.3
7.1
8.0
9.0
10.0

Figure 19. **Walford plots of *M. balthica* - 1985 transplant for MDH-1 allele. [(+) = clams with MDH allele and (-) = clams without the MDH allele]. Lines are drawn to cover the range of data actually used. The 95% confidence bands are shown.**

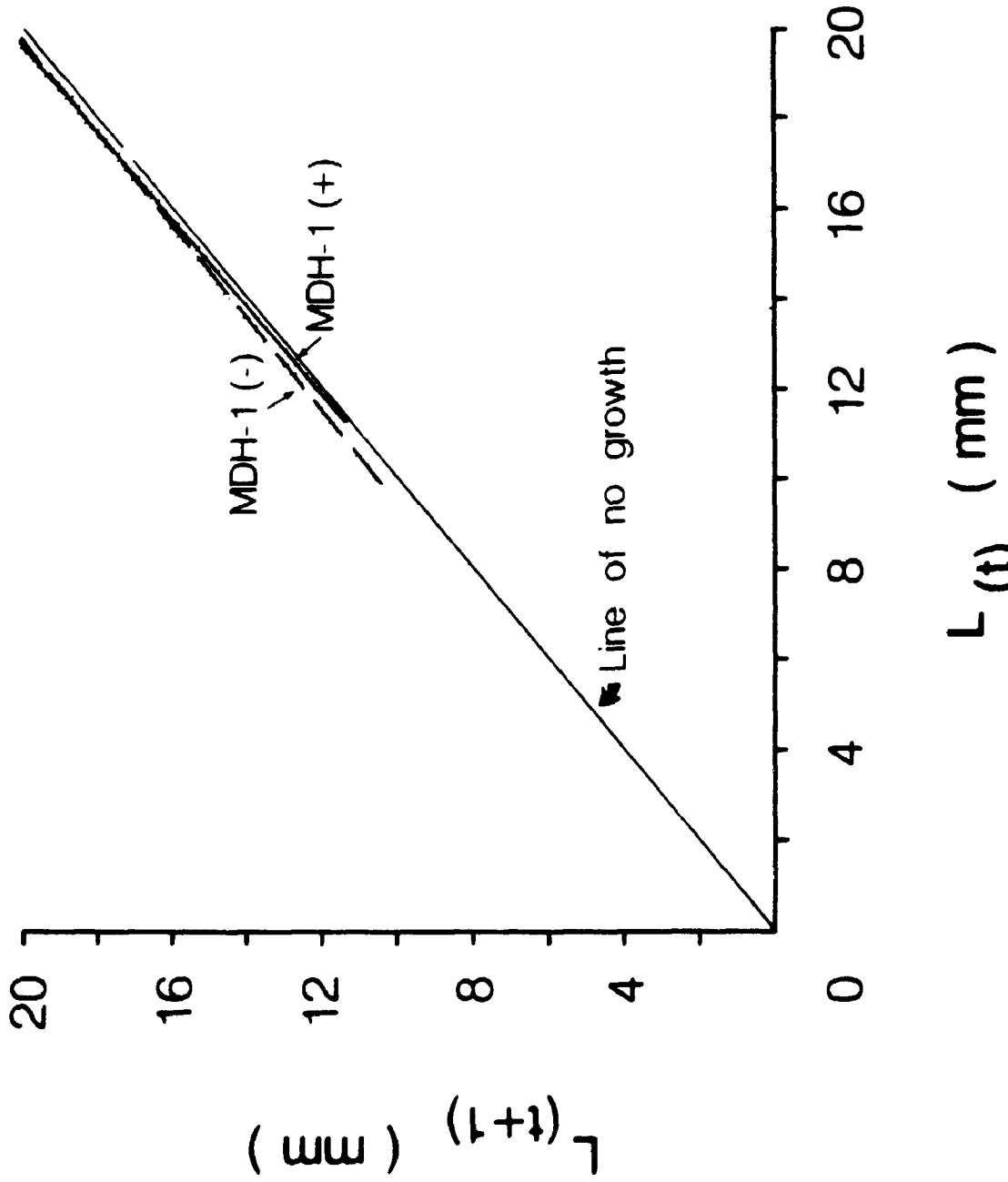
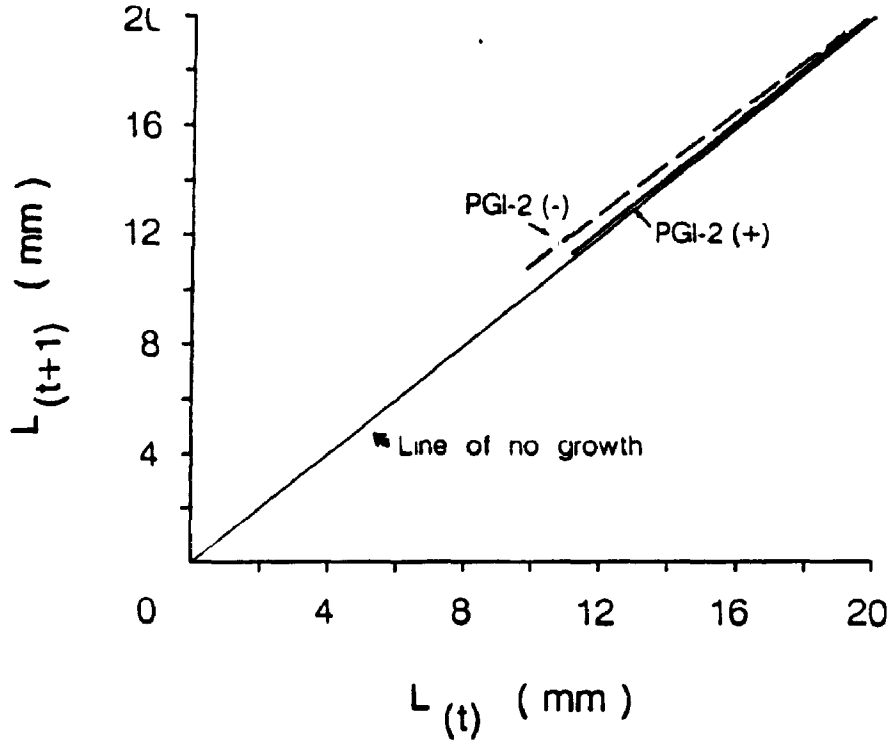
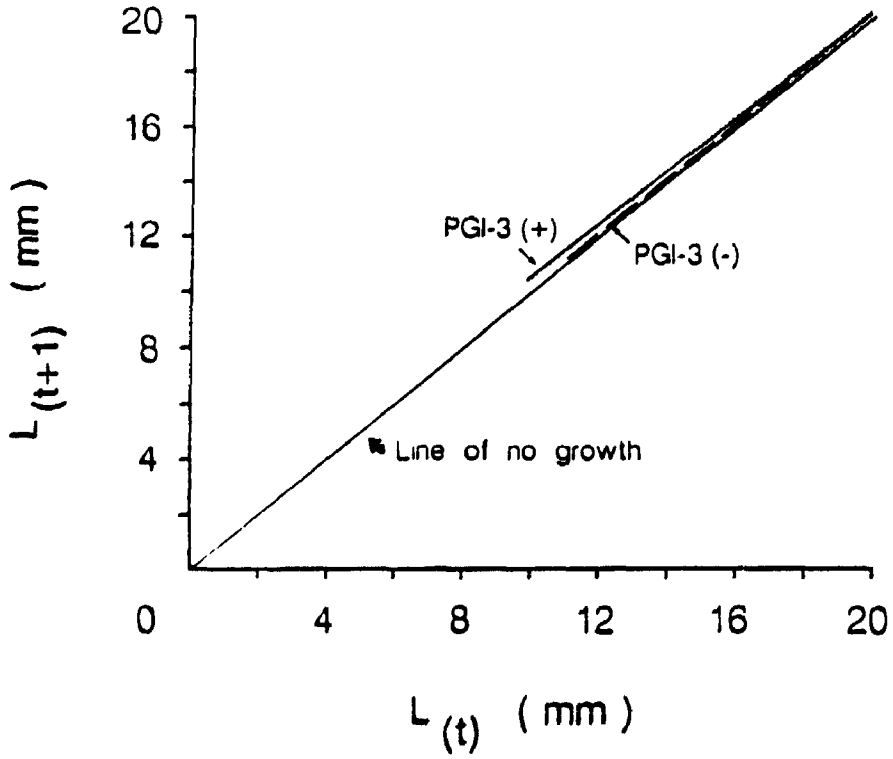

MDH-1 (-)
MDH-1 (+)
Line of no growth
0
4
8
12
16
20
$L_{(t)}$ (mm)
$L_{(t+1)}$ (mm)

Figure 20. **Walford plots of *M. balthica* - 1985 transplant for (a) PGI-2; (b) PGI-3 [(+) = clams with PGI allele and (-) = clams without the PGI allele]. Lines are drawn to cover the range of data actually used. The 95% confidence bands are shown.**

(a)


20
16
12
8
4
0
4
8
12
16
20
PGI-2 (-)
PGI-2 (+)
Line of no growth
L (t+1) (mm)
L (t) (mm)

(b)


20
16
12
8
4
0
4
8
12
16
20
PGI-3 (+)
PGI-3 (-)
Line of no growth
L (t+1) (mm)
L (t) (mm)

destroyed due to severe August storms which broke out during extreme low tides. Although some clams were recovered from the low tide area the following year, there were too few for data analysis.

Results of the ANCOVA carried out on the before transplant data showed that the growth rate of the high tide *Macoma* was significantly faster than that of the low tide ones ($p<0.01$) reconfirming the occurrence of the phenomenon before the transplant study was carried out (Table 9a). No significant difference was found when the growth of the low tide clams was similarly compared with that of the high tide clams after one summer (Table 9b). δ was calculated to be 0.05 mm. Fig. 21 shows the Walford plots for the clams 'before' and 'after' the transplant experiment. The former plots were based on the winter rings and the latter ones based on the actual length increment observed after one summer.

The summer and year long transplant clams' growth are shown in the Walford plots in Fig. 22. A significant difference was found when the growth of one summer was compared with that which occurred during one year ($p<0.05$). The Walford plot regressions for the clams left out for the summer of 1986 and for the year long transplant were : $L_1 = 0.21 + 0.99\ L$ and $L_1 = 0.78 + 0.96\ L$ respectively.

An examination of the mean daily temperatures at Churchill during 1985, 1986 and 1987 (Fig. 23) showed no appreciable difference among the years. A probable reason for the difference between the summer and year's growth could be that since the sampling and marking effort of clams in the summer of 1986 was four times higher than that of 1985, by the time that clams were marked and released

Table 9. ANCOVA results of *M. balthica* recovered after summer 1986 transplant (a) before transplant experiment; (b) after transplant experiment; (c) after 1 year - between high tide clams only.

	Source of clams	p	
(a)	Before transplant		
	High tide level Low tide level	p=0.0001	**
(b)	After transplant		
	High tide level Low tide level	p=0.923	n.s.
(c)	After 1 year		
	Between high tide clams only	p=0.022	*

Figure 21. Walford plots of *M. balthica* before and after transplant (1986).

— — — = H-BT (High tide level, before transplant)

———— = L-BT (Low tide level, before transplant)

— — — — = H-AT (High tide level, after transplant)

———— = L-AT (Low tide level, after transplant)

Lines are drawn to cover the range of data actually used.

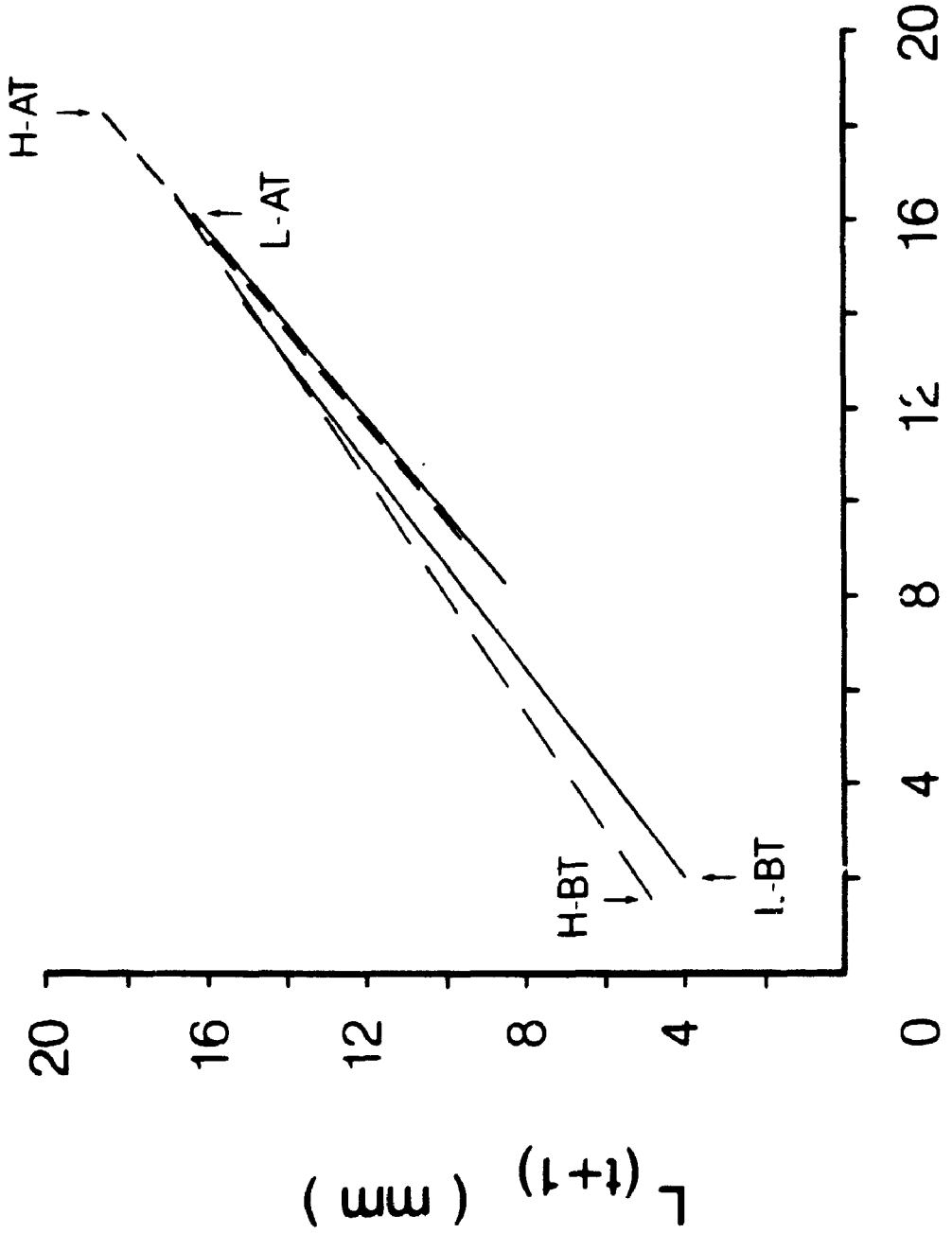

H-AT
L-AT
H-BT
L-BT
20
16
12
8
4
0
4
8
12
16
20
L (t+1) (mm)
L (t) (mm)

Figure 22. **One year vs. one summer's growth - 1986 transplant** (SUM = summer; YR = year). **Lines are drawn to cover the range of data actually used. The 95% confidence bands are shown.**

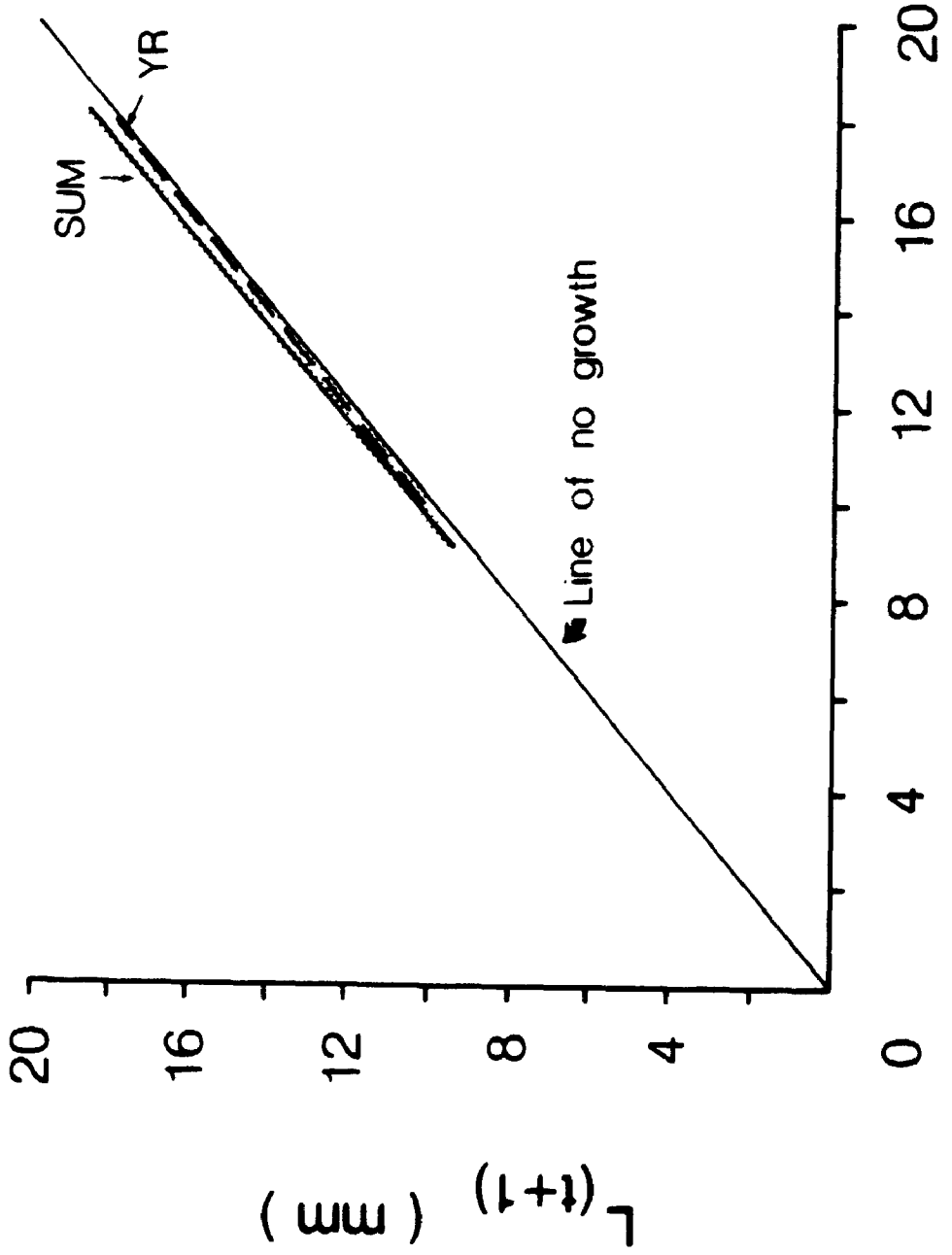
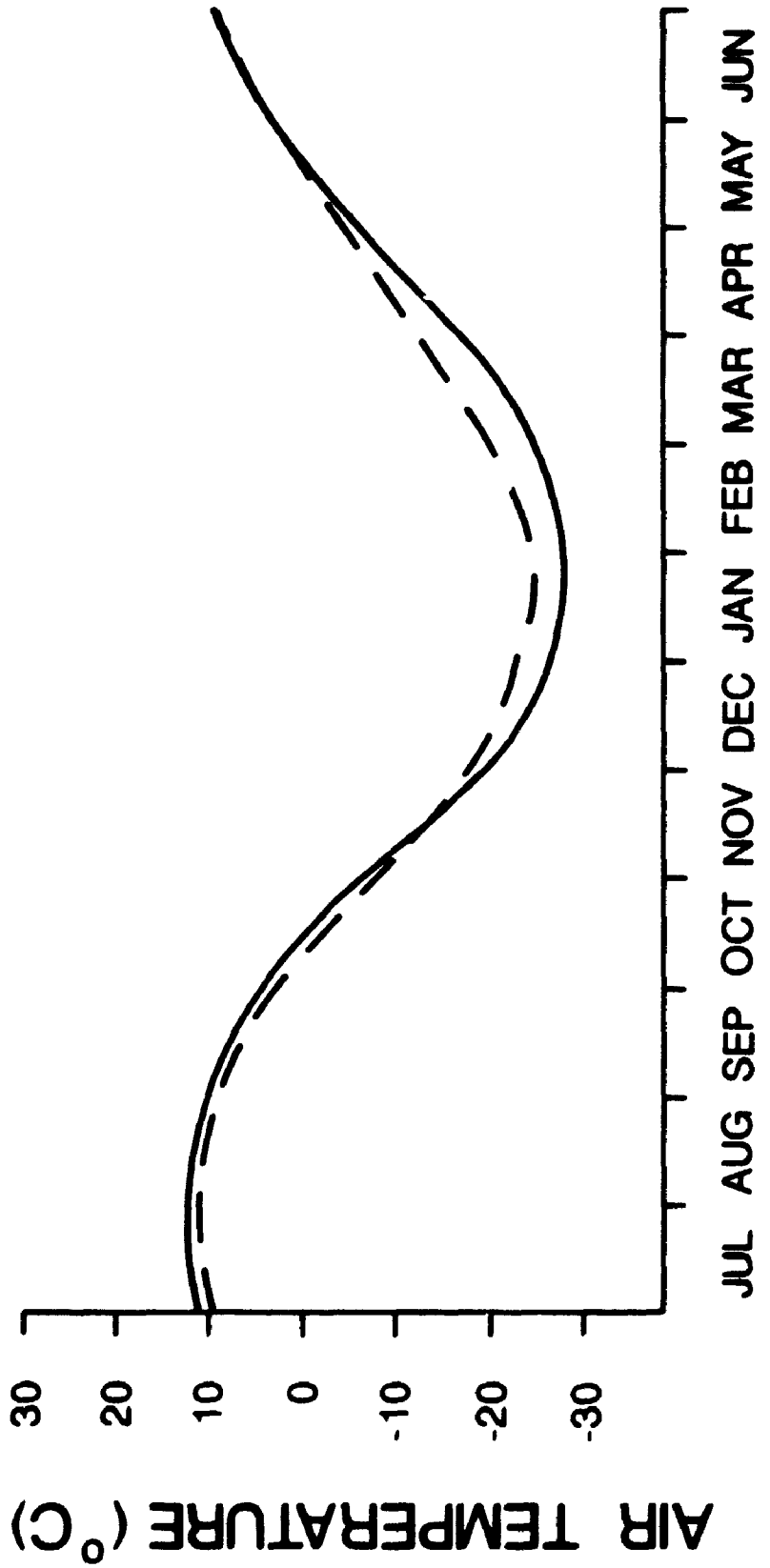

SUM
YR
Line of no growth
20
16
12
8
4
0
4
8
12
16
20
L (t+1) (mm)
L (t) (mm)

Figure 23. **Mean daily air temperatures at Churchill, Manitoba for the months of July to June for 1985-86 and 1986-87.**
(—— = 1985-86; - - - = 1986-87).


AIR TEMPERATURE (°C)
30
20
10
0
-10
-20
-30
JUL AUG SEP OCT NOV DEC JAN FEB MAR APR MAY JUN
MONTH

for the long term study, approximately two weeks of the growing season had elapsed (i.e. the summer transplant clams had already been in the bay for about 4 to 5 days before). Fig. 24 shows the mean daily temperatures for the month of July 1985 and 1986. It can be seen that the temperatures of the few days between the release of the summer transplant and the long term transplant clams were abnormally high; this could account for the greater growth of the summer transplant clams.

Results of the electrophoretic analysis showed that the frequency of the alleles of PGM, MDH and PGI were generally in accordance with the Hardy-Weinberg equilibrium (Table 10). The only exception was LAP. These results differ slightly from the previous year's results in that it is the LAP locus this time instead of the PGI locus (see Table 6). All D values are negative with the low tide clams more deficient in heterozygotes again (Table 10).

Table 11 shows the ANCOVA results of the test of slopes of the Walford plots for homozygous and heterozygous clams. Low tide clams heterozygous at the MDH locus grew significantly slower than the homozygotes (Table 11b, Fig. 25); all the rest of the pair-wise comparison of slopes were not significant (Table 11a).

ANCOVA results testing for difference in slopes of the Walford plots between clams with and without a specified allele are presented in Table 12. The presence or absence of any one of the alleles of MDH, LAP and PGI did not affect the growth rate of the high or low tide clams significantly. Only PGM-1's presence significantly increased the growth rate within the high tide clams and the Walford plot for this allele is shown in Fig. 26.

Figure 24. **Mean daily air temperatures at Churchill, Manitoba for the month of July in 1985 and 1986. [●——● : 1985; o - - - o : 1986; S85 and S86 = release of summer 1985 and summer 1986 transplant clams respectively; Y85 and Y86 = release of overwintering clams of 1985 and 1986 respectively].**

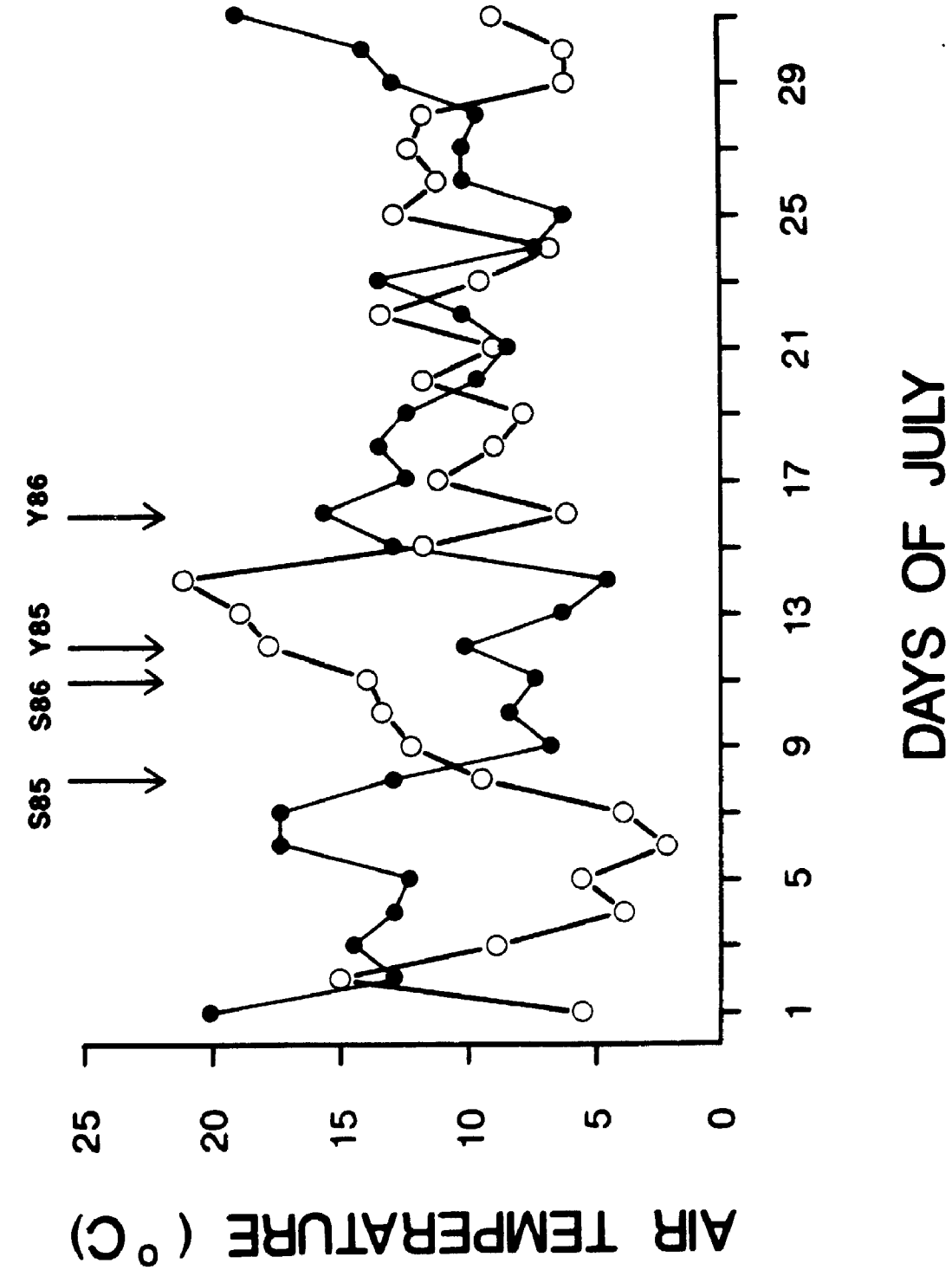

AIR TEMPERATURE (°C)
25
20
15
10
5
0
1
5
9
13
17
21
25
29
DAYS OF JULY
S85
S86
Y85
Y86

Table 10. Genetic parameters for four enzyme loci of *M. balthica* recovered in the 1986 summer transplant. ($n_{High\ tide} = 218$, $n_{Low\ tide} = 82$)

p (H.W.) is the chance probability for Hardy-Weinberg equilibrium;
D = (#Heterozygotes observed - #Heterozygotes expected)/(#Heterozygotes expected)

Locus	Allele	Tide level	
		High	Low
		(Allele frequency)	
PGM	1	0.53	0.59
	2	0.47	0.41
	p (H.W.)	>0.01	>0.01
	D	-0.162	-0.269
MDH	1	0.47	0.52
	2	0.53	0.48
	p (H.W.)	>0.01	>0.05
	D	-0.171	-0.218
LAP	1	0.52	0.51
	2	0.48	0.49
	p (H.W.)	<0.001	<0.001
	D	-0.265	-0.414
PGI	1	0.060	0.080
	2	0.539	0.518
	3	0.401	0.402
	p (H.W.)	>0.001	>0.001
	D	-0.268	-0.371

Table 11. (a) Comparison of slopes of the Walford plots (by ANCOVA) of *M. balthica* - homozygous versus heterozygous clams (summer 1986 transplant); (b) Walford plot equations of low tide homozygous and heterozygous clams for the MDH locus. LT and LT_1 are the length of the clam before and after the transplant experiment respectively.

(a)

Enzyme locus	High tide level		Low tide level	
	p		p	
PGM	0.0533	n.s.	0.7095	n.s.
MDH	0.1511	n.s.	0.0265	*
LAP	0.2673	n.s.	0.4959	n.s.
PGI	0.2291	n.s.	0.9660	n.s.

(b)

	n	Walford plot equation	r^2
Homozygotes	128	$LT_1 = 0.13 + 1.00\,LT$	0.99
Heterozygotes	90	$LT_1 = 0.37 + 0.98\,LT$	0.99

Figure 25. **Walford plots of *M. balthica* - 1986 transplant for clams homozygous and heterozygous at the MDH locus. HOMO = homozygous clams; HET = heterozygous clams.**
(- - - = Homozygous clams; —— = Heterozygous clams). Lines are drawn to cover the range of data actually used. The 95% confidence bands are shown.

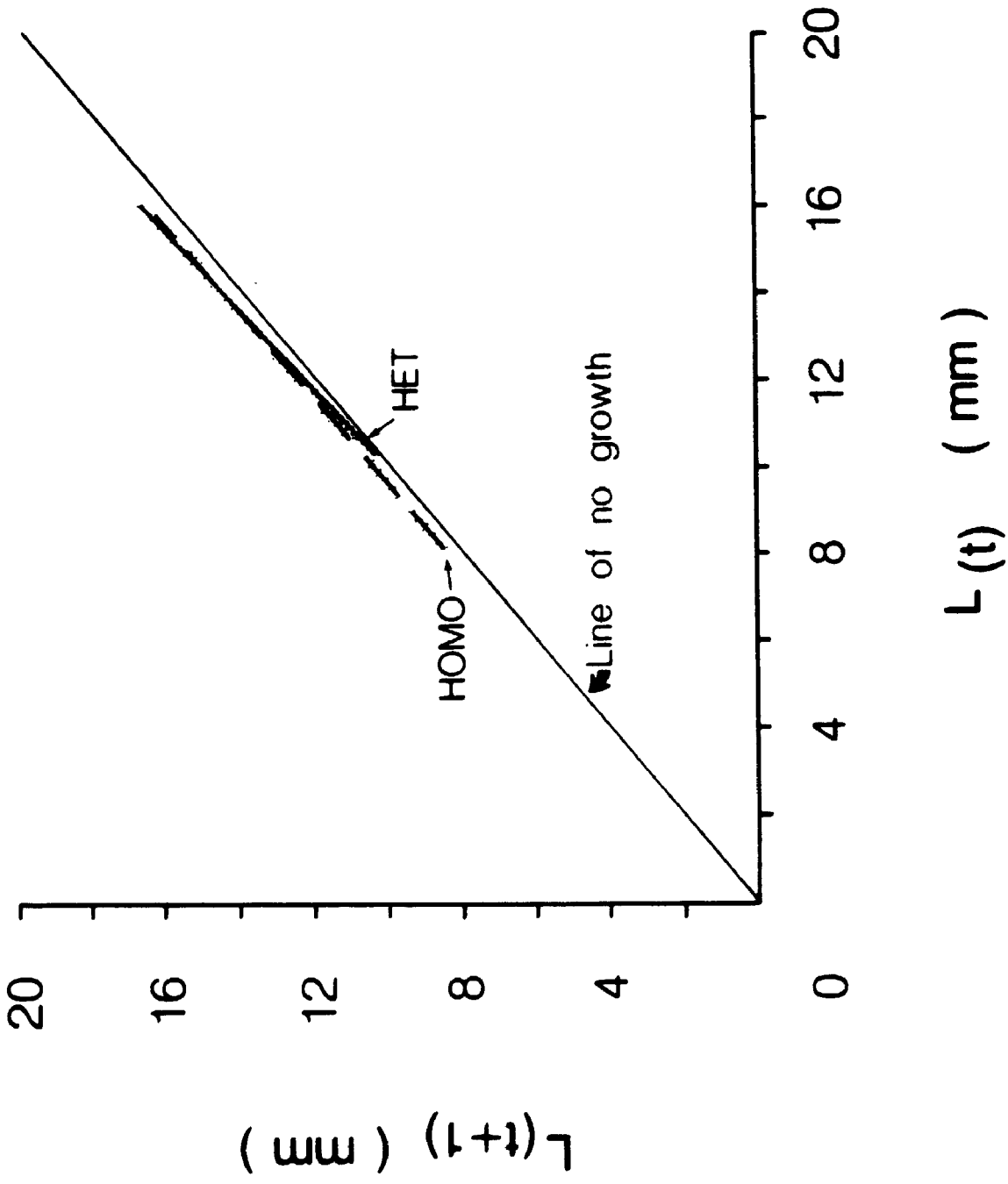

L(t+1) (mm)
L (t) (mm)
0
4
8
12
16
20
HET
HOMO
Line of no growth

Table 12. Comparison of slopes of the Walford plots (by ANCOVA) of *M. balthica* with and without the alleles of four enzyme loci (summer 1986 transplant). W.P. (±allele) is the equation for the Walford plots with and without the allele specified respectively. LT and LT_1 are the length of the clam before and after the transplant experiment respectively.

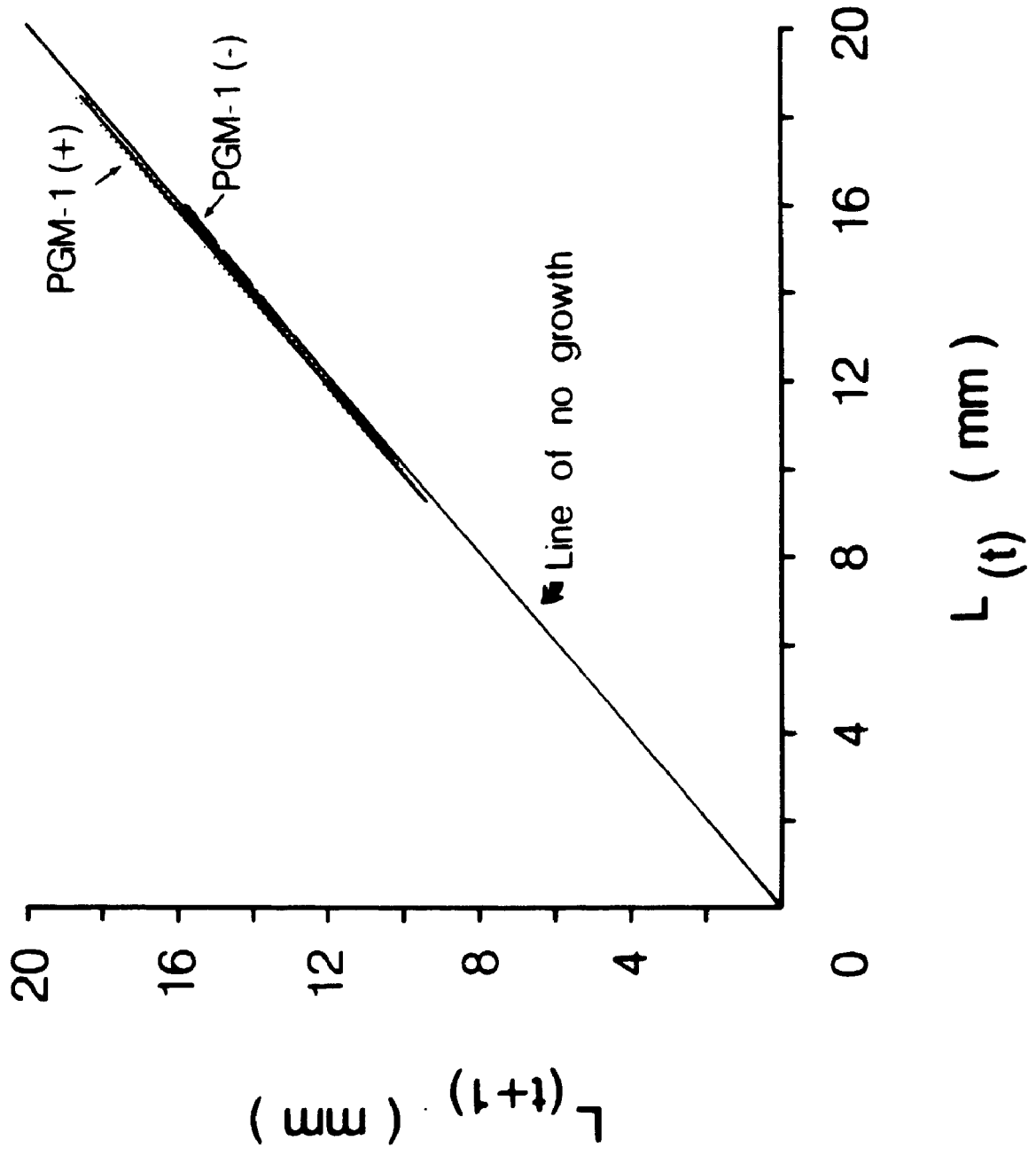
Tide level	Effect tested	Allele 1	Allele 2
(a) PGM			
High	Slope difference	p=0.018*	p=0.917 n.s.
	W.P. (- allele)	LT_1=0.61 + 0.97LT	LT_1=0.19 + 0.97LT
	n	58	69
	r^2	0.985	0.993
	W.P. (+ allele)	LT_1=0.09 + 1.00LT	LT_1=0.22 + 0.99LT
	n	160	149
	r^2	0.993	0.990
Low	Slope difference	p=0.126 n.s.	p=0.086 n.s.
	W.P. (-allele)	LT_1=-0.16 + 1.03LT	LT_1=0.66 + 0.96LT
	n	19	34
	r^2	0.986	0.990
	W.P. (+ allele)	LT_1=0.45 + 0.98LT	LT_1=0.18 + 1.00LT
	n	63	48
	r^2	0.986	0.985
(b) MDH			
High	Slope difference	p=0.783 n.s.	p=0.229 n.s.
	W.P. (- allele)	LT_1=0.17 + 0.99LT	LT_1=0.07 + 1.00LT
	n	70	58
	r^2	0.995	0.988
	W.P. (+ allele)	LT_1=0.24 + 0.99LT	LT_1=0.27 + 0.99LT
	n	148	160
	r^2	0.988	0.992
Low	Slope difference	p=0.133 n.s.	p=0.409 n.s.
	W.P. (-allele)	LT_1=0.79 + 0.96LT	LT_1=0.46 + 0.98LT
	n	23	27
	r^2	0.981	0.991
	W.P. (+ allele)	LT_1=0.17 + 1.00LT	LT_1=0.25 + 1.00LT
	n	59	55
	r^2	0.991	0.980

Table 12. (cont'd)

Tide level	Effect tested	Allele 1	Allele 2
(c) LAP			
High	Slope difference	p=0.753 n.s.	p=0.384 n.s.
	W.P. (- allele)	LT_1=0.22 + 0.99LT	LT_1=0.32 + 0.99LT
	n	65	73
	r^2	0.996	0.989
	W.P. (+ allele)	LT_1=0.20 + 0.99LT	LT_1=0.15 + 0.99LT
	n	153	145
	r^2	0.990	0.999
Low	Slope difference	p=0.546 n.s.	p=0.606 n.s.
	W.P. (-allele)	LT_1=0.49 + 0.98LT	LT_1=0.22 + 1.00LT
	n	28	30
	r^2	0.969	0.993
	W.P. (+ allele)	LT_1=0.26 + 0.99LT	LT_1=0.40 + 0.99LT
	n	54	52
	r^2	0.991	0.980

Tide level	Effect tested	Allele 1	Allele 2	Allele 3
(d) PGI				
High	Slope difference	p=0.266 n.s.	p=0.183 n.s.	p=0.374 n.s.
	W.P. (-allele)	LT_1=0.18 + 0.99LT	LT_1=0.05 + 1.00LT	LT_1=0.32 + 0.99LT
	n	198	57	94
	r^2	0.991	0.995	0.987
	W.P. (+allele)	LT_1=0.53 + 0.97LT	LT_1=0.30 + 0.99LT	LT_1=0.15 + 0.99LT
	n	20	161	124
	r^2	0.987	0.988	0.993
Low	Slope difference	p=0.669 n.s.	p=0.531 n.s.	p=0.861 n.s.
	W.P. (-allele)	LT_1=0.32 + 0.99LT	LT_1=0.49 + 0.98LT	LT_1=0.39 + 0.99LT
	n	74	25	36
	r^2	0.985	0.995	0.975
	W.P. (+allele)	LT_1=0.642 + 0.96LT	LT_1=0.27 + 0.99LT	LT_1=0.28 + 0.99LT
	n	8	57	46
	r^2	0.985	0.982	0.993

Figure 26. Walford plots of *M. balthica* - 1986 transplant for PGM-1 allele [(+) = clams with PGM allele and (-) = clams without the PGM allele]. Lines are drawn to cover the range of data actually used. The 95% confidence bands are shown.


PGM-1 (+)
PGM-1 (-)
Line of no growth
20
16
12
8
4
0
4
8
12
16
20
L (t+1) (mm)
L (t) (mm)

3.4 Temperature-related experiments

3.4.1 Oxygen consumption

The results of the four-way factorial analyses are given in Table 13. It can be seen that the 4-way and all of the 3-way interaction terms are non-significant (Table 13a).

Table 13(b) shows the results of the analysis after the 4- and 3-way interaction terms have been discarded from the model. All the two-way interaction terms were also non-significant. It should be noted that the last 2-way interaction term ('lntotal by tide level') is on the margin of significance. This could be due to the confounding factor of differing parasite loads between the two tide levels as shown in Section 3.2.

Table 13(c) shows that the rate of oxygen consumption is not significantly related to the number of parasites that the clams harbour. In the final model (Table 13d), all the three remaining effects, ln(dry weight), temperature at which oxygen uptake was measured and the source of the clams were found to be highly significant ($p<0.0001$ for all three effects).

The final common slope model obtained by regressing the ln of dry weight, temperature and tide level on the ln of oxygen consumption is given below:

$$\text{Ln(oxygen)} = 4.236 + 0.645\text{Ln(dry weight)} + 0.041\text{Temp} + 0.2223\text{(Tide)}$$

The common Q_{10} for the clams of the two tide levels calculated by taking the antilog of (10*0.041) is 1.506. Thus oxygen consumption goes up by 50% for each 10° C rise in temperature. Fig. 27 shows the

Table 13. Four-way factorial analysis of *M. balthica* (oxygen experiment): (a) saturated model; (b) without 4 and 3-way interaction terms; (c) all main effects; (d) significant main effects only. Lndrywt = Ln(dry weight of clam); Temp = temperature of experiment; Tide = tide level from which clams came; Lntotal = Ln(total number of parasites+1).

Source	df	F	p	
(a)				
Lndrywt	1	6.99	0.009	
Temp	1	2.69	0.102	
Tide	1	0.00	0.954	
Lntotal	1	0.09	0.758	
Lndrywt*Temp	1	0.87	0.352	
Lndrywt*Tide	1	0.01	0.911	
Lndrywt*Lntotal	1	0.07	0.789	
Temp*Tide	1	0.53	0.467	
Temp*Lntotal	1	0.49	0.485	
Lntotal*Tide	1	0.41	0.521	
Lndrywt*Temp*Tide	1	0.39	0.531	n.s.
Lndrywt*Lntotal*Tide	1	0.49	0.483	n.s.
Temp*Lntotal*Tide	1	1.35	0.246	n.s.
Lndrywt*Temp*Lntotal*Tide	2	0.66	0.518	n.s.
(b)				
Lndrywt	1	12.97	0.0004	
Temp	1	3.65	0.057	
Tide	1	0.98	0.322	
Lntotal	1	0.03	0.873	
Lndrywt*Temp	1	0.95	0.329	n.s.
Lndrywt*Tide	1	2.29	0.131	n.s.
Lndrywt*Lntotal	1	0.03	0.864	n.s.
Temp*Tide	1	1.35	0.246	n.s.
Temp*Lntotal	1	0.00	0.972	n.s.
Lntotal*Tide	1	3.48	0.063	n.s.
(c)				
Lndrywt	1	139.60	0.0001	
Temp	1	143.62	0.0001	
Tide	1	16.95	0.0001	
Lntotal	1	0.78	0.379	n.s.
(d)				
Lndrywt	1	152.29	0.0001	***
Temp	1	147.98	0.0001	***
Tide	1	20.67	0.0001	***

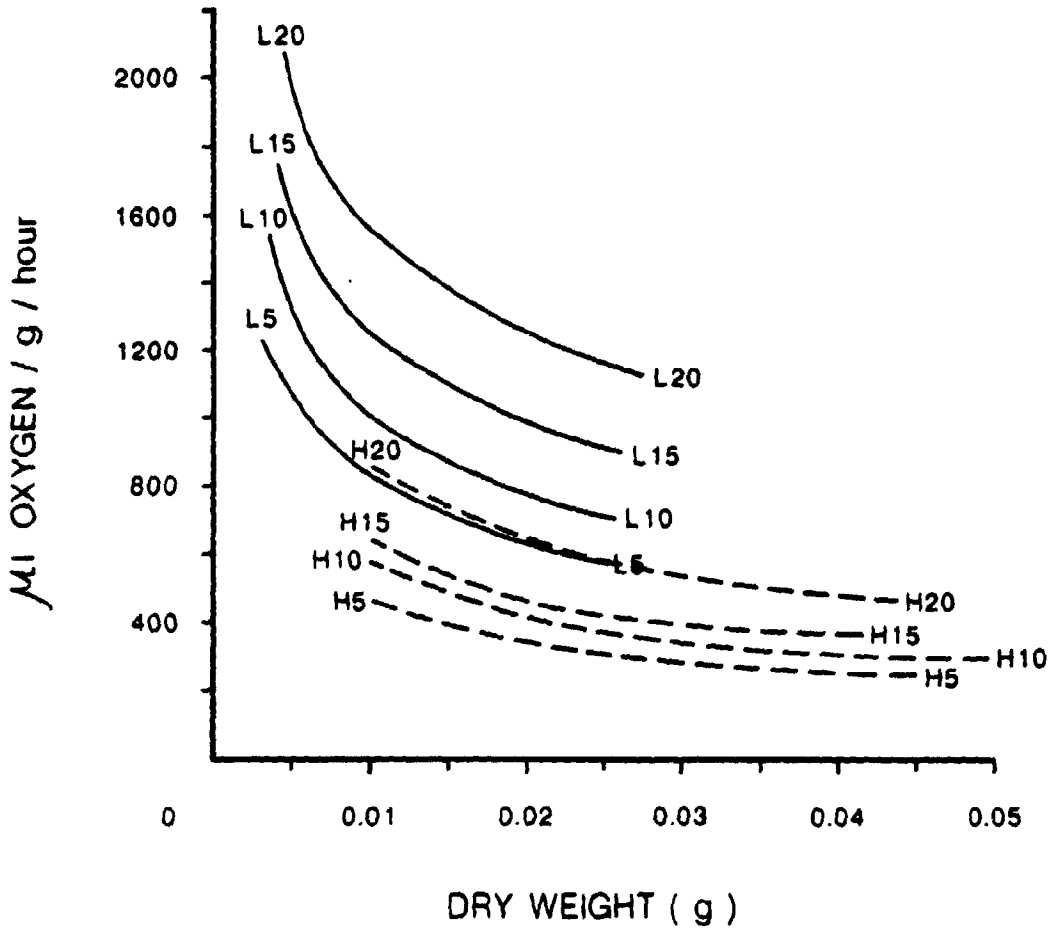
Figure 27. **Oxygen metabolic rate curves of high and low tide *M. balthica* at four temperatures.**

H5 = High tide level, 5° C; L5 = Low tide level, 5° C

H10 = High tide level, 10° C; L10 = Low tide level, 10° C

H15 = High tide level, 15° C; L15 = Low tide level, 15° C

H20 = High tide level, 20° C; L20 = Low tide level, 20° C.


L20
L15
L10
L5
H20
H15
H10
H5
2000
1600
1200
800
400
0
0.01
0.02
0.03
0.04
0.05
µl OXYGEN / g / hour
DRY WEIGHT (g)

series of oxygen metabolic rate curves that were generated for *Macoma* from both tide levels. The curves were drawn to cover the range of data used only. It can be seen that oxygen consumption per gram dry weight is inversely related to size. For clams of a given size and temperature, low tide *Macoma* consumed more oxygen than high tide ones. It should be noted that the metabolic curve for the high tide *Macoma* at 20° C is almost exactly the same as for the low tide *Macoma* at 5° C. These temperatures approximate the highest temperatures that they will experience in the field, at each tide level.

An examination of the D value of the clams used in this experiment showed that low tide clams were slightly more deficient in heterozygotes than high tide ones (Table 14). Although allelic frequencies of the three enzymes were generally similiar to those observed for the retrieved transplant clams (see Tables 6 and 10), significant deviations from the Hardy-Weinberg equilibrium were observed for both high and low tide clams at the PGM and PGI loci.

Table 15 shows the results of the three-way factorial analyses to examine the effects of degree of heterozygosity on the rate of oxygen uptake. The three-way interaction terms for both tide levels were not significant. In high tide clams, the degree of heterozygosity affected oxygen uptake rate differently in small and large animals ('lndrywt by numhet' term significant, $p<0.05$). Fig. 28 shows this interaction as clam length increases, the rate of oxygen consumption of the totally heterozygous clams (for the three loci examined) increased more than those of the other clams. All other two-way interaction terms for both tide levels were not significant (Table 16).

Table 14. Genetic parameters for three enzyme loci of *M. balthica* used in the oxygen consumption experiment. ($n_{High\ tide} = 200$, $n_{Low\ tide} = 200$)

p (H.W.) is the chance probability for Hardy-Weinberg equilibrium;
D = (#Heterozygotes observed - #Heterozygotes expected)/(#Heterozygotes expected)

Locus	Allele	Tide level	
		High	Low
		(Allele frequency)	
PGM	1	0.51	0.57
	2	0.49	0.45
	p (H.W.)	<0.001	<0.001
	D	-0.27	-0.30
MDH	1	0.51	0.52
	2	0.49	0.48
	p (H.W.)	>0.001	<0.001
	D	-0.23	-0.24
PGI	1	0.052	0.067
	2	0.528	0.523
	3	0.420	0.410
	p (H.W.)	<0.001	<0.001
	D	-0.20	-0.38

Table 15. Three-way factorial analysis of *M. balthica* (oxygen experiment) using only the significant main effects from previous analysis and incorporating number of heterozygous loci for three enzymes - saturated model, tide levels analysed separately. Lndrywt = Ln(dry weight of clam); Temp = temperature of experiment; Numhet = number of heterozygous loci for three enzymes per clam.

(a) High tide *M. balthica*

Source	df	F	p	
Lndrywt	1	0.02	0.8765	
Temp	1	3.07	0.0812	
Numhet	1	3.83	0.0518	
Lndrywt*Temp	1	1.29	0.2579	
Lndrywt*Numhet	1	3.85	0.0511	
Temp*Numhet	1	0.93	0.3366	
Lntotal*Temp*Numhet	1	0.80	0.3733	n.s.

(b) Low tide *M. balthica*

Source	df	F	p	
Lndrywt	1	0.03	0.8744	
Temp	1	2.62	0.1070	
Numhet	1	2.69	0.1028	
Lndrywt*Temp	1	1.72	0.1908	
Lndrywt*Numhet	1	2.65	0.1053	
Temp*Numhet	1	1.54	0.2163	
Lntotal*Temp*Numhet	1	1.46	0.2282	n.s.

Figure 28. Least squares regression lines of ln(ml of oxygen consumed) against ln (dry weight of clam) for *M. balthica* of varying heterozygosities for the three enzymes studied. 0 = Clams totally homozygous at all three enzyme loci; 1 = Clams heterozygous at 1 enzyme locus; 2 = Clams heterozygous at 2 enzyme loci and 3 = Clams totally heterozygous at three enzyme loci. Lines are drawn to cover the range of data actually used. The 95% confidence bands are shown.

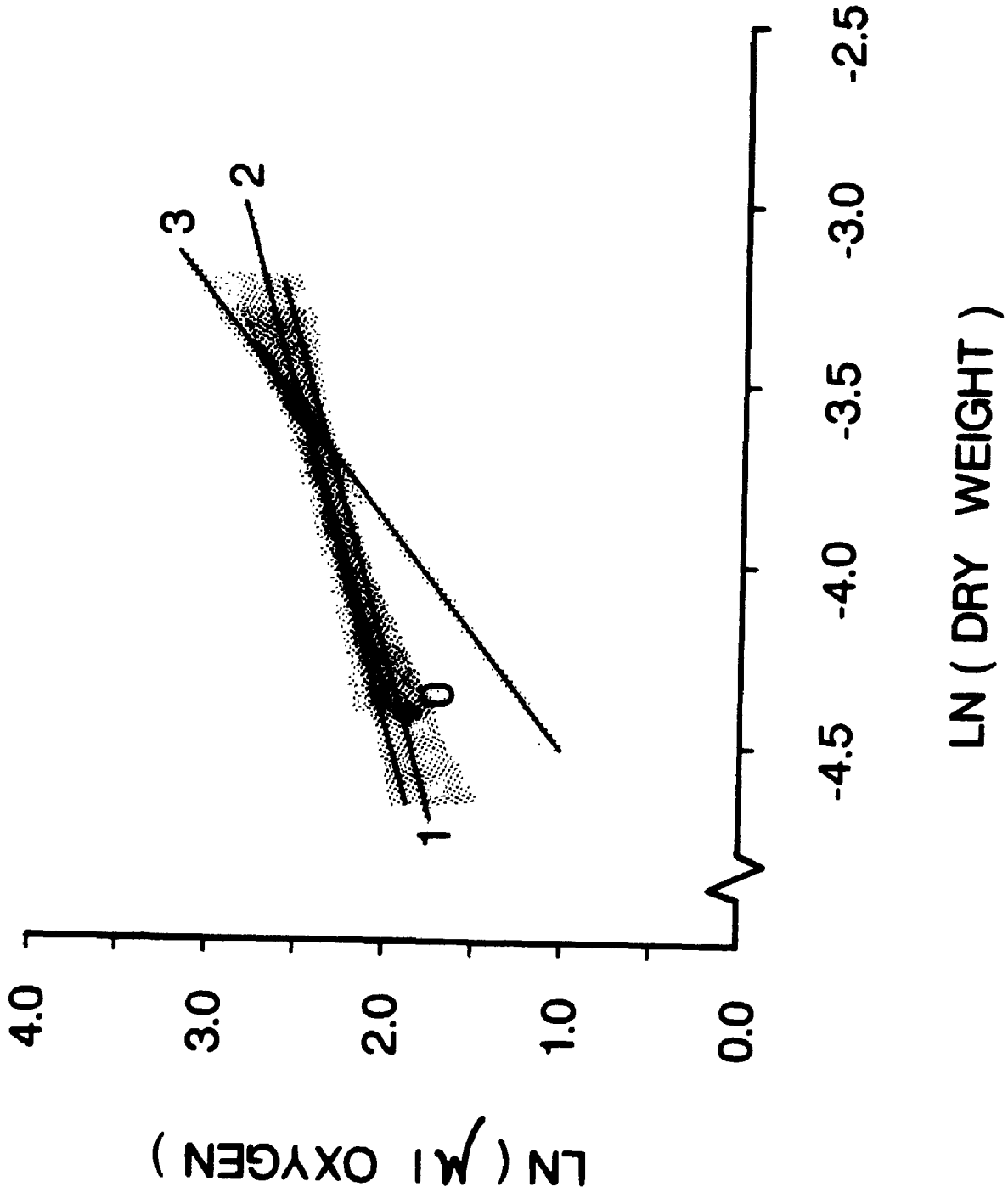

LN (µl OXYGEN)
4.0
3.0
2.0
1.0
0.0
-4.5
-4.0
-3.5
-3.0
-2.5
LN (DRY WEIGHT)
0
1
2
3

Table 16. Three-way factorial analysis of *M. balthica* (oxygen experiment) using only the significant main effects from previous analysis and incorporating number of heterozygous loci for three enzymes - without 3-way interaction, tide levels analysed separately. Lndrywt = Ln(dry weight of clam); Temp = temperature of experiment; Numhet = number of heterozygous loci for three enzymes per clam.

(a) High tide *M. balthica*

Source	df	F	p	
Lndrywt	1	1.99	0.1597	
Temp	1	3.56	0.0606	
Numhet	1	8.40	0.0042	
Lndrywt*Temp	1	0.51	0.4751	n.s.
Lndrywt*Numhet	1	8.90	0.0032	**
Temp*Numhet	1	0.75	0.3890	n.s.

(b) Low tide *M. balthica*

Source	df	F	p	
Lndrywt	1	2.96	0.0868	
Temp	1	1.25	0.2655	
Numhet	1	1.78	0.1833	
Lndrywt*Temp	1	0.29	0.5889	n.s.
Lndrywt*Numhet	1	1.75	0.1876	n.s.
Temp*Numhet	1	0.24	0.6240	n.s.

At the low tide level, clam heterozygosity had no effect on the rate of oxygen consumption whereas its size and the temperature treatment did; since 'numhet' effect was not significant ($p>0.05$) and the other two were highly significant ($p<0.01$ for both) (Table 17b). All three main effects as well as the 'lndrywt by numhet' interaction term were significant for the high tide *Macoma* (Table 17a).

The ANCOVA comparing rate of oxygen uptake among the four temperatures with weight of the clams as covariates are given in Table 18. No significant difference in rates of oxygen uptakes between homozygotes and heterozygotes was found for the various temperatures by enzyme locus combination. The only exception was at the MDH locus (low tide clams, 10^{0} C) where the homozygotes consumed less oxygen than the heterozygotes (Table 18b, Fig. 29). However, only 0.05% of the variance was explained by the regression for the homozygotes (see Table 18b).

Table 19 summarises the GLM analyses using the significant main effects found in the previous analyses with the addition of presence or absence of specified alleles of the three enzymes studied. For PGM, all 3-way and 2-way interactions involving alleles 1 and 2 were not significant for both high and low tide clams. Also the presence or absence of these alleles did not affect the rate of oxygen uptake significantly. Similarly, for PGI, all interaction terms were not significant for the 3 alleles and the presence or absence of these alleles makes no significance difference to the rate of oxygen consumption. In MDH-1, however, oxygen uptake is influenced by the presence/absence of the allele in a way that differs with the length of the clam. This was observed for the high tide clams only ($p<0.01$).

Table 17. Three-way factorial analysis of *M. balthica* (oxygen experiment) using only the significant main effects from previous analysis and incorporating number of heterozygous loci for three enzymes - without 2-way interactions, tide levels analysed separately. Lndrywt = Ln(dry weight of clam); Temp = temperature of experiment; Numhet = number of heterozygous loci for three enzymes per clam.

(a) High tide *M. balthica*

Source	df	F	p	
Lndrywt	1	8.86	0.0033	**
Temp	1	161.36	0.0001	**
Numhet	1	8.07	0.0050	**
Lndrywt*Numhet	1	9.02	0.0030	**

(b) Low tide *M. balthica*

Source	df	F	p	
Lndrywt	1	62.18	0.0001	**
Temp	1	35.76	0.0001	**
Numhet	1	0.34	0.5618	n.s.

Figure 29. **Least squares regression lines of ln(ml of oxygen consumed) against ln (dry weight of clam) for low tide *M. balthica* - homozygous and heterozygous at MDH locus. HOMO = homozygous clams; HET = heterozygous clams.**

(- - - = Homozygous clams; —— = Heterozygous clams). Lines are drawn to cover the range of data actually used. The 95% confidence bands are shown.

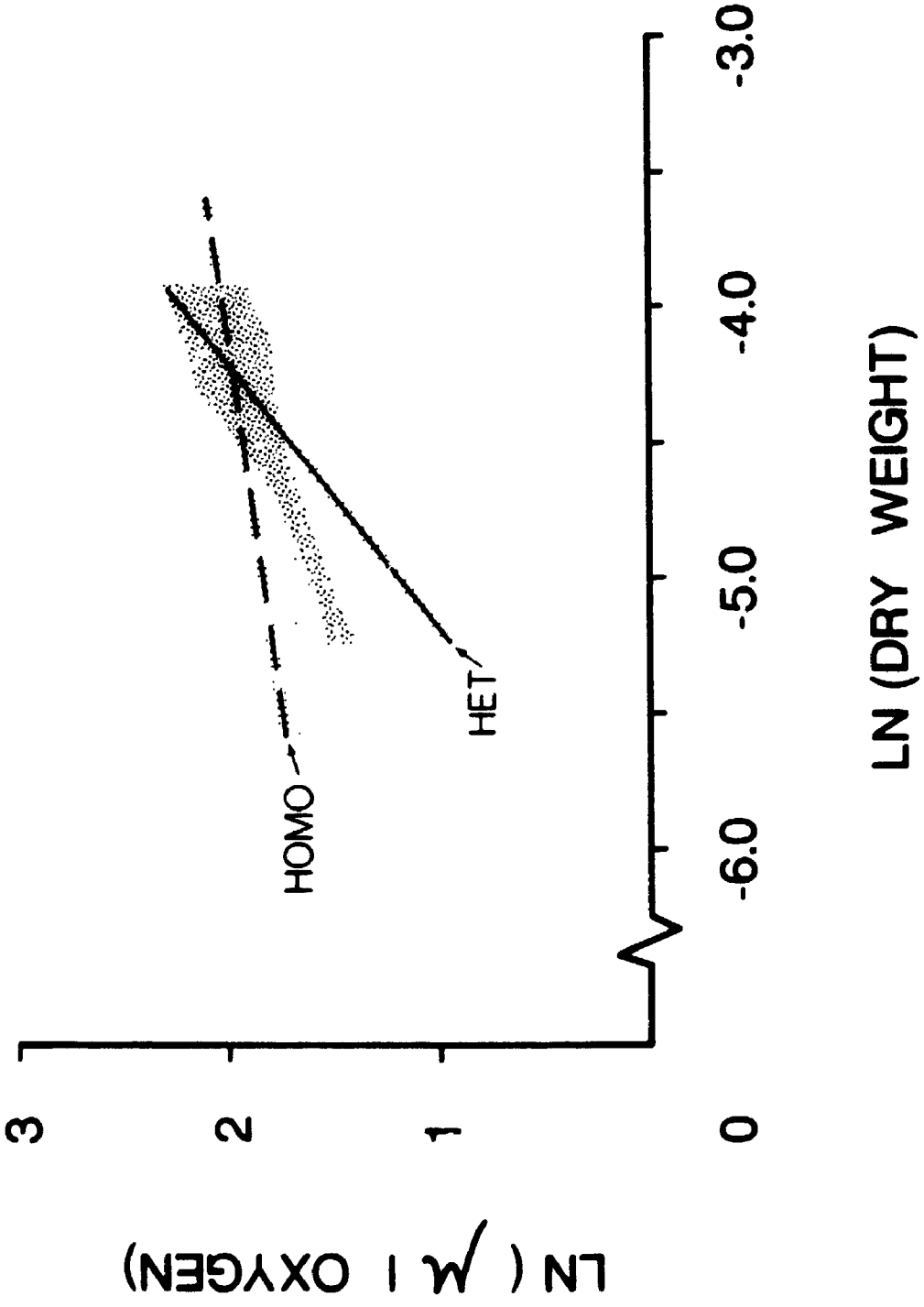

LN (μ) OXYGEN)
3
2
1
0
HOMO
HET
-6.0
-5.0
-4.0
-3.0
LN (DRY WEIGHT)

Table 18. (a) Comparison of slopes of the Walford plots (by ANCOVA) of *M. balthica* - homozygous versus heterozygous clams; (b) Walford plot equations of low tide homozygous and heterozygous clams for the MDH locus. $Ln(O_2)$ = Ln(volume of oxygen consumed per clam); Ln(DW) = Ln(dry weight of clam).

(a)

Tide level	Temperature (°C)	Enzyme locus PGM		Enzyme locus MDH p		Enzyme locus PGI	
High	5	0.2131	n.s.	0.2383	n.s.	0.6158	n.s.
	10	0.3712	n.s.	0.2837	n.s.	0.1084	n.s.
	15	0.7771	n.s.	0.8262	n.s.	0.1117	n.s.
	20	0.2544	n.s.	0.8786	n.s.	0.8386	n.s.
Low	5	0.7191	n.s.	0.3498	n.s.	0.2013	n.s.
	10	0.6090	n.s.	0.0203	n.s.	0.8682	n.s.
	15	0.5728	n.s.	0.8746	n.s.	0.2486	n.s.
	20	0.8118	n.s.	0.4421	n.s.	0.2390	n.s.

(b)

	n	Equation	r^2
Homozygotes	30	$Ln(O_2) = 2.74 + 0.18\ Ln(DW)$	0.005
Heterozygotes	20	$Ln(O_2) = 6.43 + 1.05\ Ln(DW)$	0.37

Table 19. Sytematic examination of effects in GLM analysis (oxygen experiment) involving Ln(dry weight), temperature and presence or absence of allele of 3 enzymes.

Tide level	Effect tested	Allele 1 p	Allele 2
(a) PGM			
High	LnDW*Temp*PGM	0.479 n.s.	0.964 n.s.
	LnDW*Temp	0.658 n.s.	0.189 n.s.
	LnDW*PGM	0.204 n.s.	0.436 n.s.
	Temp*PGM	0.529 n.s.	0.164 n.s.
	LnDW	0.0001 **	0.0001 **
	Temp	0.0001 **	0.0001 **
	PGM	0.143 n.s.	0.516 n.s.
Low	LnDW*Temp*PGM	0.395 n.s.	0.344 n.s.
	LnDW*Temp	0.692 n.s.	0.628 n.s.
	LnDW*PGM	0.478 n.s.	0.834 n.s.
	Temp*PGM	0.807 n.s.	0.870 n.s.
	LnDW	0.0001 **	0.0001 **
	Temp	0.0001 **	0.0001 **
	PGM	0.389 n.s.	0.849 n.s.
(b) MDH			
High	LnDW*Temp*MDH	0.465 n.s.	0.393 n.s.
	LnDW*Temp	0.326 n.s.	0.345 n.s.
	LnDW*MDH	0.012 *	0.713 n.s.
	Temp*MDH	0.503 n.s.	0.581 n.s.
	LnDW	0.0001 **	0.0001 **
	Temp	0.0001 **	0.0001 **
	MDH	0.014 *	0.103 n.s.
	LnDW*MDH	0.014 *	----------
Low	LnDW*Temp*MDH	0.968 n.s.	0.700 n.s.
	LnDW*Temp	0.687 n.s.	0.697 n.s.
	LnDW*MDH	0.678 n.s.	0.017 *
	Temp*MDH	0.562 n.s.	0.382 n.s.
	LnDW	0.0001 **	0.0001 **
	Temp	0.0001 **	0.0001 **
	MDH	0.209 n.s.	0.016 *
	LnDW*MDH	----------	0.017 *

Table 19. (cont'd)

Tide level	Effect tested	Allele 1	Allele 2	Allele 3
			p	
(c) PGI				
High	LnDW*Temp*PGI	0.068 n.s.	0.996 n.s.	0.993 n.s.
	LnDW*Temp	0.316 n.s.	0.395 n.s.	0.317 n.s.
	LnDW*PGI	0.251 n.s.	0.250 n.s.	0.786 n.s.
	Temp*PGI	0.414 n.s.	0.378 n.s.	0.462 n.s.
	LnDW	0.0001 **	0.0001 **	0.0001 **
	Temp	0.0001 **	0.0001 **	0.0001 **
	PGI	0.980 n.s.	0.840 n.s.	0.358 n.s.
Low	LnDW*Temp*PGI	0.167 n.s.	0.879 n.s.	0.060 n.s.
	LnDW*Temp	0.503 n.s.	0.697 n.s.	0.768 n.s.
	LnDW*PGI	0.205 n.s.	0.184 n.s.	0.360 n.s.
	Temp*PGI	0.877 n.s.	0.894 n.s.	0.731 n.s.
	LnDW	0.0001 **	0.0001 **	0.0001 **
	Temp	0.0001 **	0.0001 **	0.0001 **
	PGI	0.281 n.s.	0.657 n.s.	0.362 n.s.

Fig. 30 shows this interaction. For MDH-2, it was the low tide clams that had a significant 'ln(dry weight) by presence/absence MDH allele' interaction term. Fig. 31 shows this relationship.

3.4.2 Freezing tolerance

Results of the 2*8 contingency table analysis are given in Table 20. The interaction term of 'tide by duration' was significant ($p<0.01$). Thus, the tolerance of the clams to cold exposure was different for high and low tide *Macoma*. This interaction of tide level and duration of cold exposure can be seen in Fig. 32. High and low tide clams were analysed separately because of this interaction.

Table 21 presents the genetic parameters calculated for the clams used for the freezing tolerance experiment. A general deficiency of heterozygotes was observed for both high and low tide clams. Allelic frequencies more often than not, deviated from the Hardy-Weinberg equilibrium.

All interaction terms for both high and low tide clams were not significant (Tables 22-25). This means that survival of the clams varies consistently across these effects (whichever ones were specified in the interaction terms). At the high tide level, survival of *Macoma* was not significantly influenced by its size, degree of heterozygosity (at the three enzymes) nor by the presence or absence of parasites ($p>0.05$ for all 3 variables). The only significant influence on survival was the duration of freezing treatment ($p<0.01$); more clams lived when the treatment time was shorter.

For the low tide clams, however, two of the main effects were significant in affecting survival: the presence or absence of parasites

Figure 30. Least squares regression lines of ln(ml of oxygen consumed) against ln (dry weight of clam) for *M. balthica* from the 1.0 m above mean low water level, with and without the MDH-1 allele. [(+) = clams with MDH allele and (-) = clams without the MDH allele]. Lines are drawn to cover the range of data actually used. The 95% confidence bands are shown.

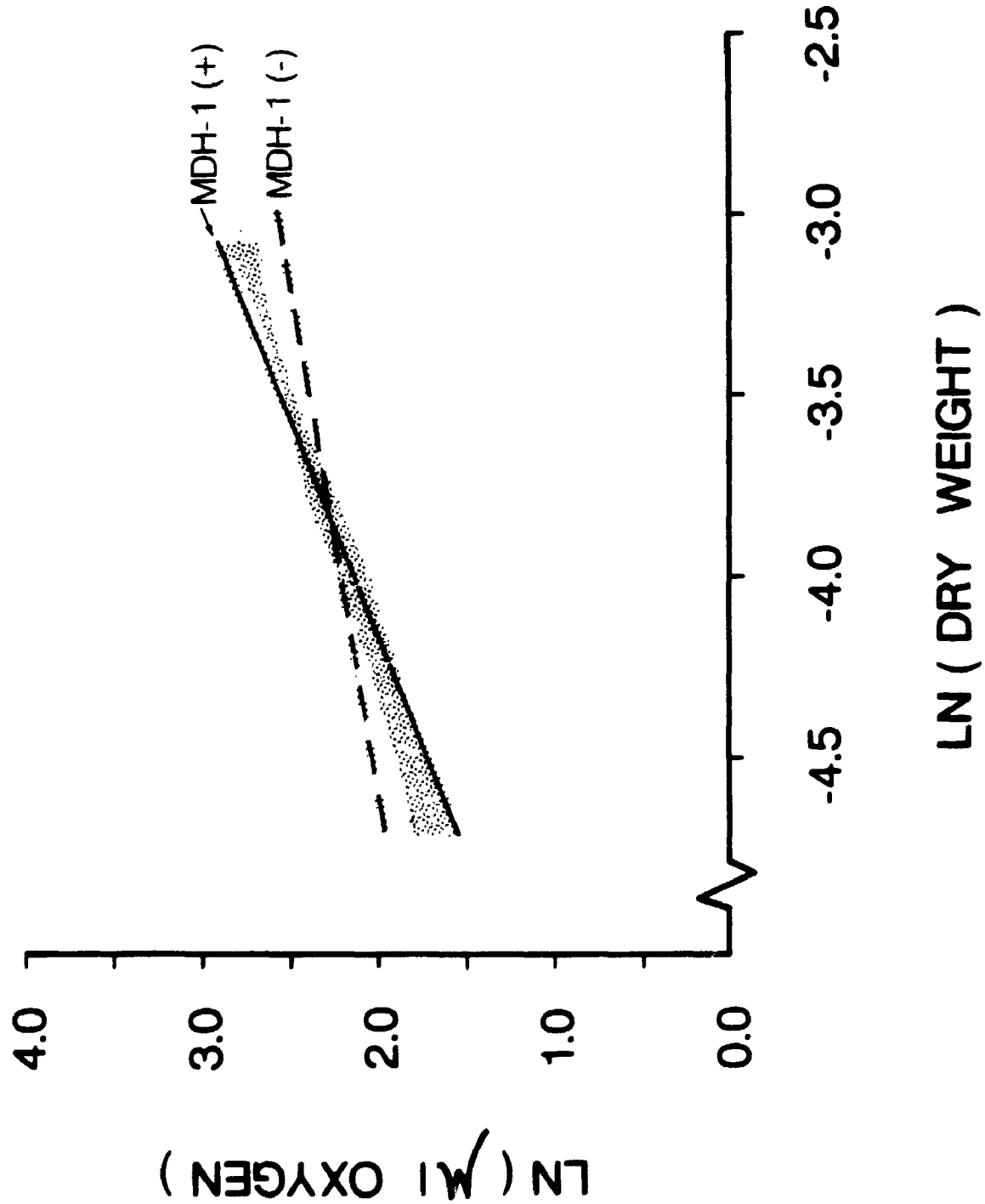

MDH-1 (+)
MDH-1 (-)
4.0
3.0
2.0
1.0
0.0
LN (µl OXYGEN)
-4.5
-4.0
-3.5
-3.0
-2.5
LN (DRY WEIGHT)

Figure 31. Least squares regression lines of ln(ml of oxygen consumed) against ln (dry weight of clam) for *M. balthica* from mean low water level, with and without the MDH-2 allele. [(+) = clams with MDH allele and (-) = clams without the MDH allele]. Lines are drawn to cover the range of data actually used. The 95% confidence bands are shown.

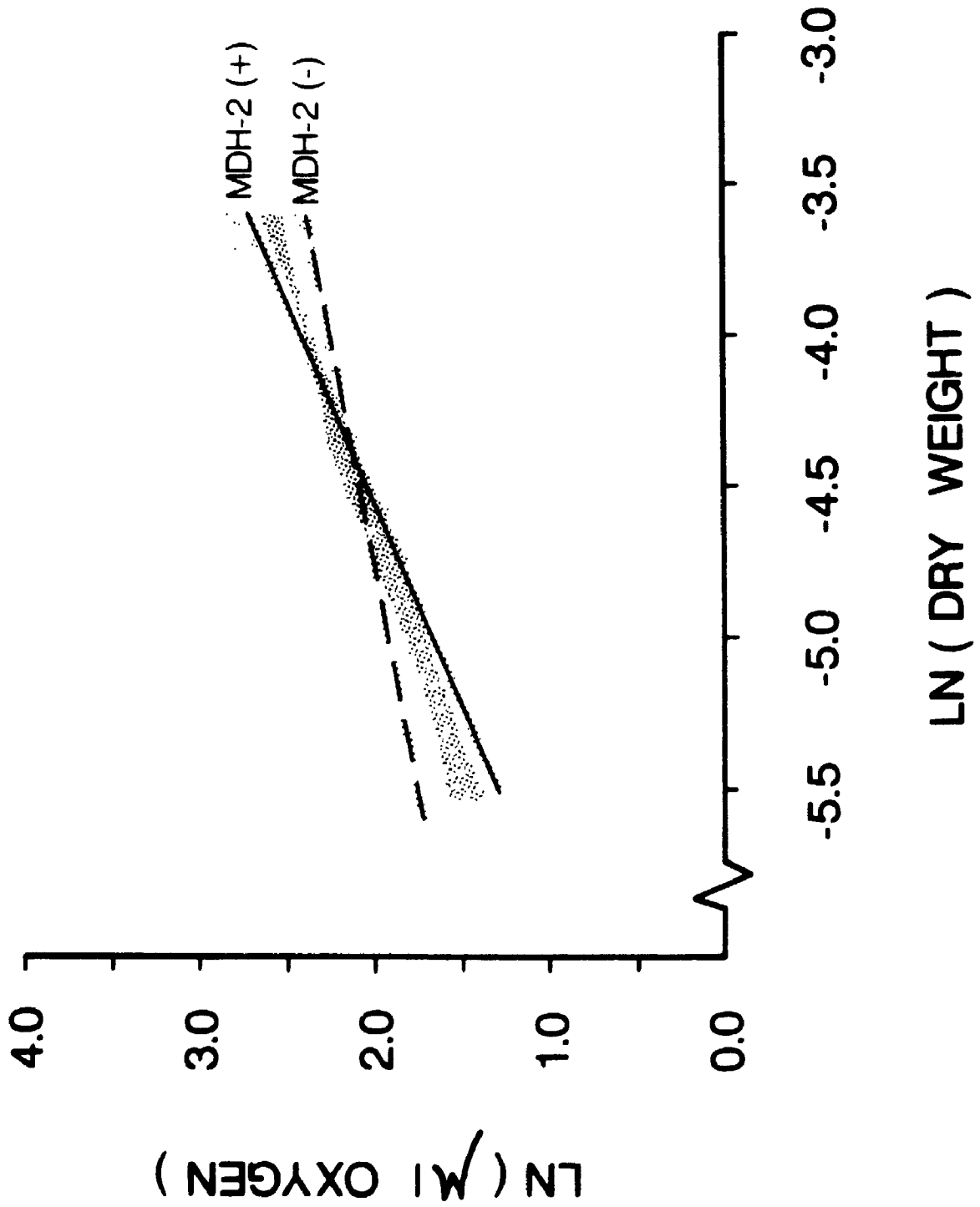

MDH-2 (+)
MDH-2 (-)
4.0
3.0
2.0
1.0
0.0
LN (µl OXYGEN)
-5.5
-5.0
-4.5
-4.0
-3.5
-3.0
LN (DRY WEIGHT)

Figure 32. Percentage survival of high and low tide *M. balthica* over a duration of six hours of freezing exposure.

(◇---◇ : High tide level; ●- - -● : Low tide level)

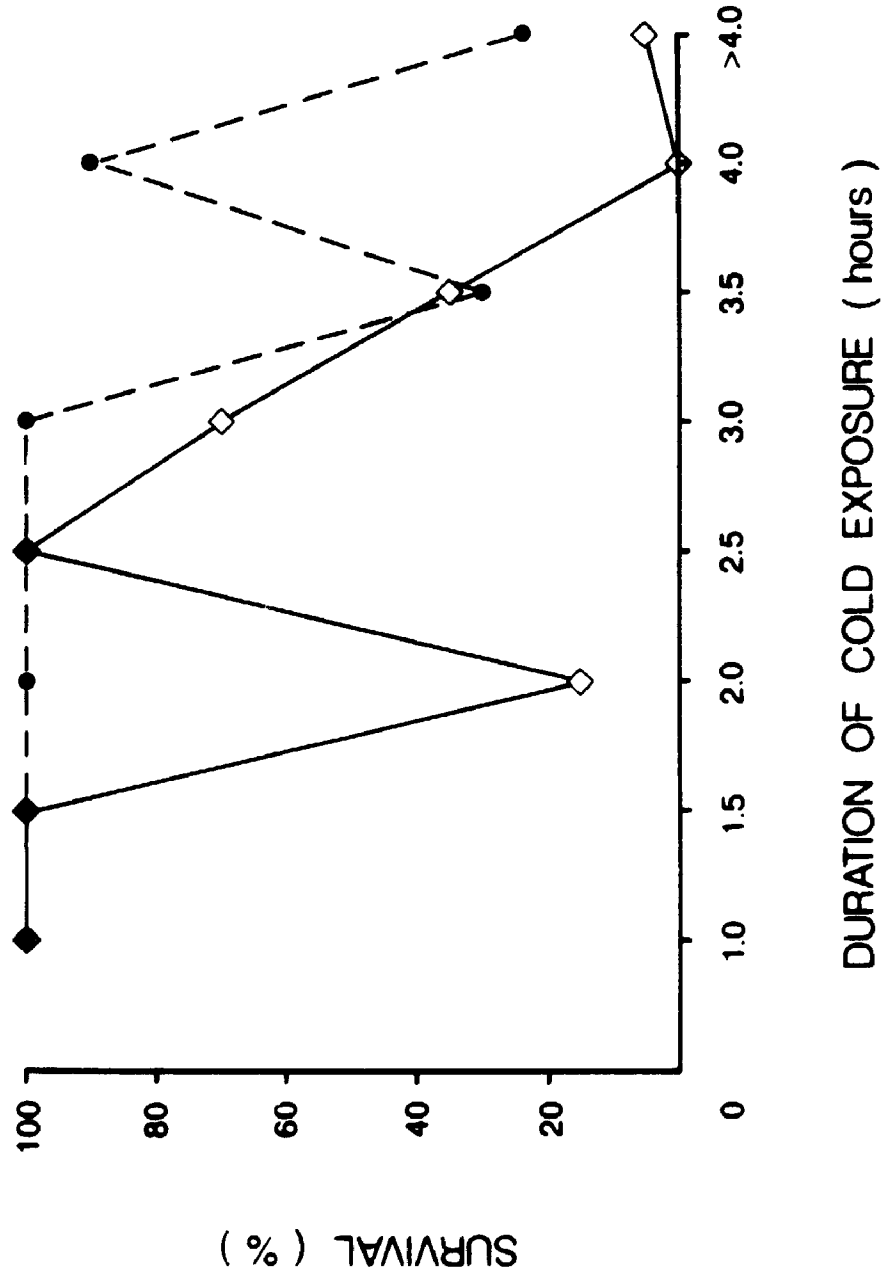

SURVIVAL (%)
100
80
60
40
20
0
1.0
1.5
2.0
2.5
3.0
3.5
4.0
>4.0
DURATION OF COLD EXPOSURE (hours)

Table 20. Summary of the results of the 2*8 contingency table analysis for the freezing experiment.

Source	df	χ^2	p	
Intercept	1	24.50	0.0001	
Duration	7	94.88	0.0001	
Tide level	1	11.01	0.0009	
Duration*Tide level	7	22.52	0.0021	**

Table 21. Genetic parameters for three enzyme loci of *M. balthica* used in the freezing experiment. ($n_{High\ tide} = 220$, $n_{Low\ tide} = 220$)

p (H.W.) is the chance probability for Hardy-Weinberg equilibrium;
D = (#Heterozygotes observed - #Heterozygotes expected)/(#Heterozygotes expected)

Locus	Allele	Tide level	
		High	Low
		(Allele frequency)	
PGM	1	0.51	0.57
	2	0.49	0.43
	p (H.W.)	<0.001	<0.001
	D	-0.26	-0.27
MDH	1	0.50	0.51
	2	0.50	0.49
	p (H.W.)	>0.005	<0.001
	D	-0.18	-0.32
PGI	1	0.075	0.066
	2	0.516	0.534
	3	0.409	0.400
	p (H.W.)	<0.001	<0.001
	D	-0.66	-0.38

Table 22. ANOVA table for the log-linear analysis of the saturated model (freezing experiment): (a) High tide *M. balthica*; (b) Low tide *M. balthica*. Dur = Duration of freezing treatment; Sizecl = size class of clam; Totalcl = total class of parasites; Numhet = number of heterozygous loci of three enzymes per clam.

Source	df	χ^2	p	
(a)				
Intercept	1	0.45	0.5030	
Dur	2	5.23	0.0733	
Sizecl	1	1.36	0.2436	
Totalcl	1	1.18	0.2781	
Numhet	3	1.16	0.7630	
Dur*Sizecl	2	0.84	0.6557	
Dur*Totalcl	2	0.80	0.6688	
Dur*Numhet	6	1.50	0.9595	
Sizecl*Totalcl	1	1.18	0.2781	
Sizecl*Numhet	3	1.46	0.6912	
Totalcl*Numhet	3	0.78	0.8534	
Dur*Sizecl*Totalcl	2	1.42	0.4909	
Dur*Sizecl*Numhet	6	3.97	0.6802	
Dur*Totalcl*Numhet	4	1.57	0.8133	
Sizecl*Totalcl*Numhet	2	1.64	0.4397	
Dur*Sizecl*Totalcl*Numhet	1	0.81	0.6663	n.s.
(b)				
Intercept	1	1.82	0.1768	
Dur	2	7.49	0.0236	
Sizecl	1	0.06	0.8117	
Totalcl	1	0.03	0.8590	
Numhet	3	0.49	0.9210	
Dur*Sizecl	2	0.11	0.9472	
Dur*Totalcl	2	0.18	0.9118	
Dur*Numhet	6	1.50	0.9597	
Sizecl*Totalcl	1	0.01	0.9283	
Sizecl*Numhet	3	0.11	0.9912	
Totalcl*Numhet	2	0.60	0.7401	
Dur*Sizecl*Totalcl	2	0.20	0.9059	
Dur*Sizecl*Numhet	5	0.43	0.9946	
Dur*Totalcl*Numhet	4	0.86	0.9297	
Sizecl*Totalcl*Numhet	2	0.67	0.7156	
Dur*Sizecl*Totalcl*Numhet	3	0.78	0.8544	n.s.

Table 23. ANOVA table for the log-linear analysis without the 4-way interaction term (freezing experiment): (a) High tide *M. balthica*; (b) Low tide *M. balthica*. Dur = Duration of freezing treatment; Sizecl = size class of clam; Totalcl = total class of parasites; Numhet = number of heterozygous loci of three enzymes per clam.

Source	df	χ^2	p	
(a)				
Intercept	1	0.00	0.9754	
Dur	2	4.79	0.0911	
Sizecl	1	0.65	0.4191	
Totalcl	1	0.53	0.4677	
Numhet	3	0.53	0.9133	
Dur*Sizecl	2	0.33	0.8495	
Dur*Totalcl	2	0.26	0.8800	
Dur*Numhet	6	0.96	0.9870	
Sizecl*Totalcl	1	0.44	0.5048	
Sizecl*Numhet	3	0.81	0.8476	
Totalcl*Numhet	3	0.14	0.9870	
Dur*Sizecl*Totalcl	2	1.70	0.4271	n.s.
Dur*Sizecl*Numhet	6	4.03	0.6732	n.s.
Dur*Totalcl*Numhet	4	1.15	0.8869	n.s.
Sizecl*Totalcl*Numhet	2	0.86	0.6500	n.s.
(b)				
Intercept	1	2.24	0.1341	
Dur	2	20.17	0.0001	
Sizecl	1	0.01	0.9238	
Totalcl	1	0.03	0.8609	
Numhet	3	0.43	0.9339	
Dur*Sizecl	2	0.02	0.9894	
Dur*Totalcl	2	0.35	0.8401	
Dur*Numhet	6	1.86	0.9319	
Sizecl*Totalcl	1	0.12	0.7287	
Sizecl*Numhet	3	0.06	0.9966	
Totalcl*Numhet	2	0.54	0.7632	
Dur*Sizecl*Totalcl	2	0.85	0.6527	n.s.
Dur*Sizecl*Numhet	5	0.39	0.9957	n.s.
Dur*Totalcl*Numhet	4	1.02	0.9064	n.s.
Sizecl*Totalcl*Numhet	2	0.92	0.6318	n.s.

Table 24. ANOVA table for the log-linear analysis without the 4-way interaction term and all 3-way interaction terms (freezing experiment): (a) High tide *M. balthica*; (b) Low tide *M. balthica*. Dur = Duration of freezing treatment; Sizecl = size class of clam; Totalcl = total class of parasites; Numhet = number of heterozygous loci of three enzymes per clam.

Source	df	χ^2	p	
(a)				
Intercept	1	0.15	0.6975	
Dur	2	10.37	0.0056	
Sizecl	1	0.69	0.4058	
Totalcl	1	0.16	0.6893	
Numhet	3	0.98	0.8062	
Dur*Sizecl	2	0.50	0.7803	n.s.
Dur*Totalcl	2	0.74	0.6896	n.s.
Dur*Numhet	6	2.10	0.9098	n.s.
Sizecl*Totalcl	1	0.63	0.4263	n.s.
Sizecl*Numhet	3	0.32	0.9567	n.s.
Totalcl*Numhet	3	0.10	0.9922	n.s.
(b)				
Intercept	1	5.41	0.0201	
Dur	2	28.45	0.0001	
Sizecl	1	0.49	0.4857	
Totalcl	1	0.00	0.9601	
Numhet	3	0.36	0.9490	
Dur*Sizecl	2	0.93	0.6291	n.s.
Dur*Totalcl	2	0.21	0.9016	n.s.
Dur*Numhet	6	1.40	0.9658	n.s.
Sizecl*Totalcl	1	0.11	0.7390	n.s.
Sizecl*Numhet	3	0.55	0.9087	n.s.
Totalcl*Numhet	2	1.46	0.4815	n.s.

Table 25. ANOVA table for the log-linear analysis with main effects only (freezing experiment): (a) High tide *M. balthica*; (b) Low tide *M. balthica*; (c) Low tide *M. balthica* with significant effects. Dur = Duration of freezing treatment; Sizecl = size class of clam; Totalcl = total class of parasites; Numhet = number of heterozygous loci of three enzymes per clam.

Source	df	χ^2	p	
(a)				
Intercept	1	0.45	0.5030	
Duration	2	29.91	0.0001	**
Size class	1	0.32	0.5715	n.s.
Total class	1	0.68	0.4092	n.s.
Numhet	3	1.45	0.6941	n.s.
(b)				
Intercept	1	5.81	0.0160	
Duration	2	53.03	0.0001	**
Size class	1	0.03	0.8662	n.s.
Total class	1	3.81	0.0509	
Numhet	3	0.19	0.9794	n.s.
(c)				
Intercept	1	0.00	0.9870	
Duration	2	43.22	0.0001	**
Total class	1	6.51	0.0107	*

as well as the duration of freezing treatment. Non-parasitized *Macoma* survived better than infected ones when subjected to increasing duration of freezing treatment.

For both MDH and PGI, survival of clams from both tide levels did not depend on whether clams were homozygous or heterozygous (Table 26b and c). As for PGM, the significance of the 2-way interaction term, 'sizeclass*hetPGM', in the high tide clams indicates that survival of homozygotes and heterozygotes is dependent on the size of the clam (Table 26a). Generally the results are very similar to the previous analysis with duration of cold exposure as the main factor that affects survival of the clams in the high tide region. For low tide clams, in addition to the length of cold treatment, there is a negative association between parasite load and survival.

3.4.3 Burrowing behaviour

Table 27 shows a summary of the results of the repeated measures ANOVA. The interaction term of 'tide level by temperature' was significant ($p<0.01$), indicating a different trend of response of high and low tide *Macoma* to the three temperatures. This can be seen in Fig. 33: low tide clams burrowed to a greater depth than high tide ones at 10° and 20° C. At 30° C, clams from both tide levels remained near the surface of the substrate.

Fig. 34 shows the relationship between length of the clams and burrowing depth at the three temperatures. For low tide *Macoma*, ANCOVA results showed that the slopes differed significantly ($p<0.01$). Burrowing depth generally decreased as length of shell

Table 26. Systematic examination of effects in log-linear analysis (freezing experiment) involving four variables. Dur = Duration of freezing treatment; Sizecl = size class of clam; Totalcl = total class of parasites; HetPGM = Homozygous or Heterozygous at PGM.

Tide level	Effect tested	df	χ^2	p	
(a) PGM					
High	Dur*Sizecl*Totalcl*HetPGM	1	0.00	0.999	n.s.
	Dur*Sizecl*Totalcl	2	0.00	1.000	n.s.
	Dur*Sizecl*HetPGM	2	0.66	0.717	n.s.
	Dur*Totalcl*HetPGM	2	0.00	1.000	n.s.
	Sizecl*Totalcl*HetPGM	1	0.00	0.999	n.s.
	Dur*Sizecl	2	1.06	0.589	n.s.
	Dur*Totalcl	2	0.00	0.999	n.s.
	Dur*HetPGM	2	0.08	0.963	n.s.
	Sizecl*Totalcl	1	0.00	0.987	n.s.
	Sizecl*HetPGM	1	5.32	0.021	*
	Totalcl*HetPGM	1	0.27	0.603	n.s.
	Dur	2	47.18	0.0001	**
	Sizecl	1	0.52	0.471	n.s.
	Totalcl	1	1.48	0.223	n.s.
	HetPGM	1	0.40	0.530	n.s.
	Sizecl*HetPGM	1	5.75	0.017	*
Low	Dur*Sizecl*Totalcl*HetPGM	1	0.00	1.000	n.s.
	Dur*Sizecl*Totalcl	2	0.08	0.962	n.s.
	Dur*Sizecl*HetPGM	2	4.69	0.096	n.s.
	Dur*Totalcl*HetPGM	2	0.00	1.000	n.s.
	Sizecl*Totalcl*HetPGM	1	0.68	0.411	n.s.
	Dur*Sizecl	2	2.03	0.362	n.s.
	Dur*Totalcl	2	0.01	0.994	n.s.
	Dur*HetPGM	2	0.96	0.620	n.s.
	Sizecl*Totalcl	1	0.37	0.542	n.s.
	Sizecl*HetPGM	1	2.77	0.096	n.s.
	Totalcl*HetPGM	1	0.07	0.793	n.s.
	Dur	2	43.11	0.0001	**
	Sizecl	1	0.41	0.521	n.s.
	Totalcl	1	6.20	0.013	*
	HetPGM	1	0.00	0.990	n.s.

Table 26. (cont'd)

Tide level	Effect tested	df	χ^2	p	
(b) MDH					
High	Dur*Sizecl*Totalcl*HetMDH	1	0.00	0.998	n.s.
	Dur*Sizecl*Totalcl	2	0.00	1.000	n.s.
	Dur*Sizecl*HetMDH	2	0.12	0.941	n.s.
	Dur*Totalcl*HetMDH	2	0.00	1.000	n.s.
	Sizecl*Totalcl*HetMDH	1	0.00	0.999	n.s.
	Dur*Sizecl	2	1.86	0.395	n.s.
	Dur*Totalcl	2	0.06	0.972	n.s.
	Dur*HetMDH	2	2.28	0.320	n.s.
	Sizecl*Totalcl	1	0.00	0.987	n.s.
	Sizecl*HetMDH	1	0.01	0.904	n.s.
	Totalcl*HetMDH	1	1.14	0.285	n.s.
	Dur	2	45.14	0.0001	**
	Sizecl	1	1.70	0.192	n.s.
	Totalcl	1	1.60	0.206	n.s.
	HetMDH	1	0.34	0.559	n.s.
Low	Dur*Sizecl*Totalcl*HetMDH	1	0.00	1.000	n.s.
	Dur*Sizecl*Totalcl	2	0.66	0.719	n.s.
	Dur*Sizecl*HetMDH	2	1.20	0.549	n.s.
	Dur*Totalcl*HetMDH	2	1.08	0.583	n.s.
	Sizecl*Totalcl*HetMDH	1	0.31	0.580	n.s.
	Dur*Sizecl	2	1.44	0.488	n.s.
	Dur*Totalcl	2	0.03	0.984	n.s.
	Dur*HetMDH	2	0.02	0.992	n.s.
	Sizecl*Totalcl	1	0.29	0.593	n.s.
	Sizecl*HetMDH	1	0.00	0.967	n.s.
	Totalcl*HetMDH	1	0.96	0.326	n.s.
	Dur	2	43.10	0.0001	**
	Sizecl	1	0.58	0.447	n.s.
	Totalcl	1	6.25	0.012	*
	HetMDH	1	0.64	0.424	n.s.

Table 26. (cont'd)

Tide level	Effect tested	df	χ^2	p	
(c) PGI					
High	Dur*Sizecl*Totalcl*HetPGI	1	0.00	0.998	n.s.
	Dur*Sizecl*Totalcl	2	0.00	1.000	n.s.
	Dur*Sizecl*HetPGI	2	0.15	0.926	n.s.
	Dur*Totalcl*HetPGI	2	0.00	0.999	n.s.
	Sizecl*Totalcl*HetPGI	1	0.00	0.999	n.s.
	Dur*Sizecl	2	1.77	0.413	n.s.
	Dur*Totalcl	2	0.00	0.999	n.s.
	Dur*HetPGI	2	5.76	0.060	n.s.
	Sizecl*Totalcl	1	0.00	0.987	n.s.
	Sizecl*HetPGI	1	0.01	0.934	n.s.
	Totalcl*HetPGI	1	0.96	0.328	n.s.
	Dur	2	44.81	0.0001	**
	Sizecl	1	1.53	0.216	n.s.
	Totalcl	1	1.75	0.186	n.s.
	HetPGI	1	0.68	0.409	n.s.
Low	Dur*Sizecl*Totalcl*HetPGI	1	0.00	0.999	n.s.
	Dur*Sizecl*Totalcl	2	0.64	0.725	n.s.
	Dur*Sizecl*HetPGI	2	0.75	0.686	n.s.
	Dur*Totalcl*HetPGI	2	0.00	1.000	n.s.
	Sizecl*Totalcl*HetPGI	1	0.79	0.374	n.s.
	Dur*Sizecl	2	1.73	0.422	n.s.
	Dur*Totalcl	2	0.04	0.979	n.s.
	Dur*HetPGI	2	0.90	0.638	n.s.
	Sizecl*Totalcl	1	0.25	0.617	n.s.
	Sizecl*HetPGI	1	0.0	0.867	n.s.
	Totalcl*HetPGI	1	0.58	0.447	n.s.
	Dur	2	42.91	0.0001	**
	Sizecl	1	0.40	0.525	n.s.
	Totalcl	1	6.21	0.013	*
	HetPGI	1	0.01	0.906	n.s.

Table 27. Repeated measures ANOVA table for burrowing experiment.

Source	df	F	p	
Tide level	1	69.90	0.0001	
Clam # (Tide level)	438	1.46	0.0001	
Temperature	2	288.17	0.0001	
Tide level*Temperature	2	24.40	0.0001	**
Error	876			
Total	1319			

Figure 33. **Plot of mean burrowing depth of *M. balthica* against temperature.**

(o—o : High tide level; ●- - -● : Low tide level). Standard error bars were calculated based on the EMS from the ANOVA table.

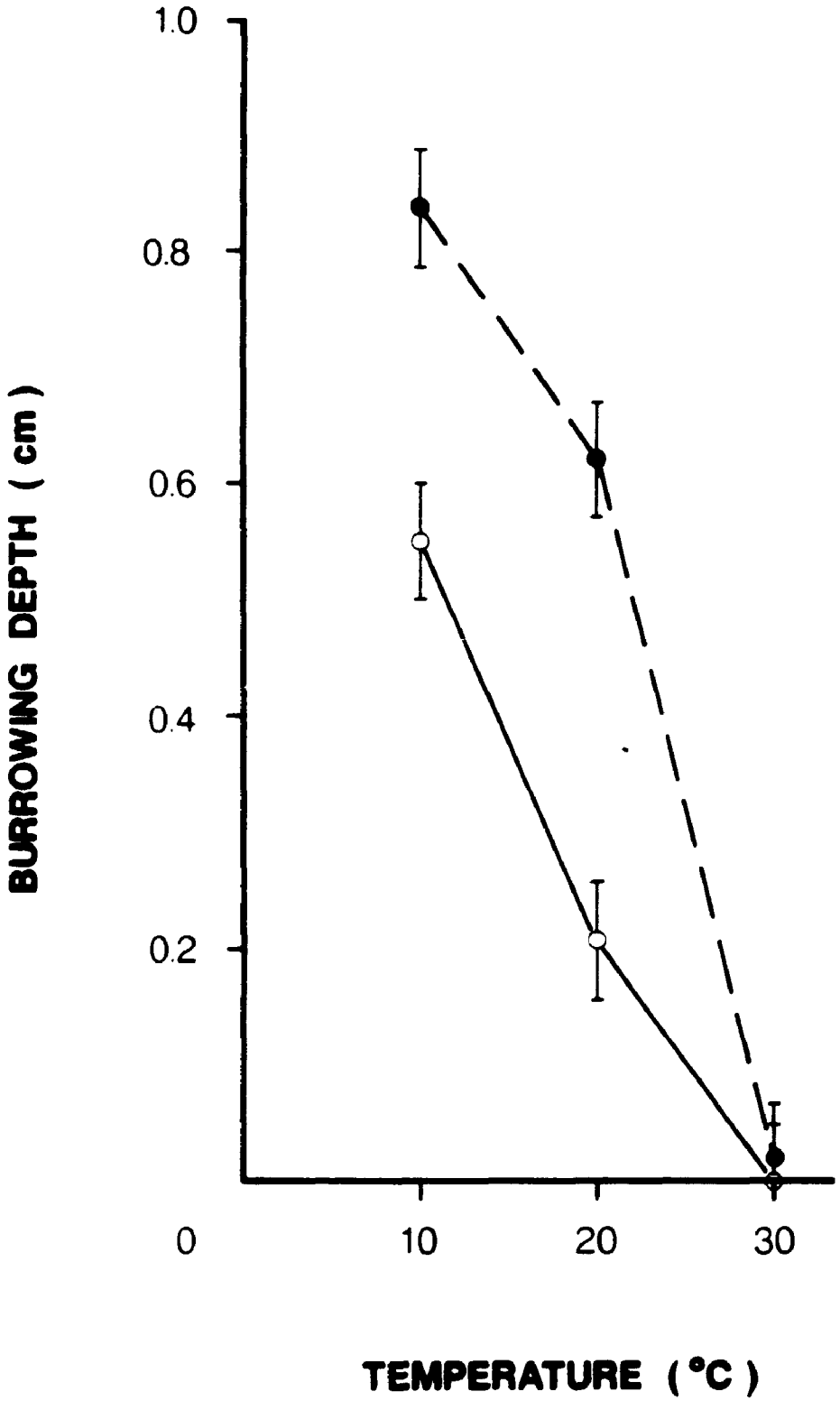
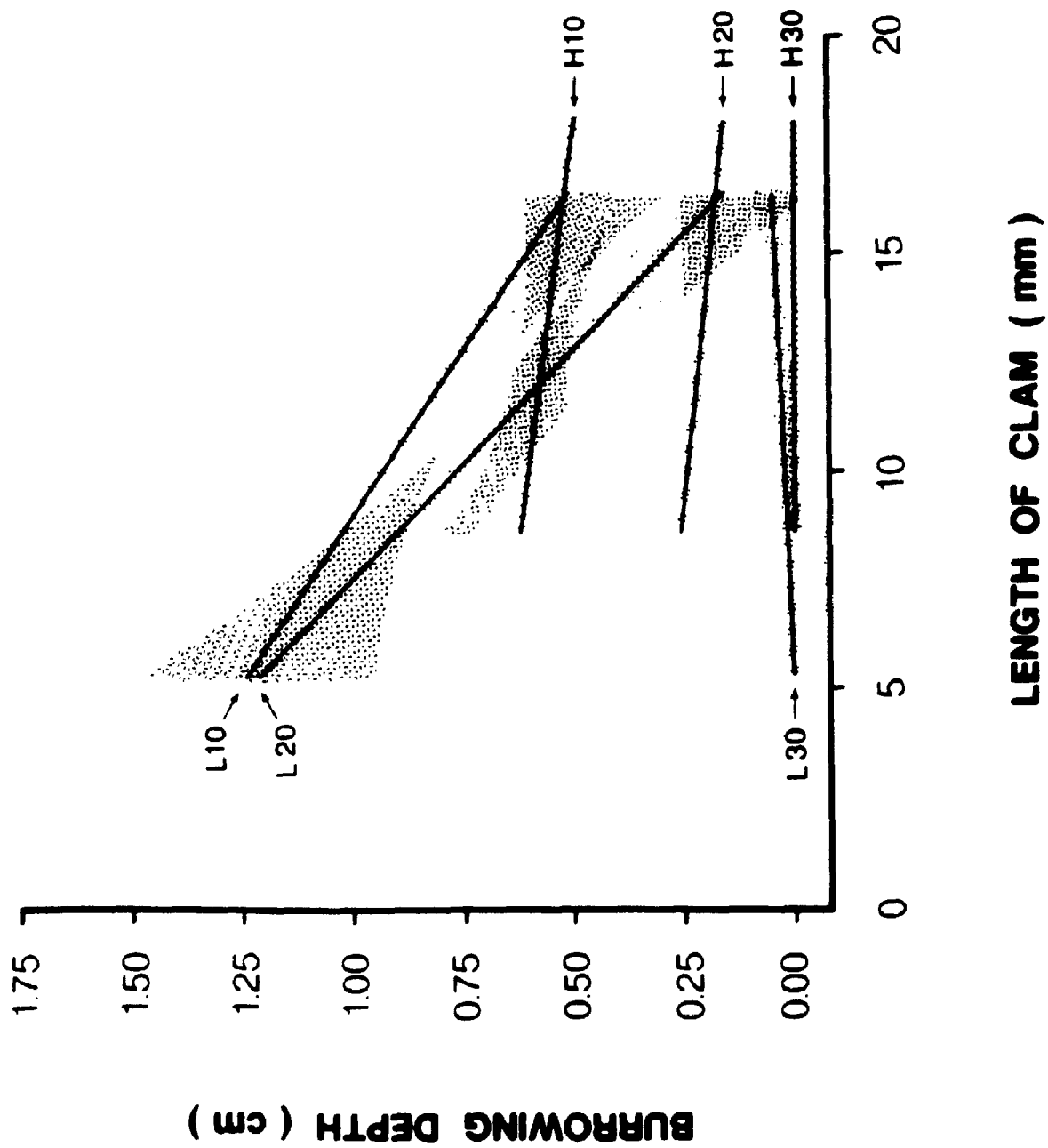

1.0
0.8
0.6
0.4
0.2
0
10
20
30
BURROWING DEPTH (cm)
TEMPERATURE (°C)

Figure 34. **Least squares regression of burrowing depth of *M. balthica* against length of clam. 95% confidence bands on the regressions are shown.**

H10 = High tide level, 10° C; L10 = Low tide level, 10° C

H20 = High tide level, 20° C; L20 = Low tide level, 20° C

H30 = High tide level, 30° C; L30 = Low tide level, 30° C.


BURROWING DEPTH (cm)
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
5
10
15
20
LENGTH OF CLAM (mm)
H10
H20
H30
L10
L20
L30

increased for 10° and 20° C whereas at 30° C, burrowing depth was independent of *Macoma* length and almost equal to 0 mm.

The behaviour of high tide *Macoma* was rather different. ANCOVA results showed that there was no significant difference in the slopes ($p>0.05$). From Fig. 34, it can be seen that at all three temperatures, burrowing depth of high tide clams was independent of clam length. *Macoma* were found to bury themselves deeper into the substrate at 10° than 20° C. At 30° C, clams did not burrow into the substrate at all.

Results of the ANCOVA on the effect of temperature on burrowing depth using number of metacercaria(e) per clam as covariate showed that slopes were not significantly different within the low tide clams ($p>0.05$). From Fig. 35, it can be seen that at 10° and 20° C, the more metacercariae the clam had, the closer it remained near the surface. At 30° C, however, the clams remained at or near the surface regardless of their respective metacercariae load.

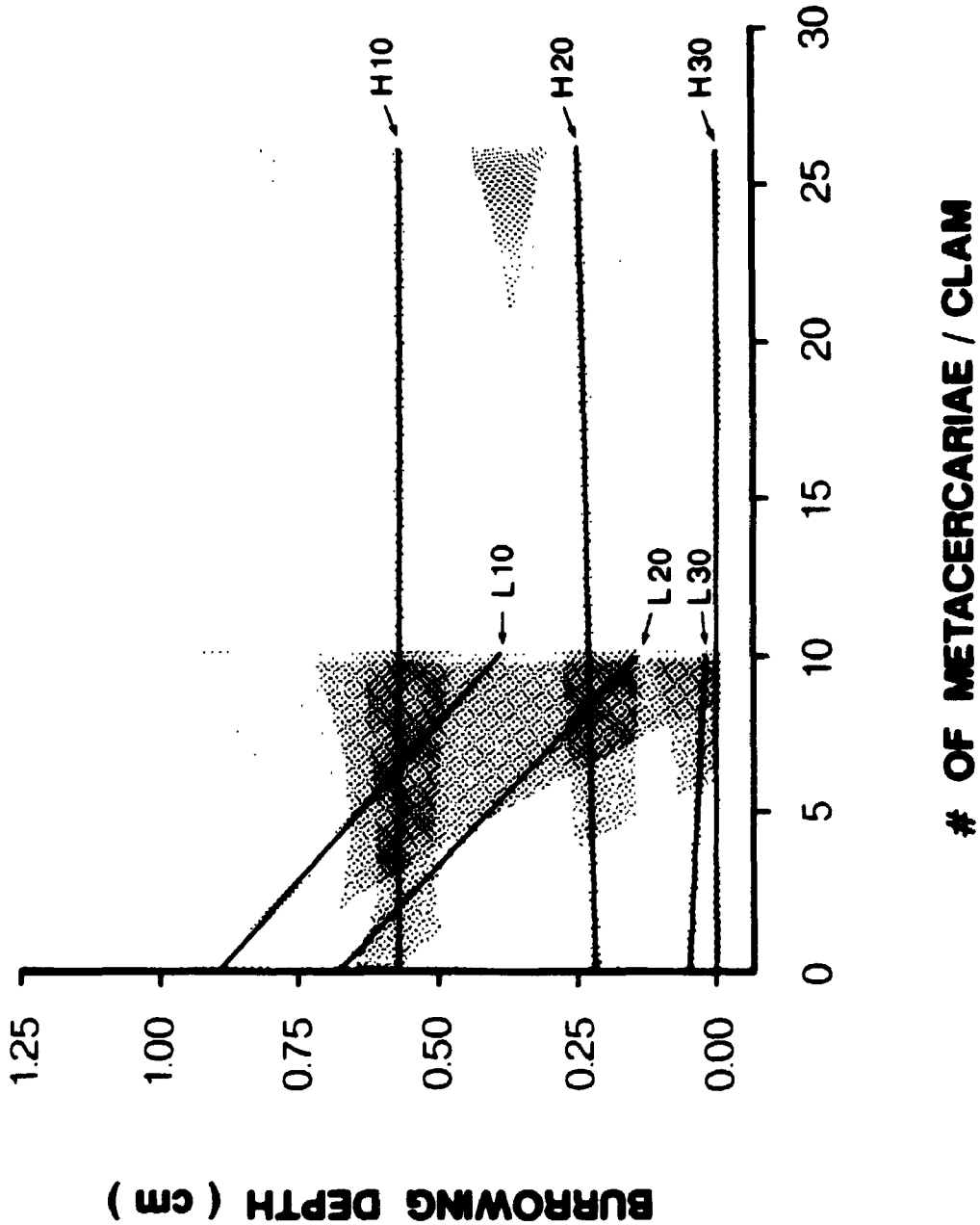
No significant difference was found among the slopes of the high tide clams using ANCOVA ($p>0.05$). For all three temperatures, burrowing depth was independent of the number of metacercaria per clam. *Macoma* burrowed the deepest at 10° C followed by those at 20° C. At 30° C, they remained inactive at the surface of the substrate.

Figure 35. Least squares regression of burrowing depth of *M. balthica* against total number of metacercariae per clam. 95% confidence bands on the regressions are shown.

H10 = High tide level, 10° C; L10 = Low tide level, 10° C

H20 = High tide level, 20° C; L20 = Low tide level, 20° C

H30 = High tide level, 30° C; L30 = Low tide level, 30° C.


BURROWING DEPTH (cm)
1.25
1.00
0.75
0.50
0.25
0.00
0
5
10
15
20
25
30
OF METACERCARIAE / CLAM
H10
H20
H30
L10
L20
L30

4. DISCUSSION

4.1 Geographical variation in growth rate with respect to tide levels

4.1.1 Growth rate

The faster growth rate in the upper intertidal zone (i.e. the "Churchill phenomenon") was not observed at sites in the St. Lawrence estuary, the upper and lower Bay of Fundy and James Bay (south of Cape Henrietta Marie). At these sites, *Macoma* either grew faster in the lower intertidal or there was no growth rate variation among the tide levels. These results agree with literature regarding the growth of *M. balthica* at various locations in North America, e.g. Baie des Roses, St. Lawrence estuary (Vincent et al. 1987, Vincent et al. 1989) and Europe, e.g. Dutch Wadden Sea (Beukema et al. 1977).

It can be inferred that the "Churchill phenomenon" is confined to sites along the Hudson Bay coast east of Churchill but disappears as one moves down the James Bay shoreline southwards to Moosonee. This could be due to the unique climate that exists in the south-west corner of Hudson Bay to Cape Henrietta Marie; where a warm, sometimes extremely hot, summer continental terrestrial climate adjoins an arctic marine environment. In contrast, shallow James Bay waters warm up substantially in summer. Table 28 shows the mean daily temperatures±S.D. for the 12 months of the year for weather stations situated near the sampling sites. It can be seen that the mean temperatures at Churchill and Winisk (both on the coast of Hudson Bay) for the winter and summer months are appreciably colder than those of the other weather stations. These

Table 28. Mean daily temperature ± S.D. (1951-1980) for weather stations near to sampling sites (from Environment Canada 1982). Temp.=Temperature (° C); $\bar{x}$ = Mean temperature; S.D.= Standard deviation.

For sampling sites at	Weather station	Temp.	Months Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.
Hudson Bay	Churchill	$\bar{x}$	-27.5	-25.9	-20.4	-10.1	-1.5	6.2	11.8	5.4	5.4	-1.5	-12.1	-22.2
		S.D.	2.6	3.2	2.9	2.5	2.2	1.5	1.3	1.2	1.7	2.2	2.7	3.4
Hudson Bay	Winisk	$\bar{x}$	-25.1	-24.0	-18.5	-8.5	0.9	7.2	11.0	10.8	6.7	0.6	-8.3	-19.2
		S.D.	2.8	2.3	3.3	1.5	1.1	1.3	1.2	1.6	1.2	2.1	2.1	1.9
James Bay	Moosonee	$\bar{x}$	-20.4	-18.5	-12.3	-2.3	5.7	11.9	15.3	14.3	9.5	4.1	-4.5	-16.0
		S.D.	2.8	3.2	3.3	2.1	1.8	1.9	1.2	1.6	1.7	2.1	2.1	3.4
St. Lawrence River	Cap Chat	$\bar{x}$	-10.1	-9.2	-4.5	1.5	6.9	12.9	16.0	14.8	10.8	5.6	0.3	-6.4
		S.D.	2.8	2.4	1.8	1.2	0.9	1.1	1.3	1.2	0.8	1.2	1.4	2.4
St. Lawrence River	Riviere du Loup	$\bar{x}$	-12.3	-11.2	-5.5	1.6	8.6	14.5	17.4	15.8	11.0	5.5	-0.8	-9.1
		S.D.	2.1	2.0	2.2	1.2	1.5	1.3	1.3	1.1	1.2	1.4	1.7	2.2
Upper Fundy	Sackville	$\bar{x}$	-6.8	-6.7	-2.0	3.6	9.3	14.6	17.9	17.4	13.5	8.4	2.8	-4.1
		S.D.	2.3	2.1	1.7	1.4	1.1	0.8	1.0	0.8	1.2	1.2	1.5	2.8
Lower Fundy	St. Andrews	$\bar{x}$	-6.0	-5.7	-1.1	4.6	10.0	14.7	17.7	17.5	13.8	8.9	3.5	-3.3
		S.D.	2.2	1.9	1.5	1.0	1.2	1.0	1.2	1.0	1.1	1.0	1.3	2.5
Minas Basin	Clifton	$\bar{x}$	-5.6	-6.6	-1.9	3.6	9.3	14.2	18.0	17.2	13.1	8.0	3.3	-3.1
		S.D.	1.4	2.3	2.2	1.2	2.0	0.9	1.3	0.3	0.9	1.1	1.7	2.1

temperature data show that Hudson Bay has a sub-arctic climate which is much colder than the continental weather at the other sampling sites.

4.1.2 Parasite-load

The percentage of parasitized clams at the St. Lawrence River and the Bay of Fundy sites was generally lower than those of the populations of *Macoma* in Europe (see Table 29). The only exceptions were at the St. Lawrence sites : CE and SF. Metacercaria loads were similarly much lower than those cited for the Southern Baltic and south-western Finland.

At the sampling sites where there was no growth rate variation between tide levels, there was no difference in the parasite loads of high and low tide *Macoma*. The percentage of clams parasitized and the mean number of parasites per clam were higher at CE, which was the only sampling site where a difference in clam growth rates was observed, and it was a faster growth rate at low tide level. Thus, there appears to be a positive relationship between parasite load and growth rate of the clams. Further evidence of this association was also found in the *Macoma* of Churchill (see Section 4.2).

Parasites have been known to enhance the growth of their hosts. This enhanced growth, often called "gigantism", is almost exclusively limited to molluscs (Cheng 1971). Most of the information about the influence of trematode parasitism on mollusc growth comes from gastropods. Parasitized *Hydrobia ulvae* and *Littorina neritoides* were found to grow faster than non-infected ones

Table 29. Comparison of parasite loads of *M. balthica* from other parts of the world: (a) *Lacunovermis macomae*; (b) *Parvatrema affinis*; (c) identity not given. MSL = Mean sea level.

Locality	Source	Depth (m)	n	% parasitized	#/clam
(a) *Lacunovermis macomae* metacercariae					
Fenham flats (England)	Lebour (1908)	?	?	15	2-3
Southern Baltic (Poland)	Markowski (1936)	25-35	?	34	1-30
Baltic coast (Germany)	Reimer (1962)	?	?	7-46	?
Wilhelmshaven (Germany)	Loos-Frank (1970)	?	?	>50	11
Baltic Sea (S.W. Finland)	Pekkarinen (1984)	5-40	?	1-11	30/valve
(b) *Parvatrema affinis* metacercariae					
Southern Baltic (Poland)	Markowski (1936)	?	?	0.7	?
Baltic coast (Germany)	Reimer (1962)	?	?	7	?
Dutch coast (The Netherlands)	Swennen (1969)	<MSL	?	0	?
	Swennen & Ching (1974)	>MSL	?	1-20	?
Gdansk Bay (Poland)	Wenne & Klusek (1985)	5-25	750	3.7	?
(c) Identity not given - sporocysts					
Wadden Sea (The Netherlands)	Hulscher (1973)	1.8m above MLW	1381	6-18	0
		1.65m above MLW	2207	4-34	?
		0.9m above	?	0	?
Fraser River (Canada)	McGreer (1983)	3m above MLLW	643	0	0
			95	0	0

(Rothschild and Rothschild 1939, Rothschild 1941a, Rothschild 1941b). Rothschild (1936, 1938) suggested that castration of the gonads by the parasites could be the cause of this phenomenon, but Sousa (1983) pointed out that the species of snails studied to date are all relatively short-lived ones. Most have annual life cycles and snails would have reached sexual maturity by the time of initial infection or shortly thereafter. Thus, the argument of channelling energy for reproduction to somatic growth would be unfeasible as the "growing" phase of the snails is almost over. Any excess energy channelled towards growth would not make an appreciable difference as the life span of the snail is short. However, *M. balthica* is a relatively long lived bivalve: those found at the St. Lawrence River and Bay of Fundy can live up to six years.

Furthermore, as mentioned earlier, Wenne and Klusek (1985) reported that *Parvatrema affinis* either caused partial or total disappearance of the gonads in *Macoma* from the Gdánsk Bay and Pekkarinen (1987a, b and c) observed parasitic castration by all three known trematode species in the *Macoma* from south-western Finland. In this study, it was also noted that the gonadal regions of the clams were often distended with sporocysts. Thus, enhanced growth as a result of parasitic castration could be possible.

4.2 Parasite-load of Churchill *Macoma balthica*

In the present study, not all of the *M. balthica* that made crawling tracks were parasitized as they were in studies by Hulscher (1973) and Swennen and Ching (1974). It should be noted that both of those studies

used the presence of sporocysts as the criterion for parasitism whereas metacercariae were used in this present study. Also the sample sizes used in Swennen and Ching's study were smaller than that of the present study (see Table 30). The number of crawling *Macoma* used in Hulscher's study was not given. However, present results do show that crawling behaviour increases with infection rate.

A significantly higher proportion of clams was parasitized higher up the shore at Churchill. This is similar to the findings of Swennen (1969), Hulscher (1973) and Swennen and Ching (1974). They found that parasitized *Macoma* were virtually confined to the upper regions of the shore at the Dutch Wadden Sea.

At Churchill, the number of metacercariae per clam was also much higher at the upper level than at the lower. Comparison with the studies cited above was not possible as information on the density of either sporocysts or metacercariae was not given. The difference in parasite load between the tide levels at Churchill could be explained by the increased exposure of the clams at the higher tidal level to shorebirds, the final host of the trematodes. The higher tidal level is exposed to air about 37% of the time whereas the MLW level is rarely exposed except at below average low tides. Therefore the intensity of predation on *Macoma* is probably higher, and the parasite can complete its life cycle with higher frequency. Similarly, infection of *Macoma* can occur more easily at the higher tide level because birds are more frequently feeding and defecating there during periods of ebb tide.

Behaviour of the primary host may also be relevant. *Haematopus ostralegus*, the oystercatcher, was observed in the field by Hulscher (1982) to reject *Macoma* that were infected with *Parvatrema affinis* sporocysts.

Table 30. Percentage of *M. balthica* parasitized with *Parvatrema affinis* sporocysts on the Dutch coast (from Swennen and Ching 1974).

Site	Behaviour	n	% parasitized
Terschelling	burrower	52	13
	crawler	28	100
Texel	burrower	110	5
	crawler	49	100

Choice experiments conducted using a captive bird further revealed that discrimination between infected and non-infected *Macoma* occurs after the shell valves were opened, exposing the white sporocysts. The observed mean rejection rate of parasitized *Macoma* was 30%.

Further work needs to be carried out at Churchill to study the foraging behaviour of shorebirds and estimate the predation pressure exerted by them on the local *Macoma* population. Even with a third of the infected clams being rejected, the parasites can complete their life cycle with quite high frequencies, considering the number of clams shorebirds consume per day at each ebb tide.

When track-making behaviour was first described by Brafield and Newell (1961), they postulated that the crawling movement was to seek fresh resources during deposit feeding. Swennen (1969) suggested that the stimulus for crawling behaviour is a negative environmental factor such as too much sand in the substrate, insufficient food availability or a shortage of oxygen. Dineen (as cited by Swennen and Ching 1974) found that the surfacing and crawling behaviour is in response to an oxygen shortage caused by the high number of metacercariae in the *Macoma* of the Ythan estuary (North Sea).(cf. Section 4.4.1).

Parasite infestation has been known to alter the behaviour of the hosts (Holmes and Bethel 1972). *M. balthica* normally buries itself into the substratum to a depth of about 10 cm and is relatively difficult to spot. It would be disadvantageous to "advertise" its presence by having a distinct track leading to its exact location. Furthermore, Hulscher (1982) found that his captive oystercatcher located *Macoma* at a higher rate when tracks were present than when tracks were absent or not well visible. Hence,

crawling behaviour in *Macoma* could be the result of an adaptive strategy of the parasite to further enhance the successful completion of its life cycle.

The consistency of crawling behaviour should also be studied in the field. Preliminary attempts to investigate this in the laboratory were not successful as ebb tides are difficult to simulate. All the clams, regardless of whether they were crawlers or burrowers when sampled from the wild, simply burrowed out of sight and remained there for hours. Non-parasitized clams which do not make crawling tracks could be infected with cercariae and tested to see if the presence of parasites would induce their hosts to exhibit crawling behaviour.

The possibility of "gigantism" due to castration was discussed earlier in Section 4.1.2. Temperature could also be responsible for the higher growth rate observed in crawlers since crawling *Macoma* are usually found in the top 1 to 2 cm of the substratum. Similarly, the much higher temperature experienced by the clams at the high intertidal could be the reason for the high growth there as suggested by Green (1973).

4.3 Transplant experiments at Churchill, Hudson Bay

Since, the *Macoma* transplanted from MLW to 1.0 m above MLW grew at a rate similar to that of the high tide "residents", the indication is that the phenomenon is influenced by local environmental conditions at the two tide levels rather than by the genotypic differences.

It might be argued that the more heterozygous clams from the low tide level survived better at the high tide transplant area, thus giving rise to the increased growth rate which did not differ significantly from their high tide counterparts. This can be refuted on two counts. Firstly, the low tide

clams retrieved were found to be more deficient in heterozygotes than the high tide ones, which was similar to the findings of Green et al. (1983). Secondly, Green et al. (1983) reported that in MLW clams, no significant relationship between growth rate and heterozygosity was observed. Neither was there a relationship between high temperature sensitivity and growth rate at MLW. It should be noted that the growth rate parameter used in their study was the deviation of each clam's mean annual growth from the average and not a direct length increment over a known period of time as used in this present study. However, there is a similar lack of relationship between length increment and degree of heterozygosity in the low tide clams in the present study; although as stated in Section 2.5.1, the results of these analyses were not used because some sample sizes, especially the totally heterozygous group, were much smaller than the others.

The growth rate-heterozygosity relationship reported for the high tide clams by Green et al. (1983) was not found in the present study.

Vincent et al. (1987) obtained a similar response in *Macoma* in a reciprocal transplant experiment conducted in the St. Lawrence estuary. In their *Macoma* population, where the "Churchill phenomenon" does not occur, slow growing high tide clams (from 0.8m above mean water level) grew faster when transferred to 1.2m below MWL. However, no corresponding change in growth rate was observed for the low tide clams transferred to high tide. They speculated that the clams adopt an opportunistic strategy at the upper tidal level whereas a more specialised strategy, accompanied by low phenotypic variability, is associated with the relatively stable marine environment at the lower tidal level.

No significant difference between the growth rates of homozygotes and heterozygotes at both tidal levels was found generally for the four

enzymes scored. Even when a small but significant difference was detected, the homozygotes were the ones with the faster growth (see Tables 7 and 11). Previous work on plaice (McAndrew et al. 1986) and cardiff newborns (Ward et al. 1985) similarly reported a lack of correlation between heterozygosity and growth rate.

Based on the assumption that the growth rate-heterozygosity relationship is a common occurrence, a number of reasons have been proposed by many researchers to account for undetectable correlations. Firstly, strong inbreeding and/or non-random mating in the sampled animals may have occurred (Koehn et al. 1988). However, the population size of *Macoma* at Churchill is extremely large. This coupled with the fact that *Macoma* has pelagically dispersed larvae makes the possibility of inbreeding very unlikely.

The second reason is that the animals may have already experienced genotype-dependent selection prior to sampling especially if older ones are sampled (Diehl and Koehn 1985). Extreme care was taken in this study to sample and mark clams from a wide range of sizes (which implies clams of varying ages) in an attempt to reduce this bias. This would also ensure that a greater increase in length can be measured, thereby decreasing the error of measurement (since growth is much reduced in larger clams). However, larger clams were invariably recaptured. Therefore, this reason is most plausible to account for the lack of correlation.

Moreover, there is already evidence provided by Green et al. (1983) that older clams from both tidal levels tend to be more heterozygous. Attempts made during the two field seasons to collect newly settled spats to determine whether the clams from the two tide levels differ genetically from time zero or whether selection for heterozygotes occurs during the

adult lifespan on the shore, were not successful. There appeared to be a "crash" in the recruitment during 1985 and 1986. This is apparently not an uncommon phenomenon: Olafsson (1988) reported very pronounced fluctuations in recruitment over a long term basis in sheltered populations of *Macoma* at the Falsterbo peninsula (southern Sweden). Most studies which show a positive relationship between growth rate and heterozygosity have been carried out using bivalves of a given age; in this study, clams of varying ages have been used. This is due to the confounding factor of size to age difference that exists at the two tide levels.

Another reason could be that there is insufficient power to detect the correlation statistically. The last two reasons to account for the lack of correlation between growth rate and heterozygosity are two which have not been sufficiently studied. Differences in the degree to which each individual locus contributes to the correlation might occur. These could be due to either different metabolic roles of their respective gene products and/or that a different portion of the genome is marked by each gene. In this case, the specific genes used in a study would influence the magnitude of the correlation significantly. This is a potentially serious problem, especially when the effects of individual genes differ greatly as the number of loci studied is usually small (five or six in most studies). Although the number of loci used in this present study is admittedly small, the general function of the enzymes is similar, that is, mainly involved with protein catabolism and glycolysis. Heterozygosity of such enzymes has been shown by Koehn et al. (1988) to be highly correlated with growth rate of the coot clam, *Mulinia lateralis*, while heterozygosity of other enzymes involved with either the pentose shunt, redox balance or other miscellaneous metabolic roles were not.

Lastly, it was suggested that genotype could have a diminished effect on individual growth rate when resources are abundant and/or when the environment is less harsh. Low tide Churchill clams would "fit the bill" as they have a longer immersion time, a more abundant food supply and live in a more stable environment. This could account for the apparent lack of relationship detected in the clams from that tidal region in the present study as well as previous results obtained by Green et al. (1983) regarding growth rate and heterozygosity.

Conversely, if it is true that in an environment of greater energetic stress · more significant enhancement of growth will be enjoyed by the more heterozygous individuals, then a correlation should be detected in the high tide clams. However, no evidence of this is found in the present study although it was found by Green et al. (1983). As mentioned before, a different measure of growth was used in their study.

Results from the comparison of the effect of alleles on growth rates were inconclusive. From the 1985 data, it seems that high tide clams which are homozygous for PGM at allele 2 grew faster while those homozygous for PGI allele 3 grew slower. However, these results were not reproducible in the 1986 transplant.

High temperatures experienced by the high tide clams have been suggested by Green (1973) to be responsible for the "Churchill phenomenon". Vincent et al. (1987) observed greater annual growth rates of *Macoma* in tidal pools of the St. Lawrence estuary and proposed that high sediment temperatures may be responsible for it. It is unfortunate that the low tide transplant area was destroyed by violent storms thereby limiting the conclusions that one can draw from this lop-sided reciprocal transplant design. However, the two years' data from just the high tide

transplant area suggest that the faster growth rate observed in the upper intertidal is more likely a phenotypic response, perhaps to the higher temperatures there, and has little genetic basis.

4.4 Temperature-related experiments

4.4.1 Oxygen consumption

In poikilotherms, oxygen consumption usually increases with rise in temperature up to some critical level, beyond which deleterious effects are evident. Since clams are poikilotherms, it is hardly surprising that temperature affects their oxygen uptake. However, *Macoma*'s Q_{10} of 1.5 over the temperature range of 5 to 20° C is indicative of a moderately temperature dependent metabolism; it is neither entirely independent (in which case Q_{10} would be 1) nor highly temperature dependent (where Q_{10} is greater than 2). Q_{10} values of 2 to 3 are usual (Nichol 1967).

The significance of clam length, which was used as the covariate in the factorial analysis is expected; larger clams obviously consume more oxygen than smaller ones. Present results show that *Macoma* conforms to the established physiological rule: weight-standardized oxygen uptake is inversely related to size of the animal.

Since parasite load was not found to be significantly associated with oxygen uptake, the results of the present study do not support Swennen's hypothesis that the surfacing and crawling behaviour during ebb tide is in response to the oxygen shortage caused by the high density of parasites.

Instead, the source of the clams, that is , high or low tide level, is the single most important factor that affected the oxygen uptake at all temperatures. This suggests a genetic component, although phenotypic plasticity was demonstrated by the low tide clams when transferred to the high tide region in the transplant study. Further indication that some measure of genetic control is involved is provided by the similarity of the metabolic curves of the high and low tide clams at 20° C and 5° C respectively.

For clams of a given size and temperature, low tide *Macoma* consumed more oxygen than high tide clams. This implies that the high tide clams have greater metabolic efficiency: this energy "saved" by higher metabolic efficiencies in faster growing animals can be used to increase ingestion and absorption rates, thereby enhancing growth even further.

Exactly how this is achieved in the high tide clams is not clear. The totally heterozygous clams were the least metabolic efficient (see Fig. 24). At the low tide level, degree of heterozygosity does not seem to affect metabolic efficiency at all; perhaps this is evidence again that the growth rate of animals living in an "unstressed" environment is less affected by genotype. In general, at both tide levels, the metabolic efficiency of heterozygotes (at any of the three enzyme loci scored) was not better than those of homozygotes.

When oxygen consumption is related to alleles, no particular effect was exerted by the presence of either alleles in the three enzymes. The only exceptions were that in the high tide clams the presence of allele 1 of MDH decreased its metabolic efficiency. A similar effect was seen in the low tide clams when allele 2 of MDH is

present. There is not enough information to comment on the dominance of the alleles present.

4.4.2 Freezing tolerance

Most organisms can only carry on their life sustaining processes within a narrow range of temperature. This 'biokinetic' zone usually lies between 4 and 45° C. Outside of this zone, the rates of biological reactions would slow down to such an extent that life processes are seriously affected. However, many poikilotherms have been known to carry on their life processes outside of this zone by compensating for the temperature effect. These adjustments may include the evolution of enzyme variants which have high activities at low temperatures, increases in the amounts of enzymes present and/or alterations in the control of enzymes.

For animals living in cold environments, freezing is a major problem encountered. Freezing is almost always lethal and species living in the high intertidal are expected to have greater tolerance to freezing than those in the lower intertidal (Murphy 1979).

Present results show that the low tide clams are able to withstand longer periods of cold than the high tide clams, which is contradictory to Murphy's prediction. A greater percentage of survival was also observed for low tide clams. Laboratory conditions of course do not imitate those of the bay completely. If the same mortality rate were observed in the wild, then the high tide population would be severely reduced in the winter; however, the recovery rate of overwintered high tide clams at the high tide region in both years was not appreciably lower than those of the summer

transplants. Further work needs to be done to investigate this since very little is known of the physiological mechanisms governing freezing tolerance in intertidal organisms.

While the survival of high tide *Macoma* depended solely on the duration of cold exposure, that of low tide clams was affected by both the duration of the cold treatment as well as whether they were parasitized or not. Parasitized low tide *Macoma* are less able to tolerate longer periods of cold exposure than are uninfected ones.

4.4.3 Burrowing behaviour

Sensitivity and response of high and low tide clams to water temperatu. were different. Low tide *Macoma* tended to bury deeper into the substratum than high tide ones when the temperature was less than 30° C. This could indicate that the low tide clams are able to withstand anaerobic conditions better than the ones from the high. On the other hand, it could be a normal behaviour of *Macoma* since it has been documented by Hulscher (1982) that the smaller the clam, the greater the burrowing depth ...a ...e low tide clams are smaller than those of the high.

When the water temperature was 30° C, both tidal levels' clams remained at the surface. Brafield (1963) observed that *Macoma* will fully expose itself on the surface of the sand during periods of oxygen deficiency. There is less dissolved oxygen present in water that is 30° C than at the two cooler temperatures. This could explain why the *Macoma* did not burrow into the sand in the laboratory experiment. Also, 30° C is close to the lethal temperature

of the Churchill *Macoma* (determined to be 31.5° C by Green et al. 1983), and the clams could be moribund.

Although it was not found that greater metacercaria load meant increased oxygen consumption, low tide clams tended to remain closer to the surface of the substratum when they had more parasites. This supports Swennen's hypothesis that the presence of the parasites causes clams to move to the surface. However, the low tide clams had much lower parasite loads. A similar relationship between parasite load and burrowing depth was not observed for the very heavily infected high tide clams. In fact, burrowing depth was independent of parasite load.

4.5 Conclusions

4.5.1 Geographical variation in growth rate with respect to tide level

The 'Churchill phenomenon' is not widespread. Evidence from this study shows that it is confined to sites along the coast of Hudson Bay east of Churchill to Cape Henrietta Marie on James Bay.

4.5.2 Parasite load

At Churchill, high tide *Macoma* were more heavily parasitized than the ones from the low tide region. This difference could be explained by the increased exposure of the clams at the higher tidal level to shorebirds which are the final hosts of the parasite.

High tide *Macoma* grew faster than low tide ones and crawlers (more parasitized) grew faster than the buried (less parasitized) clams. Parasitic castration resulting in enhanced growth could be

an explanation for these observations. The higher temperatures experienced by the high tide clams and the crawlers could also account for the higher growth rate.

There is no evidence that the more heavily parasitized clams consumed more oxygen than those less parasitized. Therefore, present results do not support Swennen's hypothesis that the surfacing and crawling behaviour of *Macoma* during ebb tide is in response to an oxygen shortage caused by the high density of parasites.

4.5.3 Transplant experiments at Churchill, Hudson Bay

Evidence from the 1985 and 1986 transplant experiments indicate that the phenomenon is most likely to be influenced by local environmental conditions at the two tide levels rather than by genotypic differences.

4.5.4 Temperature related experiments

4.5.4.1 Oxygen consumption

The Q_{10} of high and low tide *Macoma* was 1.5. This indicates that *Macoma* has a moderately temperature dependent metabolism. For clams of a given size and temperature, low tide *Macoma* consumed more oxygen than high tide clams, implying that high tide *Macoma* have greater metabolic efficiency. However, a greater metabolic efficiency associated with heterozygosity (as reported by many researchers) was not observed in the Churchill *Macoma*.

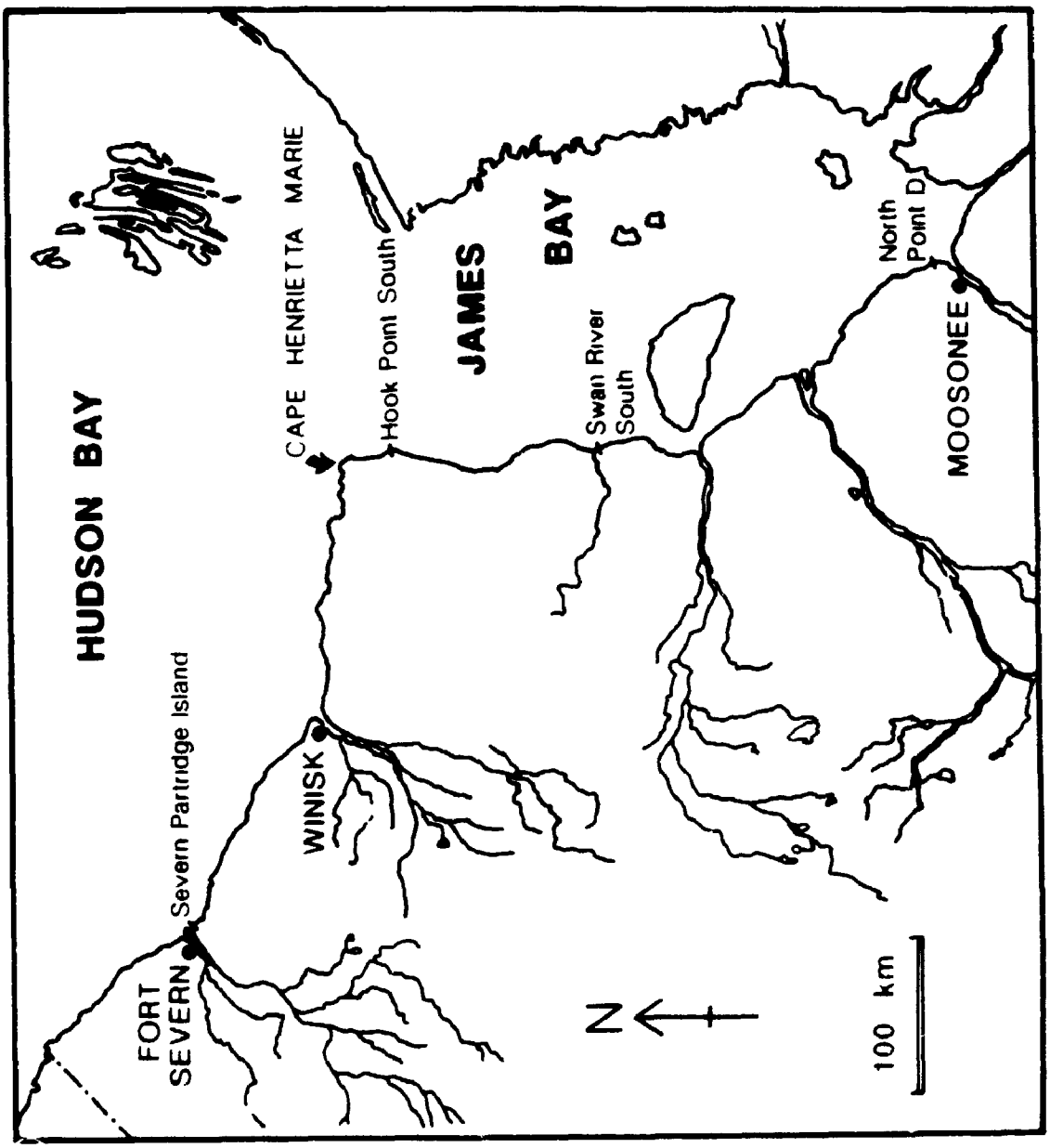
4.5.4.2 Freezing tolerance

Survival of high tide *Macoma* depended solely on the duration of cold exposure whereas that of low tide clams was affected by both the duration of cold treatment as well as the presence of parasites.

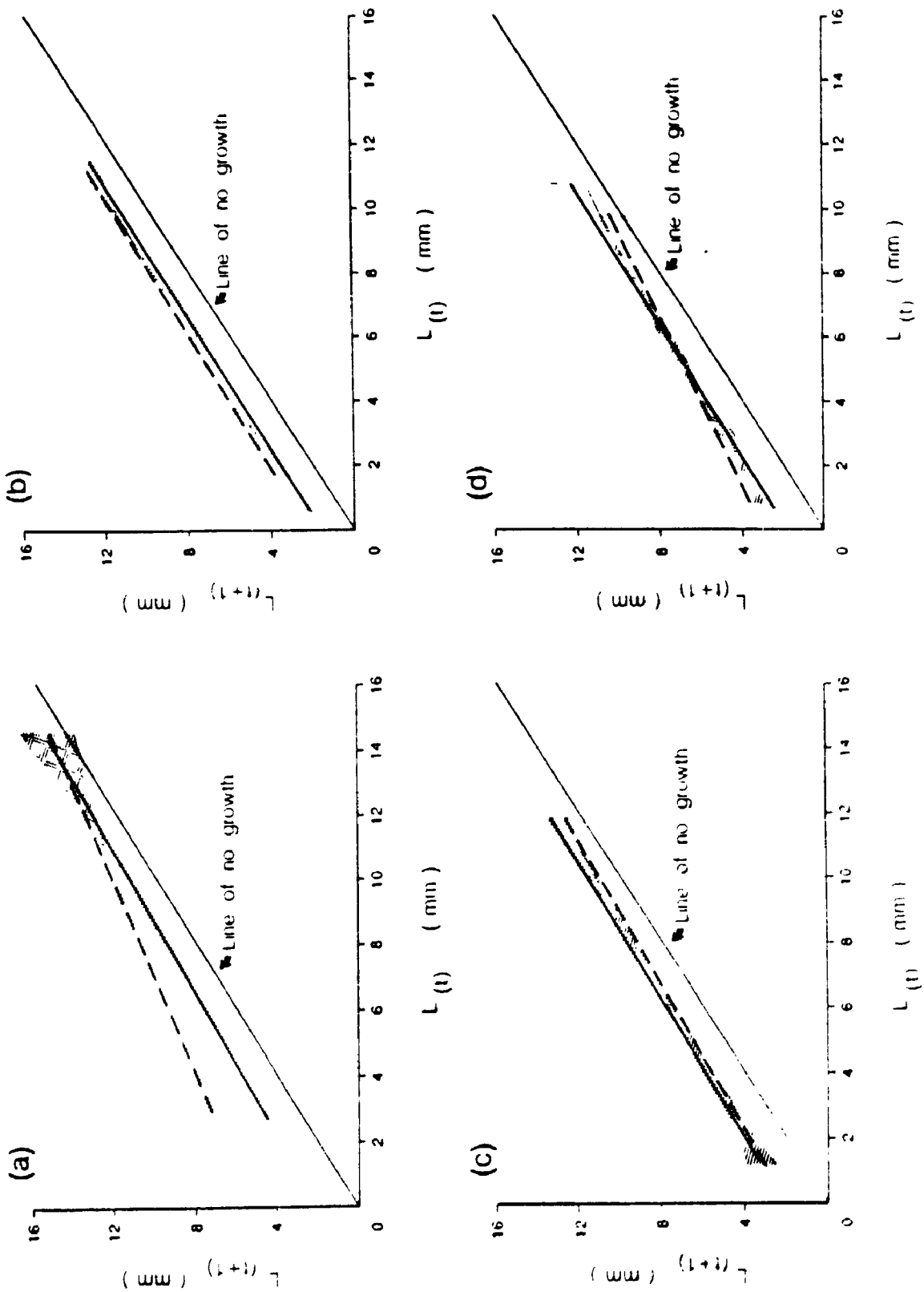
4.5.4.3 Burrowing behaviour

When the temperature was less than 30° C, low tide *Macoma* tended to bury deeper into the substratum than high tide clams. Both tide levels' clams remained at the surface when the water temperature was 30° C. This could be due to the reduced oxygen content in the water or the moribund state of the clams.

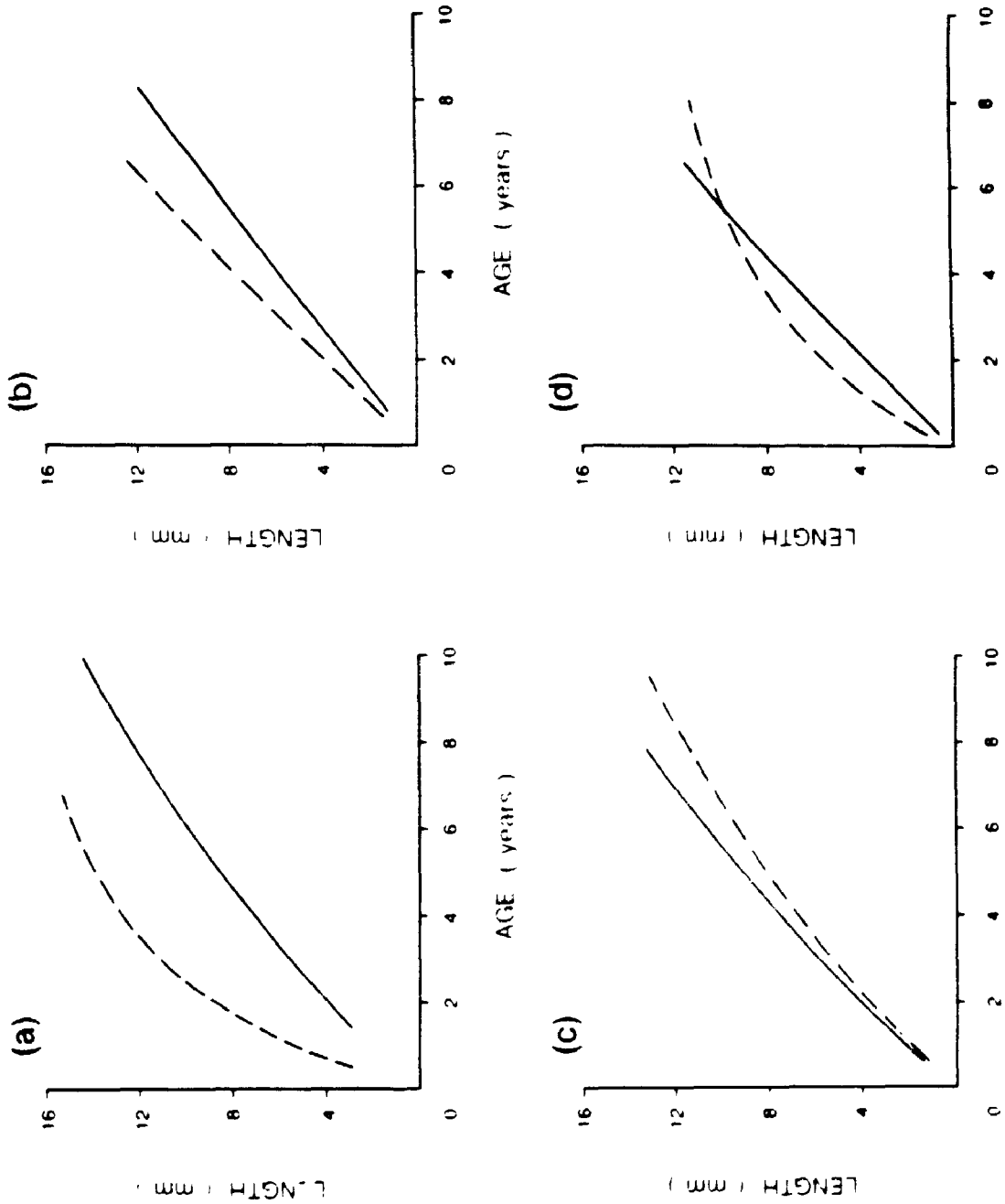
Appendix I (i) Map of Hudson Bay and James Bay region showing location of four C.W.S sampling sites: (a) Severn Partridge Island; (b) Hook Point South; (c) Swan River South; (d) North Point D.


HUDSON BAY
JAMES BAY
CAPE HENRIETTA MARIE
Hook Point South
Swan River South
North Point D
MOOSONEE
WINISK
Severn Partridge Island
FORT SEVERN
N
100 km

Appendix I (ii) Walford plots of *Macoma balthica* from: (a) Severn Partridge Island; (b) Hook Point South; (c) Swan River South; (d) North Point D. (- - - = High tide level; —— = Low tide level). Lines are drawn to cover the range of data used only. The 95% confidence bands are shown.


(a)
(b)
(c)
(d)
Line of no growth
L (t+1) (mm)
L (t) (mm)
0
2
4
6
8
10
12
14
16

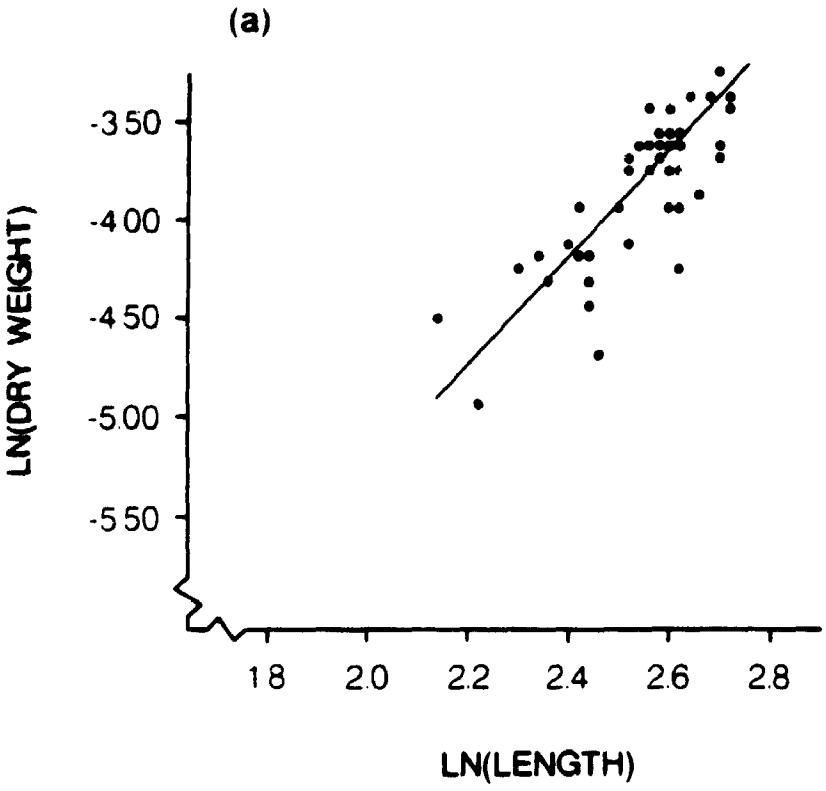
Appendix I (iii) von Bertalanffy growth curves corresponding to the Walford plots of (ii) for *Macoma balthica* from: (a) Severn Partridge Island; (b) Hook Point South; (c) Swan River South; (d) North Point D. (- - - = High tide level; —— = Low tide level). Lines are drawn to cover the range of data used only.

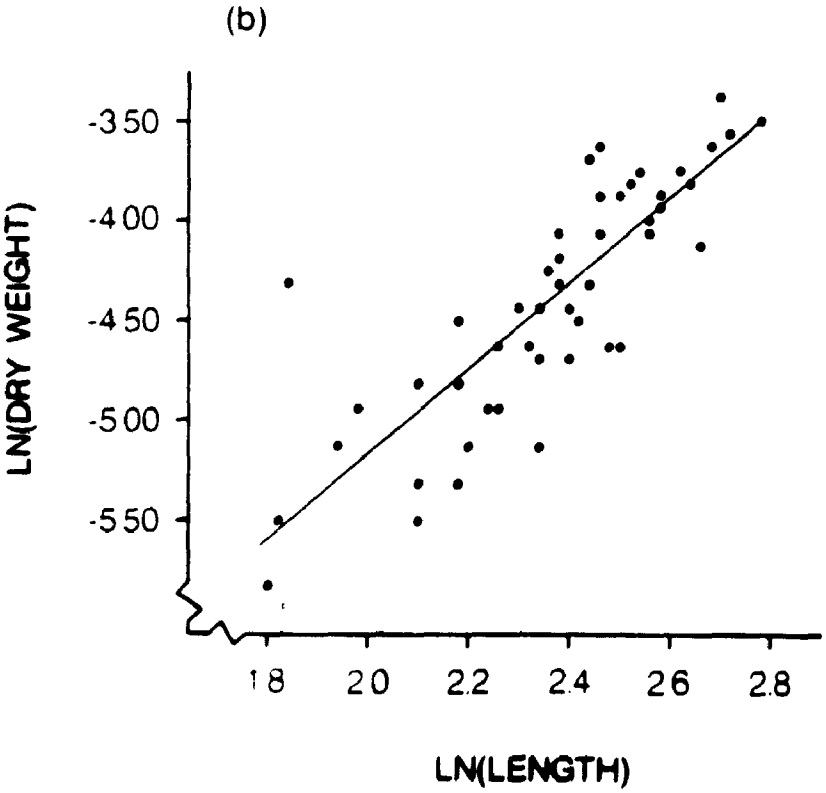

(a)
(b)
(c)
(d)
LENGTH (mm)
AGE (years)
0
2
4
6
8
10
4
8
12
16

Appendix I (iv) Tests of significance at $p<0.05$ of differences in the Walford plot slopes and intercepts between high and low tide levels for the four sites.

Sampling site	Slope	Intercept
(a) Severn Partridge Island	significant	apparently different
(b) Hook Point South	non-significant	significant
(c) Swan River South	non-significant	non-significant
(d) North Point D	significant	apparently different

Appendix II. Least squares regression lines of ln(dry weight of clam) against ln(length of clam) for: (a) High tide *Macoma* from Churchill; (b) Low tide *Macoma* from Churchill. Lines are drawn to cover the range of data used only.


(a)
-3.50
-4.00
-4.50
-5.00
-5.50
LN(DRY WEIGHT)
1.8
2.0
2.2
2.4
2.6
2.8
LN(LENGTH)


(b)
-3.50
-4.00
-4.50
-5.00
-5.50
LN(DRY WEIGHT)
1.8
2.0
2.2
2.4
2.6
2.8
LN(LENGTH)

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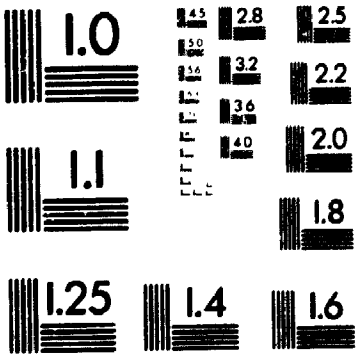

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