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The implications of heterozygosity in the scallop

Placopecten magellanicus (Gmelin)

by

Filip A.M.J. Volckaert

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Submitted in partial fulfillment of the requirements

for the degree of Ph.D.

at

Dalhousie University

Halifax, Nova Scotia

July 1988

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DALHOUSIE UNIVERSITY

FACULTY OF GRADUATE STUDIES

The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled " The Implications of Heterozygosity in the Scallop *Placopecten magellanicus* (Gmelin)"

by Filip A. M. J. Volckaert

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

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Department or School: Oceanography

Degree: Ph. D. Convocation: Fall Year: 1988

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Abstract.

In many instances, variation in phenotypic traits has been correlated with electrophoretic heterozygosity of plants, vertebrates and invertebrates. Enhanced growth of selected heterozygous molluscs has been attributed to lower energetic requirements for basal metabolism. I investigated the correlation between single- and multiple-locus heterozygosity and phenotype of the juvenile scallop *Placopecten magellanicus* (Gmelin). Two questions were being asked : do heterozygous scallops grow faster and which metabolic processes are affected by the degree of heterozygosity?

No significant correlation was observed between genotype (scored at 6 polymorphic loci) and growth in six different samples of juvenile scallops, all belonging to the same time series. This result and published records indicate that the allozyme heterozygosity of pectinids does not influence growth to the same degree as in the bivalves *Mytilus edulis*, *Crassostrea virginica* and *Mulinia lateralis*. A decrease in heterozygote deficiency with age suggests selective mortality in scallops between the ages of 2 months and 13 months.

The metabolism of 13 month old scallops switched from the catabolite carbohydrate (i.e. glycogen) to protein when they were starved for 4 weeks. Allozyme genotype had no measurable effect on oxygen uptake, excretion rate, O:N ratio and carbohydrate content, either under routine or basal metabolic conditions. Two hours after induction of muscle contractions in 20 month old scallops, the end-product octopine reached a concentration of 4 μmol$\cdot$(g wet muscle weight)$^{-1}$. Multiple-locus heterozygosity was positively correlated with octopine accumulation in the phasic part of the adductor muscle. Octopine dehydrogenase activity was on average higher among ODH homozygotes than among heterozygotes. In summary, multiple-locus heterozygosity is correlated with selected traits related to functional anaerobiosis.

A model integrates the above mentioned results with the hypotheses of "associative overdominance", of a "balanced energy pathway" and of "energetic efficiency". I argue that energy savings due to heterozygosity are used for enhanced "activity" (such as feeding and swimming) in freely moving molluscs and for enhanced "growth" (such as somatic growth in juveniles, gonadal growth in adults and resistance to starvation) in sessile molluscs.

List of abbreviations.

AAT	aspartate aminotransferase.
ADH	alcohol dehydrogenase.
AFDMU	ash free dry weight of muscle tissue.
AFDW	ash free dry weight of soft tissue.
$AFDW_{av}$	mean ash free dry weight of soft tissue.
$AFDW_{meas}$	measured ash free dry weight of soft tissue.
ANCOVA	analysis of covariance.
ANOVA	analysis of variance.
ATP	adenosine triphosphate.
D	heterozygote deficiency.
DF	degrees of freedom.
EstB	esterase B.
F-6-P	fructose-6-phosphate.
F 2,6 BP	fructose-2,6-biphosphate.
α-GPD	alpha glycerophosphate dehydrogenase.
Het	degree of heterozygosity.
LAP	leucine aminopeptidase.
LDH	lactate dehydrogenase.
MDH	malate dehydrogenase.
MPI	mannosephosphate isomerase.
NAD^+	oxidized nicotinamide adenine dinecleotide.
NADH	reduced nicotinamide adenine dinucliotide.
NS	statistically not significant ($P > 0.05$).
ODH	octopine dehydrogenase.
O:N	atomic ratio of oxygen to nitrogen.
PFK	pyruvate fructokinase.
PGD	6-phosphogluconate dehydrogenase.
PGI	glucosephosphate isomerase.

PGM	phosphoglucose mutase.
PK	pyruvate kinase.
SHDW	shell dry weight.
SS	sum of squares.
V_{cal}	rate of caloric loss.
$\dot{V}_{cal}$	weight-specific rate of caloric loss.
V_{NH_4}	ammonia excretion rate.
$\dot{V}_{NH_4}$	weight-specific ammonia excretion rate.
V_{O_2}	oxygen uptake rate.
$V_{O_2 \cdot act}$	active oxygen uptake rate.
$V_{O_2 \cdot sa}$	scope for activity.
$V_{O_2 \cdot st}$	standard oxygen uptake rate.
$\dot{V}_{O_2}$	weight-specific oxygen uptake rate.

Acknowledgements.

I thank especially Eric Mills (Oceanography) and Lefteri Zouros (Biology) for jointly supervising my research. They have taken care of my academic health by introducing me to the research community, provoking thought, gently guiding my learning, and providing research facilities. Thesis readers Ginette Robert (DFO), Mike Sinclair (DFO), Pete Wangersky (Oceanography) and especially Ron O'Dor (Biology) have looked with a careful eye over my shoulder. Jerry Hilbish (University of South Carolina, USA) served as external examiner. Mike Sinclair provided critical support in the early stages of this project. Mike Dadswell (DFO) generously provided live scallops from the Bay of Fundy and provided comments on the thesis. Becky Crowe (Biology) and Cyr Couturier (Biology) helped with culturing scallops. The seawater facilities were expertly looked after by Norval Balch, Fred (Tinker) Walsh and Ed Officia (Oceanography). Charlie Thompson (Biology) constructed the respiration chambers. Maria Romero-Dorey (Biology) introduced me to the art of starch gel electrophoresis. Tom MacRae (Biology) kindly shared his laboratory for the protein assays and David Livingstone (Plymouth, UK) suggested a neat method to assay octopine dehydrogenase. David Foltz (LSU, USA), Kathleen Fuller and Kami Ahmad (Biology) made unpublished data available. Erica Head (BIO) taught me the basics of enzyme activity assays and Sandy Shumway (DMR, Maine, USA) introduced me to the measurement of oxygen uptake, provided samples from the Gulf of Maine, and provided comments on the thesis. Jon Grant (Oceanography) and Ray Thompson (Memorial University of Newfoundland) critically read selected chapters of the thesis. Andre Mallet (BIO) made his Fortran programme available to calculate genetic statistics and provoked thought on numerous occasions. Jackie Hurst (Oceanography) guided me through the electronic typesetting maze and Craig Emerson (Oceanography) solved many emergencies on the PC.

Many more people have contributed : faculty, students and staff in the Departments of Oceangraphy and Biology at Dalhousie University, the staff of the Dalhousie Academic Computing Services and Macdonald Science Library, researchers

at the Bedford Institute of Oceanography, the Halifax Research Laboratory and the Biological Station at St. Andrews.

The Belgian-Canadian Cultural Exchange Programme (administered by the World University Service of Canada) and Dalhousie University provided scholarship support. NSERC grants to Eric L. Mills and E. Zouros, and a Dalhousie Research Development Fund to Eric L. Mills covered the research expenses.

I am very grateful to the Canadian people for their hospitality and sharing of a most fascinating country. I owe much to many friends, but especially to Myriam Bormans and Jean Braun. My parents, brother Kristof, sisters Mia and An, and Joke played key roles in the "Belgian Connection".

Chapter 1.

General introduction.

1.1 Allelic isozyme variation and life history traits.

The partitioning of phenotypic variation of a quantitative character among individuals in a population between genetic, environmental and random variation remains the focus of much research in quantitative and population genetics. The observation that growth rate of American oysters correlates positively with the individuals' degree of heterozygosity (Singh and Zouros, 1978) has been followed by numerous studies on the interaction between phenotypic variation and isozyme variation in plants, vertebrates and invertebrates (see reviews in Mitton and Grant, 1984; Singh, 1984; Zouros and Foltz, 1984; Koehn, 1985; Vrijenhoek, 1985; Watt, 1985; Zouros and Foltz, 1987).

If numerous correlations between heterozygosity and phenotype support the genetic model of heterosis, an equally large number of correlations remain inconclusive. Correlations between heterozygosity and growth rate in outbreeding populations are correlated positively on 24 occasions (explaining in each case a few percent of total phenotypic variation), negatively on 5 occasions and inconclusively in 23 cases (including *Placopecten magellanicus* and *Pecten maximus*) (Beaumont *et al*, 1985; Foltz and Zouros, 1984; Zouros and Foltz, 1987). Zouros (1987) argues that the detection of a positive correlation is promoted by several factors which affect the population such as environmental conditions, sample size, number of loci scored, background genotype and linkage disequilibrium.

In invertebrates, the effect of heterozygosity on growth rate tends to decline with age and to shift to a correlation with fertility and selected physiological traits. Growth rate of blue mussels (*Mytilus edulis*) is related to heterozygosity at a few months of age (Koehn and Gaffney, 1984). Some older mussels show no correlation between heterozygosity and growth rate (Diehl and Koehn, 1985), others do (Zouros *et al*, 1988). The strength of the correlation between heterozygosity and growth in

Crassostrea virginica decreases with age (Singh and Zouros, 1978; Zouros *et al*, 1980).

The correlation of heterozygosity with ecophysiological traits corroborates the correlation between heterozygosity and growth in molluscs. Either a higher scope for growth (Koehn and Shumway, 1982; Garton *et al*, 1984; Diehl *et al*, 1986) or a higher scope for activity (Garton, 1984) may distinguish heterozygous molluscs from homozygous conspecifics.

In this thesis I have compared the correlation between heterozygosity and phenotype at various organismal levels in the pectinid *P. magellanicus*. My logic takes the following form. Theoretical and experimental evidence suggest that a small percentage of phenotypic variation could be attributed to polymorphism at several enzyme loci and/or the adjacent genome. No correlation between heterozygosity and growth has been observed in pectinids, but suggestions of selective mortality have been made. An aspect of the biology of scallops which might be especially important from a genetic perspective is their adaptation to functional anaerobiosis. Swimming is not a common occurrence in the Mollusca; when a bivalve acquires the capacity to swim, it will affect significantly its energetic pathways.

I first examine whether juvenile scallops show a correlation between heterozygosity and growth. The genetics of growth rate are investigated in a time series of juvenile scallops from Passamaquoddy Bay, N.B., Canada in Chapter 2. Thereafter, I investigate whether the metabolic physiology of resting, starved and exercised scallops is related to allozyme variation. The link between heterozygosity and routine/basal metabolism of juvenile scallops is studied in Chapter 3. Chapter 4 focusses on the genetics of anaerobic metabolism in 20 month old scallops which are induced to snap their valves with starfish extract. Finally, the hypothesis that the function of polymorphic enzyme loci has an effect on the correlation between heterozygosity and growth rate is evaluated in Chapter 5. I present in the concluding chapter a model which explains the causality of the link between the degree

of heterozygosity and Darwinian fitness at several organismal levels. The model incorporates several hypotheses which have been proposed.

1.2 The biology of *Placopecten magellanicus* (Gmelin).

The following paragraphs summarize the published literature on the general biology of *Placopecten magellanicus.* A synopsis has been compiled by MacKenzie (1979) and a new enlarged synopsis is in preparation (S. Shumway and S. Naidu, pers. comm.).

1.2.1 Morphology.

Placopecten magellanicus (Gmelin) belongs to the Family Pectinidae, the Order Pterioida and the Class Bivalvia. Valves are subequal, slightly convex and almost equal in diameter. Animals rest on the right valve which is slightly more convex and pale in colour. The juvenile shell is subcircular, thinner and less gaping than in adults (MacKenzie, 1979). Distinct growth rings are deposited annually; in addition disturbance or shock rings may occur. The ligament and adductor muscle are especially well developed. The soft parts account for about 40% of the animal's total wet weight.

The single adductor muscle consists of a phasic part (composed of cross-striated muscle) and a catch part (composed of paramyosin muscle) (Chantler, 1983). Located next to the adductor muscle is the gonad, which matures in late spring and is coloured orange-red in females and off-white in males. Less than 1% of all scallops are hermaphrodites; a sex reversal of an all male population in the first year of reproductive activity to a 1:1 male:female population might occur later on (Worms and Davidson, 1986). The mantle is a thickened muscular structure situated along the edge of the shell. It is often pigmented with symbiotic algae (Naidu and South, 1970) and has numerous sensory tentacles and eyes. The dark-green hepatopancreas is located near the hinge. Byssal threads are produced by the byssal gland and are

glued to the substrate by the foot. The principal filaments, ordinary filaments, cilia and microvilli of the gill indicate that filter-feeding is the principal mode of feeding.

1.2.2 Ecology.

P. magellanicus occurs from the North Shore of the Gulf of St.Lawrence to off the coast of North Carolina (Cape Hatteras). In the northern part of the range scallops live in shallow water, while in the southern part they occur at depths greater than 50 m. They live on the sediment surface or in slight depressions on mud, sand, sand and pebbles, and rocky substrates. The most productive and largest populations are found on Georges Bank. Scallop concentrations coincide with high flow regimes; population growth is a function of temperature and seston supply rates (Wildish and Kristmanson, 1979). Scallops are opportunistic suspension feeders on pelagic and benthic algae, micro-invertebrates, detrital material and bacteria (Shumway *et al*, 1987). Populations growing at shallower depths experience more favourable growth conditions (MacDonald and Thompson, 1985a). Young scallops often attach by byssus threads (Caddy, 1972). A unique feature of pectinids is their ability to swim, which is especially common in young to middle-aged individuals.

Spawning time varies from area to area; it extends in the Bay of Fundy from July to September (Dadswell *et al*, 1987). Southern populations tend to spawn in summer, northern populations in early fall. Larger scallops - with correspondingly larger gonads - contribute most of the eggs. Annual variation in total production affects differences in reproductive output rather than somatic growth (MacDonald and Thompson, 1985b). A 9 year cycle characterizes the variation in recruitment (Caddy, 1979). Larval settlement occurs one month after spawning, usually during a two week peak period.

Endoparasites are not common; shell boring polychaetes (*Polydora* sp.) and sponges (*Cliona* sp.) affect primarily the health of older animals. Paralytic Shellfish Poison (PSP) accumulates in concentrations toxic for vertebrates in soft body tissues except in the adductor muscle. Predators vary with the age of the prey :

zooplankton filter-feed on larvae; cod, plaice and starfish feed on middle-aged scallops. Steroid glycosides (called saponins) of starfish induce an avoidance reaction in scallops (Mackie, 1970). Adult scallops experience a large fishing mortality.

1.2.3 Physiology and biochemistry.

Oxygen uptake and clearance rate are dependent upon gonad development and covary with temperature (MacDonald and Thompson, 1986; Shumway *et al*, 1988). Estimated scope for growth is low or negative in winter, but consistently high during the spring bloom (a period of rapid gamete maturation) (MacDonald and Thompson, 1985b). *P. magellanicus* is an oxygen regulator (Shumway *et al*, 1988) whose oxygen uptake and clearance rate are weight dependent. It is not known how salinity affects the physiology.

Valve snapping increases the heart rate, reduces the partial pressure of blood oxygen and lowers the blood pH; oxygen uptake increases after the valves reopen (Thompson *et al*, 1980). The snap response is powered by the phasic and catch part of the adductor muscle, while valve closure is controlled by the catch part. The major metabolic pathway in the adductor muscle during anaerobiosis is the breakdown of glycogen to octopine. Glycolytic pyruvate may be converted into alanine in the catch part. Transphosphorylation of stored phosphoarginine is the major energy source in the phasic part during the snap response. Anaerobic glycolysis provides ATP in the catch part during valve closure and valve snapping (de Zwaan *et al*, 1980). Octopine is formed during the first few hours of recovery, particularly in the phasic muscle, and is not transported to other tissues. Glycolysis is the major source of energy during anoxia and maintains the basal rate of energy production. The phosphoarginine pool is restored by aerobic metabolism.

Gonad development starts with the mobilization of glycogen from the adductor muscle and lipid from the digestive gland to the developing gonad (Robinson *et al*, 1981). The levels of lipid, protein, low molecular weight carbohydrate and glycogen increase in blood plasma during gonad growth and maturation (Thompson, 1977).

The protein biochemistry of *P. magellanicus* has been studied fragmentarily. The enzyme aspartate amino transferase (AAT) is more temperature labile than in intertidal bivalves and is comparable to other subtidal bivalves (Read, 1963). Low enzymatic activities of pyruvate kinase and high activities of glutamate dehydrogenase characterize stressed populations (E. Gould, NOAA, pers. comm.). Enzyme activities of phosphorylase, phosphofructokinase, pyruvate kinase and octopine dehydrogenase are considerably higher than in *Crassostrea virginica* and reflect the importance of burst metabolism in scallops (de Zwaan *et al*, 1980).

1.2.4 Population genetics.

The karyotype of *P. magellanicus* shows 19 chromosomes (Beaumont and Gruffydd, 1974). Seven soluble enzymes (α-GPD, PGI, PGD, AAT, MPI, ODH and PGM) are polymorphic. The degree of polymorphism measured at the latter six enzymes is 0.59 (Foltz and Zouros, 1984). Scallops are on average deficient in heterozygotes; locus-specific deficiencies are observed consistently at loci ODH and PGM. No strong clinal or biogeographical variation in allelic and genotypic frequencies is evident in the Northwest Atlantic, although varying selection pressures and reduced migration rates may influence gene frequencies (Gartner-Kepkay and Zouros, 1985).

Mitochondrial DNA of *P. magellanicus* measures 34 kilobasepairs (kbp), double the size of most metazoan animals (Snyder *et al*, 1987). Polymorphism in mtDNA is evident within and among populations and is largely due to the number of copies of a tandemly repeated 1.47 kbp element (J. LaRoche, Brookhaven National Laboratory, pers. comm).

1.2.5 Socio-economic aspects.

The Canadian scallop fishery totalled about 7500 metric tons in 1987, representing a market value of CN$ 75 million (G. Robert, DFO, pers. comm.). The offshore fishery on Georges Bank represents about 85% of the total scallop catch in

Canadian waters. Secondary fishing grounds are located in the Bay of Fundy, the Gulf of St.Lawrence, off the coast of Newfoundland and on the Scotian Shelf. The American fishery is concentrated primarily on Georges Bank and secondarily in the Gulf of Maine and in the Mid Atlantic Bight. Dragging the scallop grounds with steel mesh bags is the traditional way of fishing.

The economic importance of the scallop fishery on Georges Bank was at the root of the boundary dispute between the U.S. and Canada in the Gulf of Maine in 1984 (McDorman *et al*, 1985). The effect of the fishery on the economy of the Maritimes and New England is large (N.S. Department of Fisheries, 1982).

The high demand and high cash value of scallops has stimulated interest in enhancing the resource through hatchery culture, spat collection on artificial collectors in nature, bottom restocking programs and suspension culture. At present wild spat is grown with some success only in St.Pierre and Miquelon (France) and Passamaquoddy Bay (N.B.). Poor survival rates of scallop spat hamper hatchery culture.

The socio-economic importance of the species makes it imperative to understand its basic biological characteristics. Sound management of the scallop fishery in the Northwest Atlantic is founded on knowing, amongst other aspects, the population genetics and ecophysiology of *P. magellanicus*. This thesis contributes towards that end.

Chapter 2.

Multiple-locus heterozygosity and growth in several cohorts of juvenile scallops (*Placopecten magellanicus* (Gmelin)).

2.1 Introduction.

The connection between heterozygosity and fitness among individuals in populations of invertebrates, vertebrates and plants has been tested in a growing body of empirical and theoretical studies. However, this hypothesis remains to be settled, because the link between degree of heterozygosity and growth is neither general nor predictable. Especially instructive has been the study of heterozygosity and growth in molluscs (Zouros, 1987). Growth rate is consistently linked to the number of heterozygous loci in mussels (Koehn and Gaffney, 1984), coot clams (Koehn *et al*, 1988) and oysters (Zouros *et al*, 1980). The correlation has not been found among offspring of single matings (Gaffney and Scott, 1984), in whelks (Garton, 1984) and pectinids.

Four studies have tested whether or not fitness increases with the number of heterozygous loci scored in pectinids. Wilkins (1978) measured the degree of heterozygosity at a single locus (PGI) of two samples totalling 280 individuals of *Pecten maximus*. Observed heterozygosity decreased with shell height in both populations. Growth rate was not measured because scallop shells were not aged. The results suggest a reduction of viability among heterozygotes. Heterozygosity at the enzyme phosphoglucose mutase (PGM) of *Chlamys opercularis* is lower in an older age class than in a younger age class (Beaumont, 1982). Both observations are inconsistent with the general trend that heterozygosity increases with age. Foltz and Zouros (1984) scored 752 specimens of the scallop *Placopecten magellanicus* at six polymorphic enzyme loci. Ages were determined from growth rings on the shell; they varied from 4 to 11 years. The degree of heterozygosity increased significantly with age, suggesting selective viability, but there was no correlation between enzyme heterozygosity and wet weight within age groups. This might be explained by a

shift from somatic growth to reproductive output in adults (Rodhouse *et al*, 1986) since reproductive effort increases with age in scallops (MacDonald and Thompson, 1987; Langton *et al*, 1987).

In a fourth paper, Beaumont *et al* (1985) studied heterozygosity and size in outbreeding and inbreeding populations of *P. maximus*. They collected settled wild scallops during 3 consecutive years, raised the spat to the ages of 1, 2 and 3 years in lantern nets and electrophoretically scored individual scallops at 6 enzyme loci. Single-locus and multiple-locus heterozygosity of the outbreeding population were not correlated with growth rate in any of the three year classes. In a second experiment two half-sib families were raised to the age of one year and typed at the same 6 loci. Again, heterozygosity and growth rate were not correlated. Possibly, the progeny of single matings were less likely to show a correlation because of a uniform background genotype (Gaffney and Scott, 1984).

A second aspect relevant to this paper is the variation in the degree of heterozygote deficiency relative to the Hardy-Weinberg equilibrium between and within populations. In general, marine bivalve populations are deficient in heterozygotes (Koehn and Mitton, 1972; Beaumont *et al*, 1980; Zouros and Foltz, 1984b). Also, heterozygote deficiency (or homozygote excess) decreases with age. According to Singh (1982), one, two and three year old *Crassostrea virginica* are increasingly deficient in heterozygotes; this is expecially true in slower growing animals. Diehl and Koehn (1985) found that *Mytilus edulis* was deficient in heterozygotes at 2 months of age, and reached the Hardy-Weinberg equilibrium at 4 months of age.

I analyzed genotypic and phenotypic variation to examine whether there was a correlation between heterozygosity and fitness. The following questions were asked : Does a population of juvenile scallops show heterozygote deficiency? Does heterozygote deficiency vary with age? Does multiple-locus heterozygosity correlate with growth rate? Does the nature of the correlation between heterozygosity and growth rate change with age within a population?

2.2 Materials and methods.

Juvenile scallops were collected at 2 locations on 6 different occasions (22 November 1984, 12 March 1985, 19 July 1985, 7 November 1985, 28 November 1985 and 11 December 1985). The Tongue Shoal site (45°03′47"N; 67°00′45"W), having a water depth of 20 m, and the Letang site (45°04′00"N; 66°48′30"W), having a water depth of 16 m, are situated in the macrotidal Passamaquoddy Bay, New Brunswick, Canada. Samples were collected using three different methods : spat collectors (Japanese spat bags of 3 mm mesh width stuffed with monofilament gillnet); lantern nets (with a mesh width of 9 mm); and from the bottom using SCUBA divers (Table 2.1). Spatfall was restricted to a period of two months, with peak spatfall occurring during a two week period in mid-October 1984, about two months after spawning (Dadswell *et al*, 1987). The discrete size-frequency distribution of juvenile scallops guaranteed that all individuals settled in the fall of 1984.

Scallops were transported from the field to Halifax (N.S.) within 12 hours, immersed in cooled seawater (at less than 8°C). In Halifax, they were kept in running sea water for a few days at ambient temperature in the Aquatron Laboratory of Dalhousie University. Subsequently whole animals or part of the adductor tissue were frozen and stored at - 70°C. Mortality between sampling and freezing was less than 2%.

Shell height (i.e. the distance measured between umbo and the ventral edge of the shell along a straight line) of the smaller individuals was measured under a binocular microscope fitted with a measuring ocular. The left valve of larger sized animals was measured with calipers. Assuming that scallops settled within a period of two weeks in mid-October (see above), the measured shell height reflected the actual growth rate.

Samples were prepared for electrophoresis by grinding either a small piece of adductor muscle (smaller than 1 g) from each animal or the complete animal (if smaller than 7 mm) on ice with a pestle in a 0.5 to 1.5 ml centrifuge tube (after

Table 2.1 : List of samples of *Placopecten magellanicus* collected in Passamaquoddy Bay (sample number, sample site, collection date (assuming that 15 October 1984 is day 1), age, sample size, mean shell height (including standard error) and method of collecting are given).

Sample number	Sample site	Collection date	Age (days)	Sample size	Shell height (±S.E.) (mm)	Method of collecting
1	Tongue Shoal	22.11.84	38	293	3.55 (±0.10)	Spat collectors
2	Tongue Shoal	12.03.85	148	310	11.17 (±0.15)	Spat collectors
3	Tongue Shoal	19.07.85	277	218	19.00 (±0.26)	Bottom
4	Tongue Shoal	07.11.85	388	124	34.06 (±0.28)	Lantern nets
5	Letang Estuary	28.11.85	409	290	26.54 (±0.21)	Bottom
6	Tongue Shoal	11.12.85	422	236	30.52 (±0.19)	Bottom

adding an equal amount of distilled water) followed by centrifugation at $12,000 \times g$ at $5°C$. The supernatant was used as enzyme source and analyzed by horizontal starch gel electrophoresis in two electrode buffer systems. The pH 8.0 tris-citrate gel contained 10 g of Electrostarch, 65 g of Sigmastarch and 600 ml of buffer; the pH 7.0 tris-citrate gel contained 8 g of Electrostarch, 67 g of Sigmastarch and 590 ml of buffer.

All animals were scored at 6 polymorphic loci : 6-phosphogluconate dehydrogenase (PGD, EC 1.1.1.44), octopine dehydrogenase (ODH, EC 1.5.1.11), aspartate aminotransferase (also called glutamate oxaloacetate transaminase) (AAT, EC 2.6.1.1), phosphoglucomutase (PGM, EC 2.7.5.1), mannosephosphate isomerase (MPI, EC 5.3.1.8) and glucosephosphate isomerase (PGI, EC 5.3.1.9). The enzymes aspartate aminotransferase 2, phosphoglucomutase 2, α-glycerophosphate dehydrogenase, two malate dehydrogenases, leucine aminopeptidase, two malic enzymes, sorbitol dehydrogenase, glutamate dehydrogenase and two superoxide dismutases proved monomorphic. The gel and tray buffer systems used were tris-citrate pH 8.0 (Selander *et al*, 1971) for PGD, AAT, MPI and PGI (run for 10 hours at 90 mA and 170 V), and tris-citrate pH 7.0 (Ayala *et al*, 1972) for PGM and ODH (run for 4 hours at 90 mA and 240 V). Stain recipes were taken from Selander *et al* (1971), Schaal and Anderson (1974) and Siebenaller (1979).

Allozymes (electromorphs) at each polymorphic locus were assigned mobility values in accordance with Foltz and Zouros (1984). Allele frequencies, observed (H_o) and expected (H_e) number of heterozygous individuals, heterozygote deficiency ($D = \frac{H_o - H_e}{H_e}$), G-test (Sokal and Rohlf, 1981) for fit to Hardy-Weinberg expectations and t-test of mean heterozygous and homozygous growth rate were calculated. Single-locus and multiple-locus linear regression analysis were done using the SPSSX statistical package.

2.3 Results.

The age of the scallops at scoring (counted from the date of peak settlement of 15 October 1984) varied between 38 days and 422 days. Insufficient staining of the zymograms considerably reduced sample size of sample 1 (22 November 1984) and sample 2 (12 March 1985); 28% and 42% respectively of the original sample was scored successfully at the loci PGI, PGD, MPI and ODH. Sample size varied between 124 and 310 animals (Table 2.1). Average size increased from 3.55 mm on 22 November 1984 to 30.52 mm on 11 December 1985. Growth occurred in spring and summer and was minimal in late fall and winter.

Estimates of allele frequency at 6 loci of 6 different samples agreed with previous electrophoretic surveys of *Placopecten magellanicus* by Foltz and Zouros (1985), Gartner-Kepkay and Zouros (1985), and K. Fuller and K. Ahmed (Dalhousie University, pers. comm.) (Table 2.2). There was no variation in allele frequency with age.

On average, the Passamaquoddy population was slightly deficient in heterozygotes ($\overline{D}$ = -0.065) (Table 2.2). Heterozygote deficiency varied among loci; PGM (-0.111) and ODH (-0.102) were the most deficient in heterozygotes, PGI had the smallest D-value. A comparison of D-values among loci within samples showed no significant deviation from the Hardy-Weinberg equilibrium, except for locus ODH on 22 November 1984 and locus PGI on 12 March 1985. Among samples, heterozygote deficiency was never significant and decreased with age from -0.121 to -0.013. Although most samples had been collected at the same site (Tongue Shoal), there were no absolute guarantees that the bottom and water collected samples had been subjected to similar environmental conditions. Therefore I considered the spatfall on the collectors as the common source of two separate cohorts; one cultured in suspended nets and one naturally grown on the bottom, both located at Tongue Shoal (Table 2.1). I calculated heterozygote deficiency not only at all 6 loci scored, but also at 4 loci (PGI, MPI, ODH and PGM) to allow comparison with the first

Table 2.2 : Allele frequency estimates, observed proportion of heterozygotes, D-values, G-test for fit to Hardy-Weinberg proportions (statistical significance, degrees of freedom at 6 polymorphic loci and number of individuals are given (* $P < 0.05$)).

Locus	Allele	22.11.84	12.03.84	19.07.85	07.11.85	28.11.85	11.12.85	$\overline{D}$ (±S.E.)
PGI	87	0.000	0.006	0.000	0.008	0.002	0.000	
	100	0.970	0.958	0.966	0.948	0.955	0.958	
	115	0.030	0.036	0.034	0.044	0.043	0.042	
	H_o	0.059	0.069	0.069	0.105	0.090	0.085	
	D	0.031	-0.142	0.036	0.048	0.045	0.044	0.010 (±0.031)
	G-test	0.526	10.328*	0.538	0.719	1.250	0.886	
	DF	1	3	1	3	3	1	
	N	287	275	218	124	289	236	
PGD	80	0.000	0.000	0.002	0.008	0.000	0.000	
	100	0.897	0.823	0.806	0.790	0.802	0.808	
	130	0.000	0.000	0.000	0.000	0.000	0.005	
	150	0.103	0.177	0.192	0.202	0.196	0.187	
	180	0.000	0.000	0.000	0.000	0.002	0.000	
	H_o	0.161	0.284	0.278	0.282	0.326	0.321	
	D	-0.133	-0.028	-0.116	-0.157	0.026	0.031	-0.063 (±0.034)
	G-test	1.242	0.106	3.276	3.657	0.640	1.047	
	DF	1	1	3	3	3	3	
	N	87	141	216	124	288	224	
AAT	50	-	0.004	0.011	0.008	0.016	0.006	
	70	-	0.008	0.021	0.033	0.009	0.009	
	80	-	0.000	0.000	0.000	0.000	0.004	
	90	-	0.433	0.449	0.447	0.392	0.411	
	100	-	0.555	0.505	0.504	0.576	0.557	
	105	-	0.000	0.000	0.000	0.000	0.011	
	115	-	0.000	0.014	0.008	0.007	0.002	
	H_o	-	0.383	0.514	0.496	0.524	0.500	
	D	-	-0.241	-0.053	-0.090	0.021	-0.039	-0.080 (±0.044)
	G-test	-	9.302	13.292	2.390	4.388	8.404	
	DF	-	6	10	10	10	21	
	N	-	128	218	123	288	236	

(continued)

Locus	Allele	22.11.84	12.03.84	19.07.85	07.11.85	28.11.85	11.12.85	$\overline{D}$ (±S.E.)
MPI	75	0.000	0.003	0.000	0.000	0.000	0.002	
	87	0.219	0.172	0.220	0.186	0.208	0.225	
	100	0.781	0.816	0.775	0.806	0.773	0.765	
	108	0.000	0.009	0.005	0.008	0.019	0.008	
	H_o	0.299	0.259	0.330	0.331	0.356	0.377	
	D	-0.127	-0.148	-0.058	0.049	-0.006	0.035	-0.043 (±0.034)
	G-test	3.052	7.106	1.841	1.534	3.076	3.484	
	DF	1	6	3	3	3	6	
	N	201	166	218	124	289	236	
ODH	96	0.000	0.000	0.000	0.000	0.000	0.002	
	98	0.003	0.002	0.007	0.004	0.003	0.010	
	100	0.389	0.393	0.459	0.432	0.481	0.473	
	105	0.609	0.605	0.534	0.564	0.516	0.513	
	107	0.000	0.000	0.000	0.000	0.000	0.002	
	H_o	0.356	0.430	0.532	0.468	0.457	0.432	
	D	-0.255*	-0.104	0.056	-0.055	-0.092	-0.159	-0.102 (±0.042)
	G-test	13.546	3.522	0.927	1.545	5.182	17.823	
	DF	3	3	3	3	3	10	
	N	202	228	218	124	289	236	
PGM	96	-	0.000	0.005	0.000	0.004	0.002	
	98	-	0.086	0.128	0.073	0.128	0.108	
	100	-	0.650	0.612	0.673	0.646	0.653	
	102	-	0.264	0.250	0.254	0.215	0.231	
	104	-	0.000	0.005	0.000	0.007	0.006	
	H_o	-	0.472	0.481	0.355	0.482	0.483	
	D	-	-0.057	-0.119	-0.256	-0.072	-0.051	-0.111 (±0.038)
	G-test	-	5.742	10.882	12.365	8.418	5.365	
	DF	-	3	10	3	10	10	
	N	-	193	214	124	284	236	
$\overline{D}$ (S.E.)		-0.121 (±0.059)	-0.120 (±0.031)	-0.042 (±0.030)	-0.077 (±0.049)	-0.013 (±0.023)	-0.023 (±0.032)	-0.065 (±0.018)

sample (in which only 4 loci had been scored). The decrease in the deficiency of heterozygotes with age, tested under these conditions, turned out to be significant (non-parametric probability test, $P < 0.05$, $DF = 1$) (Figure 2.1).

I regressed shell height on the degree of heterozygosity in all six samples to test whether fitness (measured as growth rate) increased with the number of heterozygous loci observed. All six linear regression were positive and insignificant (Table 2.3.A) indicating that growth rate was not correlated with heterozygosity in juvenile scallops. In a slightly different analysis, mean growth rates per shell height interval were recalculated, and regressed on the average degree of heterozygosity (the procedure reduces overall variation and is useful in detecting trends). Five correlations were positive and insignificant, one (28 November 1985) was positive and significant (Table 2.3.B).

In a second test I compared the average shell height of homozygous and heterozygous scallops per locus and per sample. Only four comparisons were significant, of which three belonged to the 12 March 1985 sample. Heterozygotes had faster growth rates than homozygotes in 21 cases of the 34 cases considered ($\chi^2 = 2.94$, $DF = 1$, $0.05 < P < 0.1$) (Table 2.4).

The small number of significant correlations between heterozygosity and growth, and the comparisons of the average growth rate of hetero- and homozygous individuals provide evidence for the hypothesis that growth rate of juvenile scallops is not a function of the number of heterozygous loci. The six polymorphic loci scored do not measurably explain the variability in growth rate in my samples.

I tested for temporal variation in the frequency of the number of heterozygous loci in all samples with a $R \times C$ test of independence (Figure 2.2). Frequencies of heterozygosity classes were not independent of age ($G = 101.78$, $DF = 15$, $P < 0.005$). However, no shift of the heterozygote frequency with age was observed. Taking into consideration the different environments in which the samples were collected, I compared the samples collected on the bottom (sample 3 and 6), and the samples collected from spat collectors and lantern nets (samples 1 and 2, 2

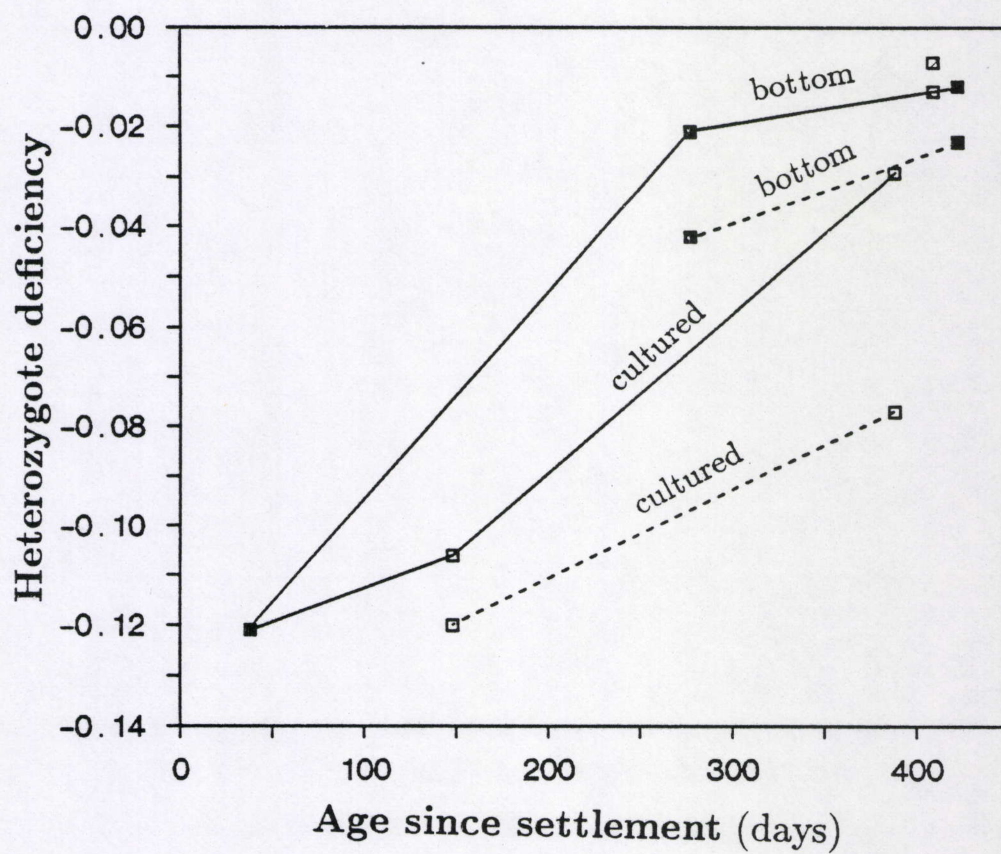


Figure 2.1 : Average heterozygote deficiency (D) and age since settlement of bottom collected and cultured *Placopecten magellanicus*; ——- 4 loci scored; - - - - 6 loci scored.

Table 2.3.A : Linear regression of shell height and degree of heterozygosity (at 4 (22.11.84) or 6 loci) (the coefficient of determination (r^2), probability (P) and degrees of freedom (DF = N-1) are given).

Heterozygosity = A + (B × Shell Height)					
Date	A (Intercept)	B (Slope)	r^2	P	DF
22.11.84	0.624	0.045	0.002	0.553	162
12.03.85	0.928	0.064	0.017	0.142	128
19.07.85	1.835	0.020	0.005	0.326	211
07.11.85	1.787	0.007	0.001	0.689	122
28.11.85	1.574	0.025	0.009	0.103	281
11.12.85	2.197	0.000	0.000	0.994	223

Table 2.3.B : Linear regression of mean shell height (averaged per shell height interval) on degree of heterozygosity (the coefficient of determination (r^2), probability (P) and degrees of freedom (DF = (number of shell height intervals) - 1) are given).

Heterozygosity = A + (B × Mean Shell Height)					
Date	A (Intercept)	B (Slope)	r^2	P	DF
22.11.84	0.561	0.057	0.057	0.480	10
12.03.85	0.793	0.083	0.439	0.073	7
19.07.85	1.854	0.020	0.093	0.426	8
07.11.85	1.867	0.004	0.016	0.765	7
28.11.85	1.417	0.032	0.512	0.030	8
11.12.85	1.786	0.010	0.036	0.625	8

Table 2.4 : Average shell height (mm) of homo- and heterozygous juvenile scallops per locus and per sample (the direction of the score (+ or −), the number of individuals sampled and the significance are given ($*\ P < 0.05$; $**\ P < 0.01$); $+\ (Height_{het} - Height_{hom}) > 0$; $-\ (Height_{het} - Height_{hom}) < 0$).

Date Locus		22.11.85	12.03.85	19.07.85	07.11.85	28.11.85	11.12.85
PGI	Homozygote	3.56	4.37	18.93	34.10	26.59	30.56
	Heterozygote	3.92	4.72	19.98	33.67	26.03	30.13
	Direction	+	+	+	−	−	−
	N	287	275	210	124	289	236
PGD	Homozygote	3.96	5.34	19.07	33.98	26.48	30.52
	Heterozygote	4.42	5.12	18.83	34.26	26.78	30.41
	Direction	+	−	−	+	+	−
	N	87	141	216	124	288	224
AAT	Homozygote	-	5.40	18.84	34.12	26.66	30.71
	Heterozygote	-	5.93	19.15	33.95	26.49	30.33
	Direction	-	+*	+	−	−	−
	N	-	128	218	123	288	236
MPI	Homozygote	3.71	4.89	19.02	33.97	26.10	30.48
	Heterozygote	3.90	5.05	18.97	34.23	27.34	30.59
	Direction	+	+	−	+	+ **	+
	N	201	166	218	124	289	236
ODH	Homozygote	3.81	4.57	18.95	34.35	26.34	30.26
	Heterozygote	3.80	5.00	19.05	33.72	26.78	30.87
	Direction	−	+*	+	−	+	+
	N	202	228	218	124	289	236
PGM	Homozygote	-	4.56	18.60	33.51	26.22	30.65
	Heterozygote	-	5.07	19.45	35.05	26.90	30.39
	Direction	-	+*	+	+	+	−
	N	-	193	214	124	284	236

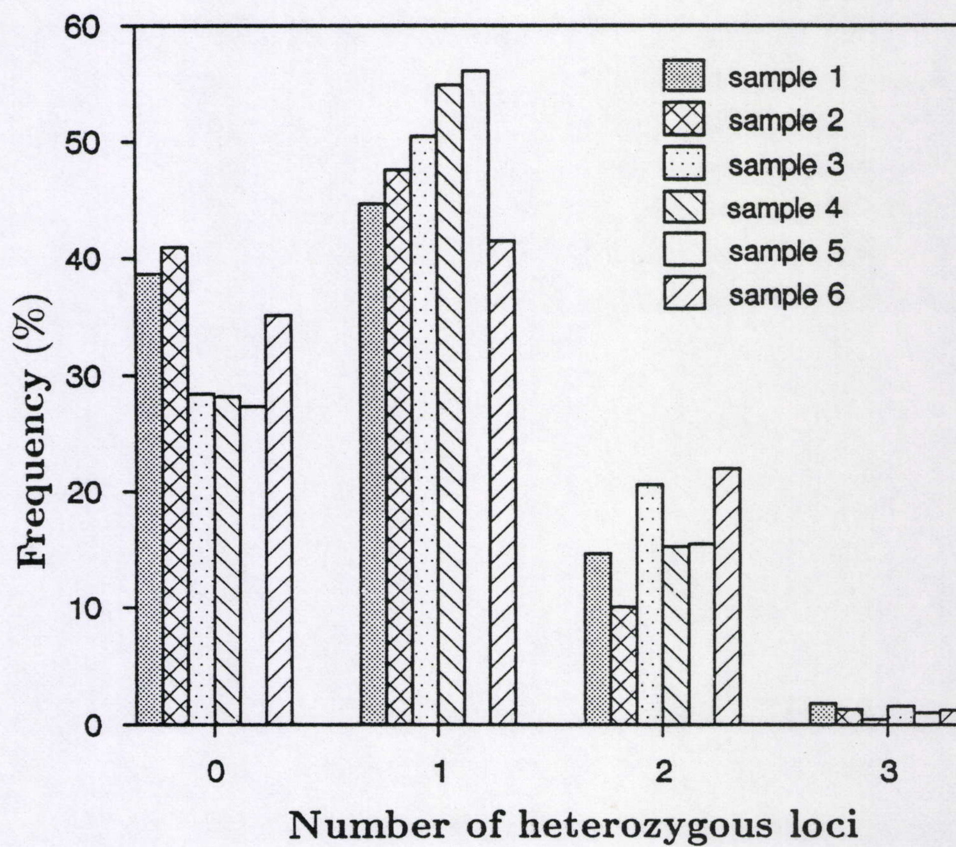


Figure 2.2 : Frequency distribution of heterozygosity classes between samples collected (loci used to calculate heterozygosity are PGI, MPI, ODH and PGM).

and 4) separately in a $R \times C$ test. No comparison was significant (respectively $G = 4.59$, $DF = 1$, $N.S.$; $G = 1.73$, $DF = 3$, $N.S.$ and $G = 5.37$, $DF = 3$, $N.S.$).

Finally, I tested whether differences in relative viability (i.e. the relative change in allele frequency between two age classes) covaried among samples. No trend similar to the one documented in *Crassostrea virginica* was observed (Zouros *et al*, 1983).

2.4 Discussion.

Important findings of this chapter were the decrease with age of heterozygote deficiency of 2 to 13 month old juvenile scallops and the absence of a correlation between the degree of multiple- or single-locus heterozygosity and growth rate. This points to a non-universal nature of the connection between heterozygosity and growth.

The experimental design did not include certain aspects which were considered critical for detecting a correlation between growth and multiple-locus heterozygosity. The following factors may have prevented the detection of a positive correlation between degree of heterozygosity and phenotype if it exists : (1) The larger the number of loci included, the stronger the correlation between growth and heterozygosity and the smaller the variance (Koehn *et al*, 1988). Heterozygosity was measured at 6 polymorphic loci (12 other loci turned out to be monomorphic), which represents a relatively small subsample of the total number of soluble proteins. (2) Non-genetic (notably environmental) factors could have affected the phenotypic trait measured. I have tried to minimize this by collecting most samples from the same site (Tongue Shoal) and, when appropriate, by differentiating between samples collected in the water column and on the bottom.

Populations of juvenile scallops in Passamaquoddy Bay were deficient in heterozygous individuals, an observation which confirms numerous similar records of

molluscs, including pectinids (Wilkins, 1978; Beaumont *et al*, 1980, 1985; Beaumont, 1982; Beaumont and Beveridge, 1984; Foltz and Zouros, 1984). A compilation of locus specific D values of *P. magellanicus* shows that there appears to be no tendency toward heterozygote deficiency since there are 49 negative values out of 88. But, 12 of the 49 negative values are significant, and only one of 39 positive values is significant. This is reflected in a mean heterozygote deficiency in 14 of 16 populations and 4 of 6 loci. ODH and MPI are nearly always deficient in heterozygotes (Table 2.5). An interspecific comparison of Mollusca generally confirms that ODH is on average deficient in heterozygotes in *Mytilus edulis* (Diehl and Koehn, 1985), *P. maximus* and *Chlamys distorta* (Beaumont and Beveridge, 1984), but not in *C. opercularis* and *C. varia* (Beaumont and Beveridge, 1984). I did not find comparable records for MPI. Other loci have variable D-values among species.

Heterozygote deficiency decreased with age in *P. magellanicus*; the older the scallop the more the observed and expected degree of heterozygosity approached the Hardy-Weinberg equilibrium. A similar result was observed in one to three year old *C. virginica* (Zouros *et al*, 1980) and two to seven month old *M. edulis* (Koehn and Gaffney, 1984; Diehl and Koehn, 1985). In the latter study the significant deficiency in heterozygotes had disappeared at the ages of 4 and 7 months.

Several mechanisms have been examined to explain heterozygote deficiency in molluscs (Zouros and Foltz, 1984a, 1984b), including inbreeding, null alleles, the Wahlund effect and self-fertilisation. (1) Self-fertilisation is not a common phenomenon in *P. magellanicus.* It is unlikely that those animals which are hermaphroditic (which is less than 1% of all mature animals (Naidu, 1970)) will spawn simultaneously. By comparison, the hermaphrodite *Pecten maximus* shows little evidence of this phenomenon (Beaumont and Beveridge, 1984). Single-locus D-values do not covary and thus do not support the possibility that inbreeding is occurring. (2) No null alleles have been found in *P. magellanicus* (Foltz and Zouros, 1984). (3) The Wahlund effect is an unlikely explanation for the observed effects. Population differences, established on the basis of allozyme genotype, seem to be

Table 2.5 : Geographical variation of heterozygote deficiency (D) between loci of *P. magellanicus* (* $P < 0.05$; ** $P < 0.01$).

Source	PGI	PGD	AAT	MPI	ODH	PGM	$\overline{D}$ (±S.E.)
This study							
	0.010	-0.063	-0.080	-0.043	-0.102	-0.111	-0.065 (±0.018)
Foltz and Zouros (1984)							
	-0.007	-0.013	0.017	-0.050	-0.264**	-0.130**	-0.075 (±0.043)
Gartner-Kepkay and Zouros (1985)							
Middle Ground	0.042	0.107	-0.091	-0.049	-0.180**	-	-0.034 (±0.050)
Browns Bank	0.062	-0.059	0.145	0.063	-0.350**	-	-0.028 (±0.087)
Bay of Fundy	0.058	0.013	0.105	-0.009	-0.204*	-	-0.007 (±0.053)
Second Peninsula	0.058	-0.033	0.067	-0.178*	-0.204**	-	-0.058 (±0.057)
Grand Manan	0.058	-0.170*	0.100	-0.022	-0.065	-	-0.020 (±0.047)
Georges Bank (S)	0.053	0.042	-0.083	-0.255*	-0.282**	-	-0.105 (±0.071)
Georges Bank (N)	-0.008	0.184*	-0.024	0.042	-0.122	-	0.014 (±0.050)
Georges Bank (NE)	0.042	0.061	0.088	-0.232*	-0.084	-	-0.025 (±0.060)
K. Fuller and K. Ahmad (pers. comm.)							
Baie des Chaleurs	0.047	-0.131	-0.160	-0.241	0.000	-0.241	-0.121 (±0.049)
Passamaquoddy B.	0.059	-0.015	0.026	0.165	-0.065	-0.252	-0.014 (±0.057)
Isle Haute	-0.299	0.055	0.092	0.092	-0.268	-0.021	-0.058 (±0.073)
St.Pierre Bank	-0.370**	-0.269	0.027	-0.121	0.014	-0.082	-0.134 (±0.065)
Georges Bank	0.054	0.123	0.213	0.075	0.074	0.145	0.114 (±0.024)
Halifax	0.000	-0.036	-0.188	-0.200	0.086	-0.141	-0.080 (±0.047)
$\overline{D}$	-0.009	-0.013	0.016	-0.060	-0.126	-0.104	
(±S.E.)	(±0.033)	(±0.029)	(±0.028)	(±0.033)	(±0.033)	(±0.045)	

small on the Scotian Shelf and in the Bay of Fundy (Gartner-Kepkay and Zouros, 1985; K. Fuller and K. Ahmad, pers.comm.). (4) Differential recruitment and non-random mating are possible mechanisms of natural selection. Spawning occurs in a series of pulses during several weeks in late summer and fall but the mechanisms influencing fertilisation are unknown. Another possible effect on heterozygote deficiency is differential survival. This study and the results of Zouros *et al* (1980), Diehl and Koehn (1985) and Mallet *et al* (1985, 1986) support the argument that under natural selection certain genotypes survive better, but do not provide sufficient proof of it. It is debatable to what extent the marker loci themselves or the associated genome are affecting natural selection (see Chapter 5).

Heterozygosity and growth rate are not correlated in adult *P. magellanicus* (Foltz and Zouros, 1984). Despite my efforts to concentrate on genotype dependent growth in juveniles, such a phenomenon was not observed. Three other studies of pectinids which addressed a similar question have been summarized in the introduction.

Nevertheless, evidence is growing that younger animals are more likely to show the effect of heterozygosity on growth. Two month old heterozygous mussels grow faster than homozygotes; growth of 4 month and 7 month old animals is less uniform and fails to show a positive correlation with growth (Diehl and Koehn, 1985; Koehn and Gaffney, 1984). It has been suggested that the increasing importance of gamete production in the metabolism of older animals determines the nature and magnitude of the correlation. Unfortunately an age specific shift in the growth-heterozygosity correlation could not be tested in juvenile *P. magellanicus* because of the total absence of a correlation.

The question arises whether there is a genetic mechanism that can explain the above observations. A survey of the literature for trends between heterozygote deficiency and the growth-heterozygosity correlation points toward a possible association between the two. A positive correlation is often associated with a deficit of

heterozygotes (Zouros, 1987). The few published time series are especially instructive in evaluating the significance of the relation between D and r. Populations of *C. virginica* are strongly deficient in heterozygotes at the age of 1 and 2 years; mean growth is correlated with the number of heterozygous loci per individual (Singh, 1982) at these ages. The growth-heterozygosity correlation of *M. edulis* changes from positive and significant at 2 months of age (when D is significantly negative) to negative and significant at 4 months and non-significant at 7 months of age (when D is not significantly different from zero) (Koehn and Gaffney, 1984; Diehl and Koehn, 1985). Populations of *P. magellanicus* had a decreasing deficit in heterozygotes with age, evolving from moderately deficient at 2 months of age to an equilibrium at 14 months of age. Growth rate - heterozygosity correlations were not significant although positive.

The observations in the literature that the expression of heterozygote deficiency and the correlation between heterozygosity and growth are time dependent prompts the question how large the heterozygote deficiency has to be before a strong positive correlation between growth and heterozygosity will be observed. According to the associative overdominance hypothesis the degree of heterozygosity adjacent to the marker enzyme is reflected by the degree of heterozygosity of the marker enzyme. The chances of an adjacent locus being homozygous increases with the degree of homozygosity of the marker locus itself, which in turn enhances the chances of detrimental effects from recessive deleterious genes. We know experimentally that heterozygous individuals are fitter than homozygotes in a number of cases. A population that is not in Hardy-Weinberg equilibrium is characterized by a discrepancy between the expected number of homozygotes and heterozygotes. The extent of the deficiency in heterozygotes enhances the effect of heterozygosity on growth. If heterozygote deficiency decreases with time, homozygotes are eliminated (i.e homozygotes which are affected by recessive detrimental genes) and the impact of homozygotes on fitness decreases proportionally; the positive correlation disappears

with time. On the other hand, if no selective mortality of homozygotes occurs, heterozygote deficiency remains high and constant with time; heterozygote deficiency remains a constant feature of the population because mortality is equally divided among all genotypes. The impact of recessive deleterious genes on the homozygotes is insignificant. Heterozygosity thus affects fitness through the detrimental effects of selected homozygous deleterious genes.

In conclusion, circumstantial evidence leads me to suspect a link between heterozygote deficiency and multiple-locus effects in molluscs. Multiple-locus heterozygosity and fitness are not related in pectinids, unlike several other molluscs, because of a low heterozygote deficiency. The driving forces behind the presence or absence of a correlation remain elusive. In the following chapter I test whether several fitness variables related to the growth physiology of scallops are linked with multiple-locus heterozygosity under routine and basal metabolic conditions.

Chapter 3.

Genetic and physiological correlates of unstressed and stressed scallops (*Placopecten magellanicus* (Gmelin)).

3.1 Introduction.

The positive correlation between the degree of heterozygosity and growth in several plant species, vertebrates and invertebrates has generated considerable interest. Nevertheless, the phenomenon is not a general one (see Chapter 2). Enzyme polymorphism has been used as a measure of genotypic variation in theoretical (Turelli and Ginzburg, 1983; Zouros *et al*, 1988), physiological (Danzmann *et al*, 1987; Mitton *et al*, 1986; Zouros and Foltz, 1987) and biochemical (Koehn, 1985; Koehn *et al*, 1988) studies that have focused on the causality of heterosis.

Studies of Mollusca have been useful in elucidating the correlation between the degree of heterozygosity and fitness (Zouros, 1987). Several factors contribute to this success : (1) growth is indeterminate, is integrated during time in the hard shell and may be unambiguously measured, (2) the general biology is reasonably well known (Wilbur, 1983), (3) physiological traits may be easily measured in a straightforward manner (Bayne *et al*, 1985), (4) polymorphic loci have a high degree of heterozygosity (Powell, 1975) and (5) molluscs are easy to collect, handle and mark.

One of the original experiments which examines the consequences of a conservative metabolic strategy for growth shows that starved oysters that have a higher degree of multiple-locus heterozygosity have lower weight-specific oxygen uptake rates (Koehn and Shumway, 1982). Under stress (i.e. at lower salinity and higher temperature) more heterozygous oysters are characterized by a proportionally lower basal metabolic loss. Different metabolic rates in heterozygous and homozygous individuals have been detected in a small number of other molluscs. Heterozygous *Mulinia lateralis* have lower standard metabolic costs, resulting in a higher scope

for growth than in homozygous conspecifics (Garton *et al*, 1984). Similarly, heterozygous *Mytilus edulis* have lower oxygen uptake rates than their homozygous conspecifics (Diehl *et al*, 1985). Decreased energy requirements for maintenance, resulting from a greater efficiency of protein synthesis, allow a higher growth rate of heterozygous *M. edulis* (Hawkins *et al*, 1986). In contrast to the higher metabolic efficiency of heterozygous *M. lateralis* and *M. edulis*, the higher scope for growth of heterozygous *Thais haemastoma* (Garton, 1984) and *T. lamellosa* (Garton and Stickle, 1985) is linked to significantly higher feeding rates. Similarly, clearance rate (i.e. feeding activity) in *Rangia cuneata* increases with the degree of heterozygosity (Holley and Foltz, 1987). Breeding experiments with bivalves show that background genotype affects the correlation between heterozygosity and growth rate (Gaffney and Scott, 1984; Mallet *et al*, 1986). Similarly, no negative correlation between heterozygosity and weight-specific oxygen uptake is visible in the progeny of a single wild caught pair of *M. lateralis* (Gaffney and Scott, 1984).

The impact of genotype on metabolism in organisms might be more pronounced under stress. I mentioned previously the proportionally lower oxygen uptake in heterozygous *C. virginica* under suboptimal temperature and salinity (Koehn and Shumway, 1982). Heterozygous *M. edulis* have a lower weight-specific oxygen uptake rate and weight loss during starvation (Diehl *et al*, 1986). In another study, the rate of weight loss of starved *C. virginica* is significantly correlated with heterozygosity (Rodhouse and Gaffney, 1984), but no correlation is observed between heterozygosity and other metabolic traits (weight-specific oxygen uptake, ammonia excretion rate, O:N ratio and carbohydrate depletion). *Macoma baltica* growing in the middle intertidal of a subarctic environment are genetically more heterozygous and grow faster than in the low intertidal (Green *et al*, 1983).

Chapter 2 focused on a genetic investigation of growth variation in juvenile scallops. This chapter concentrates on a physiological relationship between genotypic and growth variation. I have already found that growth in juvenile scallops is not significantly related to the degree of heterozygosity and that the small deficiency

in heterozygosity might be causally related to this observation. I postulate that heterozygosity affects the ecophysiology of *Placopecten magellanicus* and that the effects of heterozygosity are more easily detected in scallops subjected to stress. To test these hypotheses, I compare the metabolism of a single population of scallops before and after starvation. I examine (1) whether there is a correlation between individual heterozygosity and fitness in juvenile scallops when using a number of traits : shell height, tissue dry weight, oxygen uptake, ammonia excretion, total caloric loss, O:N ratio and carbohydrate content, and (2) whether the correlation among these traits and genotype is affected when scallops are stressed.

3.2 Materials and methods.

Thirteen month old scallops (*Placopecten magellanicus*) were collected in Passamaquoddy Bay ($45°03'47"N$; $67°00'45"W$) (N.B., Canada) by SCUBA divers in November 1985 (see Chapter 2). They were held in running seawater at the nearby Biological Station in St.Andrews (N.B., Canada) for a few days, transported in cooled seawater to Halifax (N.S.) and returned to running (sand filtered) seawater at the Aquatron Laboratory of Dalhousie University.

Fifty scallops were subsampled for allometric analysis. The remaining scallops were separated into three size classes : small (less than 21 mm), medium (21 to 33 mm) and large (larger than 33 mm). The small and large scallops were frozen immediately and not used in this study. The remaining individuals were subdivided into two groups of 100 and 110. The first was drip-fed a mixture of algae (*Isochrysis galbana* clone T-iso, *Chaetoceros gracilis* and *Chaetoceros calcitrans*) at a concentration of 10 to 30×10^3 cells$\cdot$ml^{-1}. After one day in an algae-free flow-through tank to clear their digestive systems, oxygen uptake and ammonia excretion were measured at a temperature of 10.5°(±0.5°) C. These animals were designated "unstressed". The second group was kept in 0.2 μm-microfiltered seawater without food at a temperature of 14.5°(±0.5°) C for four weeks. Fifteen (i.e 14 %) of the starved animals

died during the period. Oxygen uptake and ammonia excretion were measured at a temperature of 14.5°(±0.5°) C. The starved animals were designated "stressed".

Frozen muscle extract of each scallop was run on horizontal starch gels, stained and scored for the allozymes PGI, PGD, AAT, MPI, ODH and PGM. The methods have been described in Chapter 2.

Shell height was measured with calipers to the nearest 0.1 mm between umbo and ventral edge of the shell along a straight line. Shell weight and wet muscle weight (i.e. the phasic and catch part of the adductor muscle) (WMUW) were measured with a precision of 1 mg. The subsample of 50 individuals was used to predict the ash free dry weight (AFDW) of each unstressed and stressed scallop with the following power function :

Unstressed :

$$AFDW = 0.325 \times WMUW^{0.900} \quad (r^2 = 0.98;\ n = 30)$$

Stressed :

$$AFDW = 0.214 \times WMUW^{0.813} \quad (r^2 = 0.74;\ n = 20)$$

The AFDW of an individual is the difference between the weight of the soft tissue dried for 24 h at 60° C and the weight of the same tissue ashed in a muffle furnace at 490° C for 3 h. The condition index is the ratio of $AFDW \times 100$ to shell dry weight (Mann, 1978).

Scallops were transferred from the holding tank to individual respiration chambers under water to avoid trapping air bubbles in the mantle cavity. Direct handling of the individual scallops was kept to a minimum since they usually responded to disturbance with valve snaps, which induced functional anaerobiosis and created an oxygen debt (see Chapter 4). The oxygen saturation level was not allowed to drop below 75% because oxygen uptake of *P. magellanicus* is dependent on oxygen tension up to 70% saturation (Shumway *et al*, 1988). Triplicate incubation measurements were made in sand filtered air-saturated seawater (salinity 31 ppt) using

cylindrical acrylic chambers with lid-mounted stirring bars (Grant and Hargrave, 1987).

The beginning of the incubations involved sealing the chambers with the top lid, closing the outlet and the inlet a few minutes later, and starting the freely rotating stirring bar. Each series of 11 vessels included one control vessel without a scallop. No bacterial respiration was detected during the relatively short duration of the incubation.

Oxygen concentrations were determined with a micro-Winkler technique (Burke, 1962) on 4 ml subsamples collected in glass syringes. Shell volume was subtracted from the chamber volume when calculating animal-specific oxygen uptake rates. Ammonia was determined spectrophotometrically with a modified method of Solórzano (1969) and Grasshoff (1976).

Whole animal physiological rates (V_m) were standardized to weight-specific rate variables ($\dot{V}_m$) :

$$\dot{V}_m = V_m \times \left(\frac{AFDW_{av}}{AFDW_{meas}}\right)^b$$

(Bayne and Newell, 1983)

V_m is the animal specific physiological rate (μg-at·h^{-1}· ind^{-1}); $\dot{V}_m$ is the weight-specific physiological rate (μg-at·h^{-1}·(std ind)$^{-1}$); $AFDW_{av}$ is the average tissue weight of the scallops assayed within each treatment; $AFDW_{meas}$ is the individually estimated tissue weight of each scallop; b is a fitted parameter (i.e. slope) determined from least-squares regression of log_{10} transformed values of AFDW and the physiological rate considered ($V_m = a \times AFDW^b$). Total caloric loss was calculated as follows :

$$\dot{V}_{cal} = \dot{V}_{cal(O_2)} + \dot{V}_{cal(NH_4)}$$

with

$$\dot{V}_{cal(O_2)} = \mu g{-}at\ O_2\ consumed \cdot h^{-1} \cdot (std\ ind)^{-1} \times 0.45 \cdot J \cdot (\mu g{-}at\ O_2)^{-1}$$

(Gnaiger, 1983)

$$\dot{V}_{cal(NH_4)} = \mu mol\ NH_4 excreted \cdot h^{-1} \cdot (std\ ind)^{-1} \times 0.34 \cdot J \cdot (\mu g - at^{-1}\ NH_4)^{-1}$$

(Elliott and Davison, 1975)

The net energy exchange in the organism is described in the balanced energy equation as follows (Winberg, 1971) :

$$P = A - (R + U) = -(R + U) < 0$$

P is the energy incorporated (or lost) as growth and reproductive products. Assimilation is zero (A = 0) because no food was ingested during the preceding 24 hr (if unstressed) or during the preceding 4 weeks (if starved). R is the energy equivalent of the metabolic heat loss; U is the energy content of excreta.

The atomic ratio is the molar fraction of oxygen consumed per mol ammonia nitrogen excreted; it is an indicator of the substrate catabolised (Barber and Blake, 1985).

The carbohydrate content of the adductor muscle was determined enzymatically. A known weight of phasic adductor tissue was ground with a mortar and pestle on dry ice; 5 ml of perchloric acid (0.6 N $HClO_4$) were added per gram tissue and homogenized. A volume of 0.1 ml of homogenate, neutralized with 0.05 ml of potassium bicarbonate ($KHCO_3$), was digested with amyloglucosidase for 2 h at 40° C (pH 4.8) on a rotating wheel. The hydrolysis of glycogen to glucose was halted with 0.1 ml of $HClO_4$ and the sample centrifuged for 15 min. A solution of 1 ml ATP, $NADP^+$, glucose-6-phosphate dehydrogenase and buffer (pH 7.5) was added, the extinction of $NADP^+$ measured at 340 nm, 5 ml of hexokinase added and 30 min later the extinction of $NADP^+$ measured. The glucose liberated after hydrolysis was proportional to the increase in $NADP^+$ measured by the change in extinction (Keppler and Decker, 1974). The glucose measured was the sum of an estimated 90% tissue glycogen and 10% tissue glucose in the phasic part of the adductor muscle (wet weight), and was designated carbohydrate.

3.3 Results.

3.3.1 Shell height, shell weight, ash free dry weight and condition.

The unstressed and stressed scallops were comparable in average shell height and degree of heterozygosity, which confirmed that both subsamples were haphazardly selected from the original sample (Table 3.1). Shell dry weight decreased on average 8% during four weeks of starvation. WMUW decreased 34 % and the estimated AFDW 48% (from 105 mg to 55 mg). The condition index, which integrated the effects of a small reduction in shell weight and a larger reduction in AFDW, decreased 43%.

Shell height and AFDW were not correlated with the degree of heterozygosity, i.e. growth rate was not affected by the polymorphic loci scored (Table 3.4). Analysis of covariance showed that weight-loss was strongly affected by starvation but not by genotype (Table 3.5). Average weight-loss was not affected by the degree of heterozygosity. The difference in AFDW between stressed and unstressed individuals did not vary with the number of heterozygous loci (Table 3.6).

3.3.2 Total metabolic loss.

Oxygen uptake declined from an average of 4.47 to 2.73 μg-at$\cdot h^{-1} \cdot ind^{-1}$ (39%) after 4 weeks of starvation (Table 3.1). This change integrated a decline of V_{O_2} from the unstressed (or resting) metabolic rate (which is equivalent to the routine rate (Bayne and Newell, 1983)) to the stressed metabolic rate (which is equivalent to the basal rate (Bayne and Newell, 1983)), a higher assay temperature and a decrease in dry tissue weight. Starvation had no significant effect on the slope of the regression between oxygen uptake and shell height (Figure 3.1) or AFDW (Table 3.2) (ANCOVA, $F = 1.67$; $DF = 5$; $P > 0.05$). The excretion rate increased 38% from 0.16 to 0.22 μmol$\cdot h^{-1} \cdot ind^{-1}$. Ammonia excretion represents 60 to 90 % of all measured nitrogen excretion in bivalves (Bayne, 1976). The scaling of the excretion rate was not affected by starvation (Figure 3.2). The total caloric loss amounted to

Table 3.1 : Mean values of phenotypic traits and degree of heterozygosity of unstressed and stressed juvenile scallops (S.E., percent difference and one-tailed t-test are given) (* $P < 0.05$; ** $P < 0.001$)).

Variable (unit)	Unstressed (N = 100) (10.5°C)	Stressed (N = 95) (14.5°C)	% diff.	t-test
Shell height (mm)	27.7 (±0.3)	27.1 (±0.3)	- 2	1.47
WMUW (mg)	288 (±10)	191 (±8)	- 34	7.76**
AFDW (mg)	105 (±3)	55 (±2)	- 48	13.59**
SHDW (mg)	1397 (±46)	1278 (±43)	- 9	1.90*
Condition index (-)	7.68 (±0.12)	4.41 (±0.07)	- 43	24.09**
V_{O_2} ($\mu g-at \cdot hr^{-1} \cdot ind^{-1}$)	4.47 (±0.17)	2.73 (±0.09)	- 39	8.93**
V_{NH_4} ($\mu mol \cdot hr^{-1} \cdot ind^{-1}$)	0.16 (±0.01)	0.22 (±0.01)	+ 38	- 5.25**
$V_{cal}(J \cdot hr^{-1} \cdot ind^{-1})$	2.06 (±0.08)	1.30 (±0.04)	- 37	8.34**
O:N (-)	31.2 (±1.2)	12.7 (±0.3)	- 59	15.55**
Carboh.($\mu mol \cdot (g \cdot WMUW)^{-1}$)	31.7 (±2.4)	4.8 (±0.3)	- 85	10.99**
Heterozygosity (-)	2.26 (±0.10)	2.17 (±0.12)	- 4	0.58

Table 3.2 : Linear least squares regression of absolute oxygen uptake, absolute excretion rate, absolute total caloric loss, O:N ratio and carbohydrate content on ash free dry weight (AFDW) (mg) of unstressed and stressed juvenile *P. magellanicus*. Coefficient of determination (* $P < 0.05$; ** $P < 0.001$) and sample size are given.

Regression formula	Unit	r^2	N
Unstressed scallops :			
$V_{O_2} = 17.32 \times AFDW^{0.618}$	$\mu g{-}at \cdot h^{-1} \cdot ind^{-1}$	0.279**	100
$V_{NH_4} = 1.09 \times AFDW^{0.877}$	$\mu mol \cdot h^{-1} \cdot ind^{-1}$	0.369**	97
$V_{cal} = 8.30 \times AFDW^{0.632}$	$J \cdot h^{-1} \cdot ind^{-1}$	0.283**	97
$O : N = 16.21 \times AFDW^{-0.254}$	-	0.038	97
Carb. $= 17.09 \times AFDW^{-0.138}$	$\mu mol \cdot (g\ WMUW)^{-1}$	0.003	100
Stressed scallops :			
$V_{O_2} = 27.56 \times AFDW^{0.803}$	$\mu g{-}at \cdot h^{-1} \cdot ind^{-1}$	0.600**	93
$V_{NH_4} = 1.63 \times AFDW^{0.698}$	$\mu mol \cdot h^{-1} \cdot ind^{-1}$	0.484**	92
$V_{cal} = 13.04 \times AFDW^{0.802}$	$J \cdot h^{-1} \cdot ind^{-1}$	0.619**	91
$O : N = 16.19 \times AFDW^{0.091}$	-	0.018	91
Carb $= 26.90 \times AFDW^{0.670}$	$\mu mol \cdot (g\ WMUW)^{-1}$	0.078*	94

Table 3.3 : Weight-specific rate variables of oxygen uptake, ammonia excretion and total caloric loss.

Unstressed :	Unit
$\dot{V}_{O_2} = V_{O_2} \times (105/AFDW_{meas})^{0.618}$	$\mu g{-}at \cdot h^{-1} \cdot (std\ ind)^{-1}$
$\dot{V}_{NH_4} = V_{NH_4} \times (105/AFDW_{meas})^{0.877}$	$\mu mol \cdot h^{-1} \cdot (std\ ind)^{-1}$
$\dot{V}_{cal} = V_{cal} \times (105/AFDW_{meas})^{0.632}$	$J \cdot h^{-1} \cdot (std\ ind)^{-1}$
Stressed :	
$\dot{V}_{O_2} = V_{O_2} \times (55/AFDW_{meas})^{0.803}$	$\mu g{-}at \cdot h^{-1} \cdot (std\ ind)^{-1}$
$\dot{V}_{NH_4} = V_{NH_4} \times (55/AFDW_{meas})^{0.698}$	$\mu mol \cdot h^{-1}(std\ ind)^{-1}$
$\dot{V}_{cal} = V_{cal} \times (55/AFDW_{meas})^{0.802}$	$J \cdot h^{-1} \cdot (std\ ind)^{-1}$

Table 3.4 : Linear least squares regression of degree of multiple-locus heterozygosity (Het) and phenotypic traits (coefficient of determination, significance level and sample size are given).

Regression formula	Unit	r^2	P	N
Unstressed scallops :				
Shell height = 27.7 + (Het × 0.008)	mm	0.000	0.976	100
AFDW = 105 + (Het × 0.000)	mg	0.000	0.982	100
$\dot{V}_{O_2}$ = 4.29 + (Het × 0.092)	$\mu g{-}at \cdot h^{-1} \cdot (std\ ind)^{-1}$	0.005	0.502	100
$\dot{V}_{NH_4}$ = 0.16 + (Het × 0.002)	$\mu mol \cdot h^{-1} \cdot (std\ ind)^{-1}$	0.001	0.733	97
$\dot{V}_{cal}$ = 1.99 + (Het × 0.044)	$J \cdot h^{-1} \cdot (std\ ind)^{-1}$	0.005	0.483	97
O:N = 30.29 + (Het × 0.396)	-	0.001	0.723	97
Carb. = 34.8 - (Het × 1.347)	$\mu mol \cdot (g\ WMUW)^{-1}$	0.003	0.572	100
Stressed scallops :				
Shell height = 27.3 - (Het × 0.067)	mm	0.001	0.788	94
AFDW = 55 + (Het × 0.000)	mg	0.000	0.925	94
$\dot{V}_{O_2}$ = 2.72 + (Het × 0.003)	$\mu g{-}at \cdot h^{-1} \cdot (std\ ind)^{-1}$	0.000	0.950	92
$\dot{V}_{NH_4}$ = 0.22 + (Het × 0.000)	$\mu mol \cdot h^{-1} \cdot (std\ ind)^{-1}$	0.000	0.983	91
$\dot{V}_{cal}$ = 1.30 - (Het × 0.000)	$J \cdot h^{-1} \cdot (std\ ind)^{-1}$	0.000	0.967	90
O:N = 12.92 - (Het × 0.137)	-	0.003	0.598	90
Carb. = 5.1 - (Het × 0.158)	$\mu mol \cdot (g\ WMUW)^{-1}$	0.003	0.599	94

Table 3.5 : Analysis of covariance of selected phenotypic traits of juvenile scallops. The degrees of freedom, F-test and significance level of covariates and factor (starvation) are given (* $P < 0.05$; ** $P < 0.001$).

Variable	Covariate 1 (Size) DF = 1	Covariate 2 (Heterozygosity) DF = 1	Factor (Experiment) DF = 1
Size [a]	-	0.000	0.004
AFDW [a]	3.683**	0.012*	3.112**
Condition	0.005	0.009	2.830**
V_{O_2} [a]	1.846**	0.021	1.708**
V_{NH_4} [a]	1.553**	0.000	1.559**
V_{cal} [a]	1.744**	0.020	1.481**
O:N	0.002	0.013	6.486**
Carbohydrate	0.402	0.000	30.413**

[a] Data were log_{10} transformed to account for allometry.

Table 3.6 : Mean difference in phenotypic values between heterozygous unstressed and stressed scallops. Degree of heterozygosity (Het), the number of unstressed (N1) and stressed (N2) scallops, ash free dry weight (mg), oxygen uptake ($\mu g\text{-}at\ O_2 \cdot h^{-1} \cdot (std\ ind)^{-1}$), ammonia excretion ($\mu mol\ NH_4 \cdot h^{-1} \cdot (std\ ind)^{-1}$), caloric loss ($J \cdot h^{-1} \cdot (std\ ind)^{-1}$), O:N ratio and carbohydrate content ($\mu mol \cdot (g\ WMUW)^{-1}$) are given.

Het	N1	N2	AFDW	$\dot{V}_{O_2}$	$\dot{V}_{NH_4}$	$\dot{V}_{cal}$	O:N	Carbohydr.
0	4	4	45	2.23	-0.01	1.00	15.23	34.53
1	18	25	56	1.37	-0.09	0.59	18.08	26.47
2	37	29	39	1.67	-0.06	0.75	18.30	27.88
3	32	24	62	1.98	-0.05	0.91	19.43	25.50
4	7	11	41	2.42	-0.05	1.07	18.71	33.91
5	2	1	1	-0.42	-0.12	-0.23	14.43	8.28

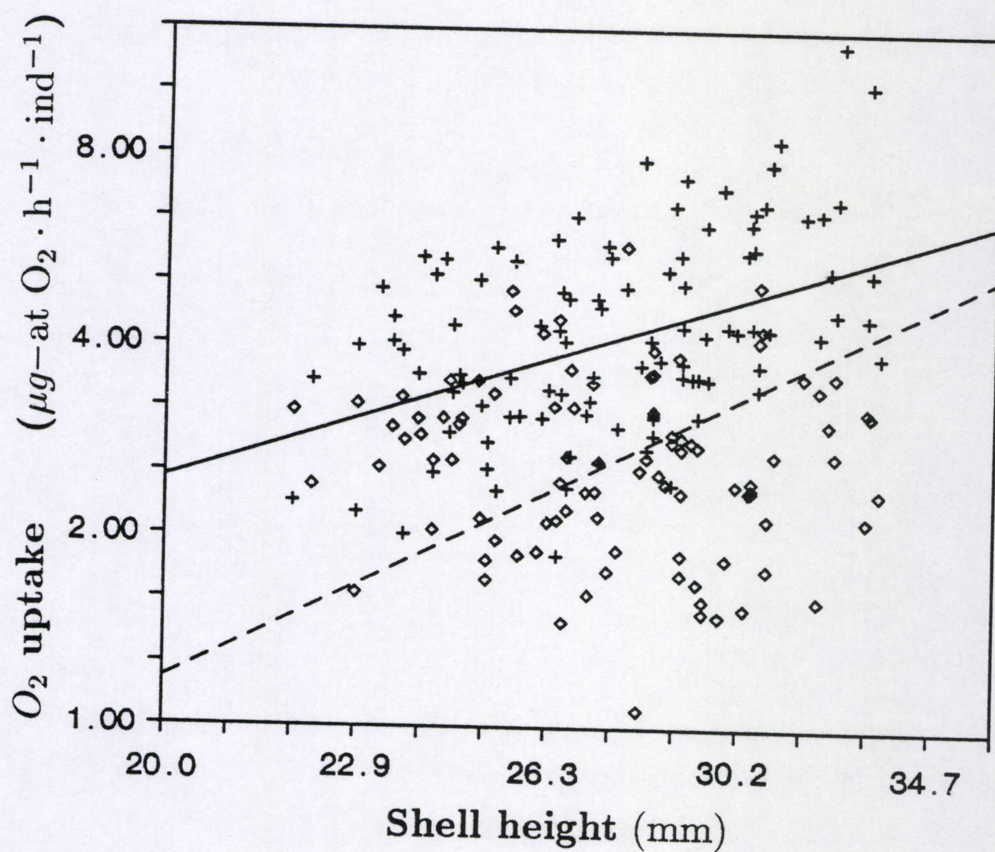


Figure 3.1 : Absolute rate of oxygen uptake (μg-at $O_2 \cdot h^{-1} \cdot ind^{-1}$) and shell height of unstressed ($Y = 18.435 \cdot 10^{-3} \times X^{1.636}$, $r^2 = 0.218$, $N = 100$) (—— and +) and stressed ($Y = 0.556 \cdot 10^{-3} \times X^{2.564}$, $r^2 = 0.586$, $N = 93$) (- - - - and ◇) juvenile *Placopecten magellanicus*. The slopes are not significantly different.

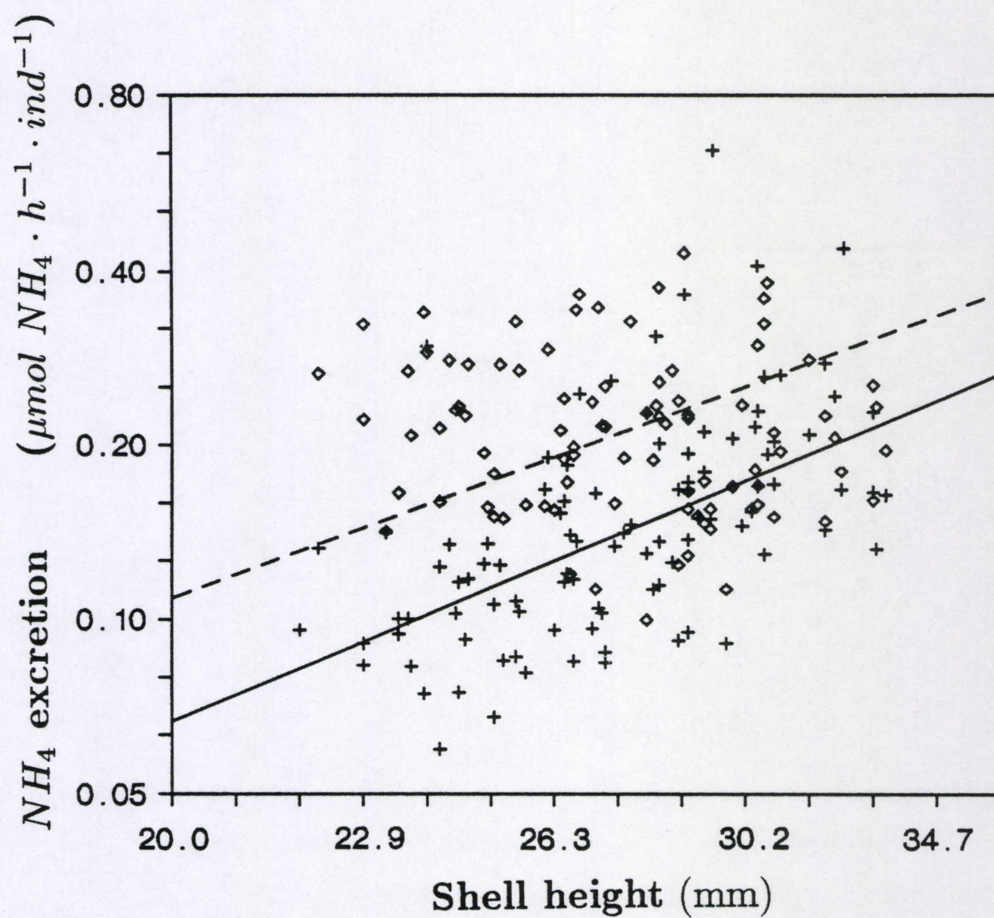


Figure 3.2 : Absolute rate of ammonia excretion (μmol $NH_4 \cdot h^{-1} \cdot ind^{-1}$) and shell height of unstressed ($Y = 0.050 \cdot 10^{-3} \times X^{2.402}$, $r^2 = 0.320$, $N = 97$) and stressed ($Y = 0.163 \cdot 10^{-3} \times X^{2.172}$, $r^2 = 0.461$, $N = 92$) juvenile *Placopecten magellanicus*. The slopes are not significantly different. Symbols are given in Figure 3.1.

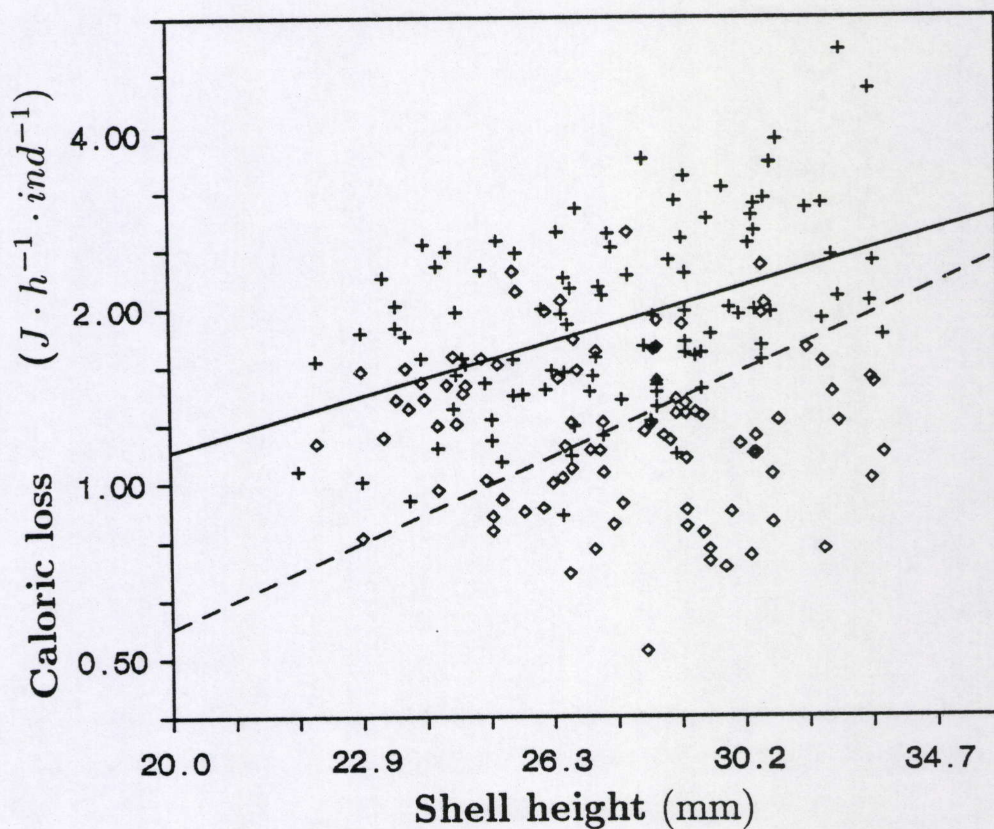


Figure 3.3 : Absolute rate of total caloric loss ($\mathrm{J}\cdot\mathrm{h}^{-1}\cdot\mathrm{ind}^{-1}$) and shell height of unstressed ($Y = 8.488 \cdot 10^{-3} \times X^{1.639}$, $r^2 = 0.221$, $N = 97$) and stressed ($Y = 0.277 \cdot 10^{-3} \times X^{2.549}$, $r^2 = 0.221$, $N = 97$) juvenile *Placopecten magellanicus*. The slopes are not significantly different. Symbols are given in Figure 3.1.

2.06 J$\cdot$h$^{-1}\cdot$ind^{-1} in the unstressed and 1.27 J$\cdot$h$^{-1}\cdot$ind^{-1} in the stressed scallops (a decrease of 38%). Although the slope of V_{cal} on shell height (Figure 3.3) or AFDW (Table 3.2) of stressed and unstressed scallops was not significantly different, a trend was evident. Respiratory losses made the largest contribution to the total caloric loss. The coefficients of determination of metabolic rate and AFDW of the starved scallops were higher than those of the unstressed scallops (Table 3.5).

A positive and significant increase of the respiration rate (and total caloric loss) was observed in the unstressed scallops during the assay period ($log_{10}V_{O_2} = 0.450 + (Day \times 0.031),\ r^2 = 0.339,\ n = 100$). Most likely, scallops arrived in the laboratory in a suboptimal nutritional condition. Those scallops assayed at a later day of the experiment had sufficient time to replenish their glycogen reserves with the algal supplement in the laboratory.

Weight-specific oxygen uptake, excretion rate and total caloric loss (Table 3.3) were not correlated with multiple-locus heterozygosity (Table 3.4). Treatment and not genotype had a significant effect on oxygen uptake, ammonia excretion and total metabolic loss (ANCOVA) (Table 3.5). No systematic trend was observed in the average metabolic loss per degree of heterozygosity (Table 3.6).

3.3.3 O:N ratio.

The O:N ratio decreased from 31.2 to 12.7 during four weeks of starvation (Table 3.1); nitrogen became proportionately a more important loss term during starvation. The O:N value was size independent; no correlation was found with AFDW (Table 3.2). Multiple-locus heterozygosity was not correlated with O:N in either treatment (Table 3.4 and 3.6).

3.3.4 Carbohydrate content.

Four weeks of starvation severely depleted the carbohydrate reserves in the adductor muscle; average carbohydrate content of the phasic part of the adductor muscle decreased 85% from 31.7 $\mu mol \cdot (g\ WMUW)^{-1}$ (or 26.9 mg$\cdot$(g AFDMU)$^{-1}$)

to 4.8 μmol $\cdot$(g WMUW)$^{-1}$ (or 4.1 mg$\cdot$(g AFDMU)$^{-1}$). Carbohydrate content of the unstressed scallops was rather low in comparison with adults assayed at the same time of the year (de Zwaan *et al*, 1980; Robinson *et al*, 1981). An artefact related to the number of days the scallops were fed (which varied between 3 and 12 days) influenced the carbohydrate content. The longer the scallops were fed, the higher the carbohydrate reserves of the adductor muscle; carbohydrate increased from 9.4 μmol$\cdot$(g WMUW)$^{-1}$ on the first day to 72.6 μmol$\cdot$(g WMUW)$^{-1}$ on the tenth day ($r^2 = 0.65$, DF = 99, $P < 0.001$).

Carbohydrate content was related to tissue weight only in the starved scallops. No correlation with heterozygosity was found (Table 3.4, 3.5 and 3.6).

3.4 Discussion.

I explored the metabolism of both unstressed and stressed juvenile scallops, and the possible influence of genotypic variation on metabolism. The instantaneous total caloric loss was considerably higher when measured at a routine rate than when measured at a basal rate; the O:N ratio decreased in parallel with a decrease in carbohydrate content of the adductor muscle. The effect of genotypic variation on the phenotype was negligible.

An essential condition of this study was the choice of a single cohort of juveniles (Chapter 2). Although the sample was collected by SCUBA divers, considerable evidence corroborated the fact that a single year class was sampled: the size-frequency distribution was unimodal; the shells carried a single growth ring (*Placopecten magellanicus* spawns in Passamaquoddy Bay once a year during a three week period in the fall (Dadswell *et al*, 1987)); divers noticed during regular field observations that scallops usually occured in homogeneous groups of a single year class.

The severity of the starvation was reflected in several measurements. The decrease in AFDW might be attributed partially to the loss of carbohydrate; stressed scallops lost 2.3% of their muscle dry weight due to carbohydrate catabolism. The

lighter shell of the starved animals suggested shell thinning. Similar observations were reported in adult *P. magellanicus* (S. Shumway, DMR, Maine, USA), the clam *Rangia cuneata* (Lane, 1986) and the fingernail clam *Sphaerium traversus* (Dietz and Stern, 1977). Shell $CaCO_3$ neutralizes the build-up of organic acids during anaerobic metabolism; the shells dissolve when the valves are closed periodically (Crenshaw, 1980). Frequent shell closure during starvation when there is no need to filter feed is a plausible explanation for shell thinning in *P. magellanicus*.

Scope for growth , i.e. the difference between the energy an organism consumes and energy loss (Bayne and Newell, 1983), was negative under starved and routine metabolic conditions. Only body reserves were utilized to maintain viable cell conditions because no food was provided. The metabolic rate of pectinids varies seasonally; oxygen uptake is linked to the seasonality of food supply in *Chlamys islandica* (Vahl, 1978), and to temperature cycles and concurrent gonad development in *C. varia* (Shafee, 1982), *Argopecten irradians* (Bricelj *et al*, 1987) and *P. magellanicus* (Shumway *et al*, 1988). The oxygen uptake rate of the scallops used in this study was probably declining to an annual minimum during the month of January.

A comparison of the exponent of the allometric equation relating oxygen uptake to body weight would be justifiable only if a full range of body weights were included. The below average b value of the routine oxygen uptake (0.618) and the expected b value of the basal oxygen uptake (0.803) only refers to juvenile scallops.

The ammonia excretion rates determined were the first to be measured in *P. magellanicus*. The average $NH_3{-}N$ excretion rate of unstressed juvenile *P. magellanicus* (1.15 μmol·h^{-1}·(g AFDW)$^{-1}$) is lower than in adult *A. irradians* (5.41 μmol·h^{-1}·(g AFDW)$^{-1}$) when measured during the same period of the year (Barber and Blake, 1985). The routine weight-dependent ammonia excretion rate (having an exponent of 0.877) is comparable to *M. edulis* (Bayne and Newell, 1983) and *M. lateralis* (Shumway and Newell, 1984).

The ratio of atomic equivalents of oxygen consumed and nitrogen excreted (O:N) is an estimate of the proportion of protein catabolized relative to carbohydrates and lipids. Its diagnostic value is higher in relative terms than in absolute terms (Bayne *et al*, 1985). The high O:N values of unstressed scallops (31.2) indicated a carbohydrate (glycogen) catabolism (Barber and Blake, 1985). Large amounts of glycogen and some lipid are stored in the adductor muscle (de Zwaan *et al*, 1980; Idler *et al*, 1964; Robinson *et al*, 1981). Glycogen reserves of adults build up during spring and are mobilised mostly in the gonads during gametogenesis in summer; glycogen reserves are at their lowest in fall. It is not clear if glycogen reserves of juvenile scallops follow a similar seasonal pattern.

The low O:N value of the starved scallops suggests a switch to a protein-based catabolism after the depletion of carbohydrate. Pectinids are known to use adductor muscle protein as energy reserve after the depletion of carbohydrate (Barber and Blake, 1985). Carbohydrate depletion in stressed *C. virginica* is related to size, unlike in *P. magellanicus*; small oysters lose relatively more glycogen than larger sized oysters (Rodhouse and Gaffney, 1984). Lipid levels in the adductor muscle of *P. magellanicus* vary seasonally (Idler *et al*, 1964; Robinson *et al*, 1981) which suggests that lipid metabolism is linked to gametogenesis. The relative importance of carbohydrate, lipid and protein in the metabolism of *P. magellanicus* seems to change somewhere between the juvenile age of 9 months and the adult age of 23 months (most scallops spawn for the first time at the age of 22 to 23 months). Lipid content of the total tissue mass decreases from 25% to 7%, and carbohydrate content increases from 11 to 23% during this period (Manning, 1985), suggesting that, as in larvae (Gabbott, 1983), lipid based metabolism plays an important role in juveniles.

Carbohydrate metabolism is in some cases scale dependent : the O:N ratios of *M. lateralis* at 10° C and 20° C (Shumway and Newell, 1984) and *M. edulis* (Bayne and Scullard, 1977) are higher in larger sized individuals. But the O:N ratio is independent of body weight in *Polinices alderi* (Macé and Ansell, 1982)

and *T. lapillus* (Stickle and Bayne, 1982) similarly to unstressed and stressed *P. magellanicus*.

Larger bivalves such as *M. edulis* (Diehl *et al*, 1986), *C. virginica* (Rodhouse and Gaffney, 1984) and *P. magellanicus* lose less weight in relative terms than smaller animals (negative allometry). The larger an animal, the more it resists suboptimal conditions. V_{O_2} is negatively scaled to weight loss in *M. edulis* (Diehl *et al*, 1986) unlike in *P. magellanicus*; in the former case, higher weight losses are typical for individuals with a high metabolic rate.

The rationale of the correlation between genotypic variation and metabolism of unstressed and stressed scallops consists of two aspects which have been detailed in the introduction. First, heterozygous bivalves make more efficient use of their resources under normal conditions. Second, the effect of multiple-locus heterozygosity might be measured more readily under stressful conditions.

Degree of heterozygosity and growth rate are not correlated in any member of the Family Pectinidae, including *P. magellanicus* (see Chapter 2). Zouros *et al* (1988) suggest that the correlation between heterozygosity and growth in other animals is always associated with an excess of homozygotes in the population. The small and insignificant deficiency of heterozygotes in *P. magellanicus* is translated into an insignificant contribution of genotypic variation to total growth variation. Before considering the impact of the genotype on physiological traits, one might ask to what degree physiological traits are a representative measure of organismal characteristics in comparison with growth rate. Unlike the variable growth rate, which integrates phenotypic variation in the shell between the time of settlement to sampling, total caloric loss, measured from the rate variables oxygen uptake and ammonia excretion, represents an immediate measure of phenotypic variation.

No co-variation of physiological traits of resting and starving *P. magellanicus* was detected either at the multiple-locus or at the single-locus level. As expected, environmental variation (e.g. starvation) was much more important than genotypic variation. Glycogen reserves in *P. magellanicus*, just as was observed in *C. virginica*

(Rodhouse and Gaffney, 1984) and *M. edulis* (Zouros *et al*, 1988), did not correlate with the degree of heterozygosity (although lack of evidence cannot be interpreted as negative evidence of the energetic interpretation of the relationship between heterozygosity and growth). Both *C. virginica* and *M. edulis*, unlike *P. magellanicus*, have a proven record of higher growth rates in more heterozygous individuals; both store in absolute terms higher amounts of energy for growth and reproduction, and both spend most mechanical energy on filter feeding. Valve movement does not require much energy in these sessile molluscs.

An important trend surfaces when comparing the relationship between metabolic rate and the number of heterozygous loci. Metabolic loss of several filter-feeding bivalves such as *C. virginica* (Koehn and Shumway, 1982) *M. edulis* (Diehl *et al*, 1985, 1986; Hawkins *et al*, 1986) and *M. lateralis* (Garton *et al*, 1984) is correlated with heterozygosity. The reduced metabolic loss of heterozygotes is channelled into an above average scope for growth. A second group, including the predators *T. haemastoma* (Garton, 1984) and *T. lamellosa* (Garton and Stickle, 1985), and the actively moving *P. magellanicus*, does not reveal a correlation of oxygen uptake and nitrogen excretion with the degree of heterozygosity. The enhanced scope for growth of heterozygous *T. haemastoma* is accounted for by the increased scope for activity (i.e. the higher consumption rate of prey). Metabolism in *P. magellanicus* might have evolved similarly to that in *T. haemastoma* : an above average scope for activity of heterozygotes. One might assume that heterozygous scallops obtained an edge over their homozygous conspecifics not through economizing on respiration and excretion, even under stressed conditions, but through enhanced grazing and swimming. A higher grazing rate might result in a higher ingestion rate and, absorption remaining below the saturation level and digestive efficiency remaining equal, a higher absorption rate would result. In general, the larger scope for growth of heterozygotes in one group of molluscs might be attributed to lower caloric loss, while in a second group it might be attributed to higher levels of activity.

Only the metabolic losses represented by oxygen uptake and ammonia excretion of *P. magellanicus* were measured in this study; other uses of mechanical energy such as filter feeding and swimming remain unaccounted for. It is not clear from the literature how an energy budget required for grazing (which is exclusively fueled by aerobic metabolism) compares with energy required for swimming (which is fueled by anaerobic metabolism). Mussels respire 8.1% of the ingested ration to maintain basal functions (Bayne and Newell, 1983); carnivorous fish require a comparable percentage of 7% (Brett and Groves, 1979). Another 24% (mussels) or 23% (carnivorous fish) is required for the cost of feeding, which is the sum of the cost of feeding proper (24% in mussels and not specified in fish) and the cost of activity. This raises the question what proportion of the cost of feeding is due to activity in motile molluscs such as *T. haemastoma* and *P. magellanicus*.

The next chapter addresses the question whether genotype and activity (i.e. swimming) of *P. magellanicus* might be linked.

Chapter 4.

Biochemical genetics of valve snapping.

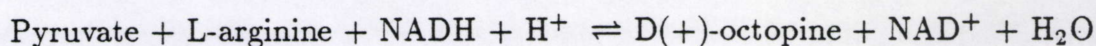
4.1 Introduction.

Predator-prey encounters and sudden changes in environmental conditions are important causes of short term stress. Stress induces both primary (neuro-hormonal and biochemical) and secondary responses (physiological and behavioural). Organisms acclimate to stimulation up to a certain threshold; once the threshold level is surpassed, a decline in condition will result although some compensation effects might occur. Environmental stress impairs thermal tolerance, the range of ration level for growth, body size and age-related fecundity in molluscs among other properties (Bayne, 1985).

The question arises as to what extent responses to short term stress are genetically controlled. What is the contribution of genetic variation to primary and secondary responses in a sudden adverse situation? The recent literature provides a few answers. Heterozygous individuals of the predatory gastropod *Thais haemastoma* have a larger scope for activity than do homozygotes. The larger scope for activity improves their chances of encountering prey and gives them a competitive advantage over homozygous conspecifics (Garton, 1984). Starved heterozygous *Crassostrea virginica* are better buffered against environmental stress (in this case to lower salinity and higher temperatures) than homozygotes (Koehn and Shumway, 1982). The most homozygous animals have a metabolic energy demand twice as high as the most heterozygous individuals. In addition, weight loss in starved heterozygous *C. virginica* is smaller than in homozygotes (Rodhouse and Gaffney, 1984). Heterozygous oysters are at an advantage over homozygotes when conditions are limiting.

Placopecten magellanicus is a most suitable organism in which to study these questions. Pectinids represent a peculiar life-style within the bivalve molluscs : they evade attacks from predators such as starfish either by swimming away quickly

(Thorburn and Gruffydd, 1979) or by valve closure. Suboptimal feeding environments may be avoided by relocating. Swimming by means of jet propulsion requires special adaptations from the standard bivalve model. The shell of *P. magellanicus* is reasonably streamlined, providing an excellent potential for overall lift and a reasonable drag potential (Gruffydd, 1976; Thorburn and Gruffydd, 1979). The phasic (smooth) part of the adductor muscle is structurally adapted to bursts of swimming (Rall, 1981; Castellani *et al*, 1983). In the early stages of swimming, metabolic energy demands are met aerobically (blood oxygen pressure drops quickly and phosphagen reserves are depleted) and at a later stage shift to anaerobic ATP production (Thompson *et al*, 1980; de Zwaan *et al*, 1980). The supply of the phosphagen arginine phosphate is probably the limiting factor in swimming (Gäde *et al*, 1978). The concomitant buildup of arginine (which inhibits cell functions) with decreasing arginine phosphate concentrations is followed by the accumulation of the end-product octopine (which is a compatible solute in the cell) (Somero and Bowlus, 1983). Octopine dehydrogenase (EC 1.5.5.11) catalyses the reductive condensation of pyruvate and arginine to octopine (Gäde, 1980) :



$$\text{Pyruvate} + \text{L-arginine} + \text{NADH} + H^+ \rightleftharpoons \text{D(+)-octopine} + \text{NAD}^+ + H_2O$$

It replaces lactate dehydrogenase in monitoring the redox balance in the muscle. *P. magellanicus* produces the glycolytic end-products octopine and alanine during recovery from swimming; no lactate or traces of other opines have been detected (de Zwaan *et al*, 1980).

The actual choice of exploring the effect of heterozygosity on the biochemical variable total ODH activity and octopine accumulation was made for a number of reasons. First, the biochemical genetics of ODH in *P. magellanicus* are well known (Foltz and Zouros, 1984; Chapter 2 and 3). A total of 5 allozymes of ODH have been scored of which two are common. Heterozygote deficiency at locus ODH is consistently negative (Chapter 2). Second, the production of a single end product (octopine) during and after swimming simplifies the experimental design (de Zwaan *et al*, 1980). Lastly, octopine remains localised in the adductor muscle and

does not appear in the blood stream (de Zwaan *et al*, 1980; Gäde, 1980). Exports of metabolites, which would otherwise require flux measurements, do not occur. The physiological and biochemical characterisation of the ODH allozymes is a logical extension of this reasonable understanding of the biochemistry of ODH and octopine, and the knowledge that ODH is highly polymorphic (Beaumont *et al*, 1980; de Zwaan *et al*, 1980; Gäde, 1980; Gäde and Grieshaber, 1986; Livingstone *et al*, 1981).

This chapter evaluates the physiological and biochemical basis of the possible link between the degree of heterozygosity and fitness phenotype at three organismal levels, namely the ecological/behavioural level (growth rate, characteristics of valve snapping), the physiological level (standard and active respiration rate, scope for activity) and the biochemical level (octopine accumulation and total ODH activity). I demonstrate that the degree of heterozygosity is correlated with scope for recovery at the ODH locus; multiple-locus heterozygosity does not correlate either with standard or active oxygen uptake, or with scope for activity. Concentrations of the end-product octopine in the adductor muscle are linked to heterozygosity at the loci PGD, AAT and MPI and to overall heterozygosity. The *in vitro* activity of the enzyme ODH is not linked to the degree of heterozygosity except at the ODH locus, where higher values are observed in homozygous scallops.

4.2 Materials and methods.

One hundred and sixty five (165) specimens of the scallop *Placopecten magellanicus* were collected by SCUBA divers at Passamaquoddy Bay (Tongue Shoal), New Brunswick, Canada on 11 July 1986. They were transported from nearby St.Andrews (N.B.) to Halifax (N.S.) in ice cooled seawater and assayed during a 2 to 3 week period after collection.

The wet muscle weight (WMUW) and total ash free dry weight (AFDW) of a subsample of thirty animals were measured to determine the allometric relation between WMUW and AFDW. Wet muscle weight is the fresh weight of the adductor

muscle, and ash free dry weight is the total weight of soft body tissue dried at 60° C minus the ashed weight at 490° C.

A total of 124 twenty month old scallops was selected for the actual testing of the hypothesis that heterozygosity and the adduction of muscle tissue are linked. The exact number of individuals used in the assays varied because of time constraints (e.g. oxygen uptake experiments) or accidental loss of tissue (e.g. octopine accumulation). Shell height was measured and age verified by interpreting external growth rings in the shell (on the external surface and in the calcareous portion of the ligament (MacDonald and Thompson, 1985a)). Shell height was defined as the distance measured between umbo and the ventral edge of the shell in a straight line. The condition index is the ratio of the $AFDW \times 100$ and the shell weight (SHDW).

Six gene loci of *P. magellanicus* (PGI, PGD, AAT, MPI, ODH and PGM) were separated, stained and scored on horizontal starch gels as described in Chapter 2.

Scallops were starved and gradually acclimated from 11° C to 15° C during a period of 10 days. During the assay each scallop was held in a chamber small enough to restrain it from swimming away but not to hinder valve snapping. Valve snapping was induced by adding extract of the starfish *Asterias vulgaris* to the incubation chambers holding the scallops. Whole starfish were homogenized in refrigerated 0.2 μm microfiltered seawater (5 ml of seawater per gram tissue) using a blender. The homogenate was centrifuged at 3500 g for 10 min and the supernatant stored at $-80°$ C (Thompson *et al*, 1980). Scallops were induced to snap their valves by repeated injections of starfish extract into the container until there were no more adductions and the scallops kept their valves tightly closed. This was regarded as a state of quiescence. The procedure was videotaped and at a later time the number of valve snaps counted and the time during which valve snapping occurred measured. The refractory time, i.e. the period between the first injection of starfish extract and the reopening of the valves, was measured during the assay.

Oxygen consumption of individual scallops was determined at $15°C$ and at 31 ppt salinity. The respiration chambers, made from clear acrylic plastic, were

similar to those described in Chapter 3. Oxygen concentration in the seawater was measured by the micro-Winkler method (Burke, 1961). Each scallop was assayed in duplicate under conditions of the least possible disturbance during an incubation period of 30 to 60 minutes. Oxygen depletion never amounted to more than 25 % of the saturation level. The calculated oxygen uptake was considered to be the standard metabolic rate because animals had been starved for 10 days (Bayne and Newell, 1983).

At the end of valve snapping, when scallops remained quiescent and kept their valves closed, container and scallop were transferred to a seawater bath to flush away the starfish extract and observed until the valves reopened. Shortly thereafter, the respiration chamber was sealed and a single measurement of oxygen uptake (the active rate) was made after an incubation of 15 to 20 min. The rate of oxygen uptake (V_{O_2}) was related to body size in the power function :

$$V_{O_2} = a \times AFDW^b$$

The fitted constants a and b were calculated from the linear regression of $log_{10} V_{0_2}$ and $log_{10} AFDW$ (a equals the intercept and b the slope of the exponential equation); V_{O2} is the oxygen uptake rate (μg-at·h^{-1}·ind^{-1}) and AFDW is the ash free dry weight of the soft tissue. Whole animal respiration rates were converted to weight-specific rate variables with the equation (Bayne and Newell, 1983) :

$$\dot{V}_{O_2} = V_{O_2} \times \left(\frac{AFDW_{av}}{AFDW_{meas}}\right)^b$$

$\dot{V}_{O_2}$ is the weight-specific oxygen uptake rate (μg-at·h^{-1}·(std ind)$^{-1}$); $AFDW_{av}$ is the body weight of a standardized scallop with a mean weight of 0.801 g and $AFDW_{meas}$ is the actual body weight measured; b is the same fitted constant as in the equation relating oxygen uptake and AFDW.

Accumulation of the end-product octopine was measured in the phasic part of the adductor muscle 2 h after injection of starfish extract. Each scallop was cut open and within seconds the complete adductor muscle was divided into 3

parts, transferred to preweighed 1.5 ml disposable centrifuge tubes, frozen in liquid nitrogen and stored at $-80°$ C. One aliquot (including the catch part of the muscle) was used for starch gel electrophoresis (see above); the second aliquot (consisting exclusively of the phasic part of the adductor muscle) was used for the determination of octopine dehydrogenase (ODH) activity (see below), and the third (consisting exclusively of the phasic part) for determination of octopine as follows. The frozen tissue was powdered in a mortar cooled with liquid nitrogen and homogenized in cold 6 % perchloric acid (1 g tissue to 3 ml of $HClO_3$). The extract was centrifuged at 12,000 g for 15 min at 5° C and neutralized with potassium carbonate (0.25 ml 5 N K_2CO_3 to 1 ml of $HClO_3$). The neutralized samples were allowed to stand for 2 h and the precipitated potassium chlorate was removed by centrifugation (12,000 g for 15 min at 5° C). The supernatant was assayed for octopine according to Gäde *et al* (1978). Octopine was determined indirectly through the reduction of NAD^+ which was monitored spectrophotometrically by the change in absorbance at 340 nm. The reaction mixture contained 500 mM glycine, 400 mM hydrazine hydrate, 230 mM Tris-HCl buffer (pH 9.3), 5 mM NAD^+, extract of the enzyme ODH, 2 $U \cdot ml^{-1}$ of MDH and 20 μl extract. The ODH extract consisted of a crude muscle extract of *P. magellanicus* which was partially purified through salting out with ammonium sulfate. The sample was treated with malate dehydrogenase (MDH) for one hour to eliminate malate, which interferes with the octopine measurement. The reaction to measure octopine was initiated by the addition of ODH. Controls with no extract and standards were run concurrently (D. Livingstone, pers. comm.).

Total activity of the enzyme ODH was determined in the following manner. Frozen tissue and buffer solution (5 ml of 50 mM potassium phosphate, 0.1 mM EDTA and 0.1 mM DTT per gram tissue at pH 7.5) were ground with mortar and pestle. The emulsion was centrifuged at 12,000 g for 15 min to recover the supernatant for the enzyme assay. ODH activity was assayed according to the method described by Gäde and Zebe (1973). The reaction medium consisted of 100 mM Tris-HCl, 0.15 mM NADH, 5 mM sodium pyruvate, 10 mM arginine hydrochloride

and 20 μl extract. Total volume was 1.0 ml and final pH 6.6. Total activity was determined at 25° C by following the change in absorbance at 340 nm due to the oxidation of NADH.

4.3 Results.

4.3.1 Valve snapping, allometry and oxygen uptake.

At $15°C$ scallops adducted their valves between 3 and 29 times (mean = 12.8) during the 8 seconds to 4 minutes (mean = 96 sec) which elapsed until the shells remained closed (Table 4.1). It took on average 28 minutes for the shell valves to reopen.

I estimated ash free dry weight of soft tissue by linear least squares regression from the measured wet muscle weight with the equation :

$$AFDW = 0.405 \times WMUW \quad (r^2 = 0.987;\ n = 30)$$

Such an approach was necessary because wet muscle tissue was required in three biochemical assays, which did not permit destructive sampling. Active oxygen uptake was measured immediately after the scallops reopened their valves. Thompson *et al* (1980) and preliminary measurements indicated that a period of increased oxygen uptake and rapid changes in oxygen uptake occurred immediately after the valves reopened.

Body weight (i.e. estimated AFDW) and oxygen uptake were logarithmically transformed, and the slope (constant b) and intercept (constant a) calculated (Table 4.2). Rates of oxygen uptake under standard ($V_{O_2 \cdot st}$) and active ($V_{O_2 \cdot act}$) metabolic conditions were scaled to body weight (Figure 4.1). The slopes of metabolic rates of bivalves are known to be affected by activity, temperature, salinity and ration level (Bayne and Newell, 1983). I tested for differences between slopes and intercepts; slopes were not significantly influenced by activity ($F(1,190) = 2.22$, $P = 0.138$) but intercepts were ($F(1,190) = 52.24$, $P < 0.001$). The exponent of the power curve of

Table 4.1 : Mean values of phenotypic traits of 20 month old scallops (unit, S.E. and sample size (N) are given).

Variable	Unit	Mean	S.E.	N
Shell height	mm	48.18	0.683	124
Shell dry weight	mg	7039	381	98
Condition index	-	12.0	0.4	98
Ash free dry weight	mg	801	41	98
Number of snaps	-	12.79	0.58	94
Snap interval	sec	96	5	94
Snap rate	$snaps\ min^{-1}$	0.16	0.01	94
Refractory time	min	28.1	1.2	96
$\dot{V}_{O_2 \cdot st}$	$\mu g{-}at \cdot h^{-1} \cdot ind^{-1}$	15.28	0.75	97
$\dot{V}_{O_2 \cdot act}$	$\mu g{-}at \cdot h^{-1} \cdot ind^{-1}$	30.49	1.98	94
Octopine accumulation	$\mu mol \cdot (g\ WMUW)^{-1}$	3.9	0.2	64
Total ODH activity	$\mu mol \cdot min^{-1} \cdot (g\ WMUW)^{-1}$	19.7	0.3	93

oxygen uptake and body weight was somewhat higher in comparison with unstressed scallops (Chapter 3), comparable to that in the study of MacDonald and Thompson (1986) on *Placopecten magellanicus* but somewhat higher than for most suspension-feeding bivalves (Bayne and Newell, 1983). The active metabolic rate (30.489 μg-at$\cdot$h$^{-1}\cdot$(std ind)$^{-1}$) was on average twice the standard respiration rate (15.248 μg-at$\cdot$h$^{-1}\cdot$(std ind)$^{-1}$) (Table 4.1). The difference in oxygen uptake between active and standard conditions is a measure of the scope for activity (Bayne and Newell, 1983) or metabolic scope (Schmidt-Nielsen, 1984), i.e. the additional metabolic output available in a stressed scallop to return to standard metabolism (Table 4.2 and Figure 4.1). AFDW accounted for 42% of within-animal total variation of scope for activity (Table 4.2). Fast changes in $V_{O_2 \cdot act}$ over time might explain why AFDW accounted for relatively little of the overall variation in oxygen uptake. Scope for activity correlated with neither the number of valve snaps (Table 4.3) nor the rate of valve snapping. The factorial aerobic scope (i.e. the ratio between $\dot{V}_{O_2 \cdot st}$ and $\dot{V}_{O_2 \cdot act}$) is 2.0, a value comparable to that in adult *P. magellanicus* (Thompson *et al*, 1980) and *Chlamys delicatula* (Mackay and Shumway, 1980).

Standard and active oxygen uptake were standardized to weight-specific variables:

$$\dot{V}_{O_2 \cdot st} = V_{O_2 \cdot st} \times \left(\frac{0.801}{AFDW_{meas}}\right)^{0.825}$$

and

$$\dot{V}_{O_2 \cdot act} = V_{O_2 \cdot act} \times \left(\frac{0.801}{AFDW_{meas}}\right)^{0.928}$$

$\dot{V}_{O_2 \cdot st}$ is the weight-specific oxygen uptake under standard conditions; $\dot{V}_{O_2 \cdot act}$ is the weight-specific oxygen uptake under active conditions; 0.801 is the average AFDW; $AFDW_{meas}$ is the measured soft tissue weight of each scallop. The purpose of the transformation is the elimination of AFDW as a source of phenotypic variation in the regression of oxygen uptake and degree of heterozygosity.

Table 4.2 : Linear regression equations (coefficient of determination, significance level and sample size are given) of oxygen uptake ($\log_{10}$ transformed) ($\mu g\text{−}at \cdot h^{-1} \cdot ind^{-1}$) and ash free dry weight (AFDW) (mg) (** P < 0.001).

Regression equation	r^2	N
$V_{O_2 \cdot st} = 18.360 \times AFDW^{0.825}$	0.801**	97
$V_{O_2 \cdot act} = 35.618 \times AFDW^{0.928}$	0.752**	94
$V_{O_2 \cdot sa} = 15.183 \times AFDW^{1.172}$	0.419**	94

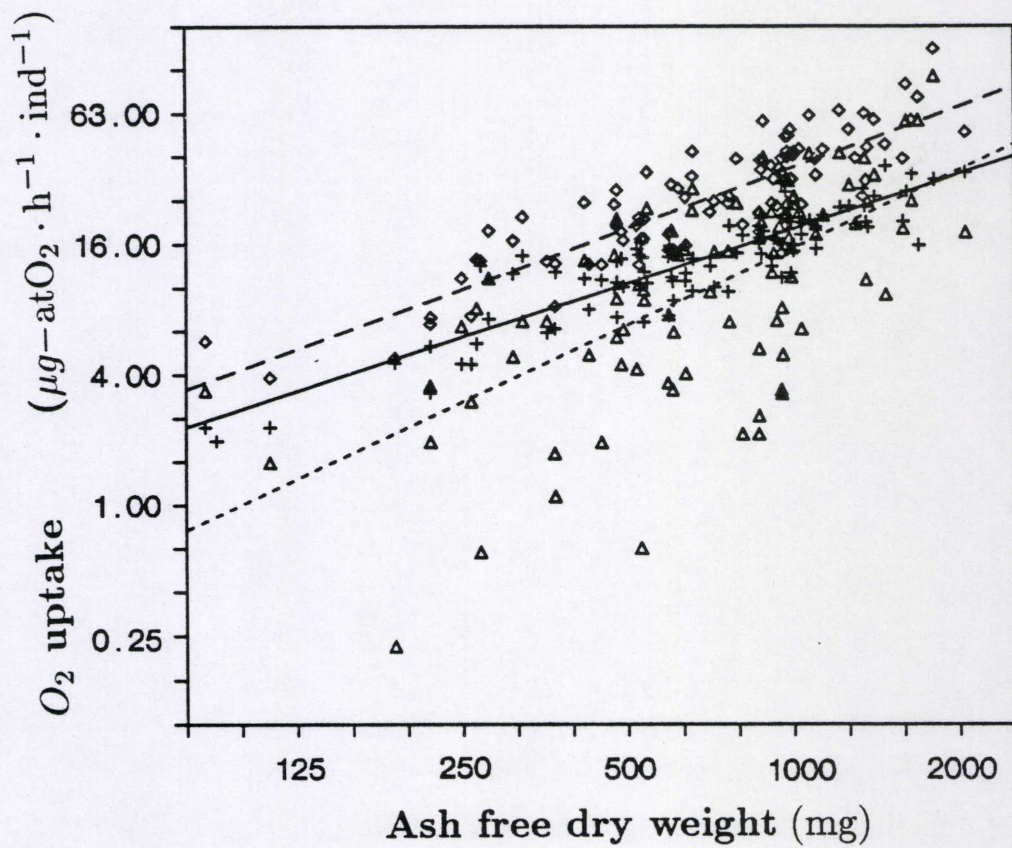


Figure 4.1 : Oxygen uptake and ash free dry weight of standard metabolic rate (——— and +), active metabolic rate (– – – – and ◇), and scope for activity (- - - - - and △) of 20 month old *Placopecten magellanicus*.

4.3.2 Octopine accumulation and ODH enzyme activity.

I measured simultaneously the accumulation of octopine and total ODH activity 2 hours after the initiation of valve snapping due to starfish extract stimulation. At that time the accumulation of octopine in adult *P. magellanicus* reaches a maximum and remains relatively stable (Livingstone *et al*, 1981). The average octopine concentration in the phasic part of the adductor muscle measured 3.9 μmol$\cdot$(g WMUW)$^{-1}$ 2 h after inducing valve snapping. This value was somewhat lower than reported for *P. magellanicus* by Livingstone *et al* (1981). My study differed from Livingstone *et al* (1981) in that younger animals were assayed at a higher temperature (15° C in comparison to 5° C). Total activity of the enzyme ODH under standard assay conditions was 19.7 μmol octopine$\cdot$min$^{-1}\cdot$(g WMUW)$^{-1}$ which is a lower level than in adult *P. magellanicus* (de Zwaan *et al*, 1980). Total ODH activity is weight dependent ($Y = 20.50 \times AFDW^{0.153}$, $r^2 = 0.279$, $P < 0.001$). The scaling of ODH activity to body weight is smaller than the scaling of V_{O_2} to body weight in scallops. Muscle tissue enzymes pyruvate kinase (PK) and lactate dehydrogenase (LDH) of fish are scaled similarly to ODH (Ewart *et al*, 1988; Somero and Childress, 1980). This might indicate that the scaling of anaerobically poised enzymes is similar to that of the body power required for burst-swimming.

I tested whether the accumulation of octopine and total ODH activity were related to the number of valve snaps (Table 4.3). Unlike the findings of Livingstone *et al* (1981), they were not correlated. It is not clear what the cause might be.

4.3.3 Allele frequencies.

Genotypes at six polymorphic loci were scored. Allele frequencies, (summarized in Table 4.4) fell within the range reported from previous collections in Passamaquoddy Bay (see Chapter 2) and the Northwest Atlantic (Foltz and Zouros, 1984; Gartner-Kepkay and Zouros, 1985; K. Fuller and K. Ahmad, pers. comm.). This sample of two year old scallops was on average not significantly deficient in

Table 4.3 : Linear regression equation of the number of valve snaps and various phenotypic traits (coefficient of determination, significance level and sample size are given) (** P < 0.001).

Equation	Unit	r^2	N
AFDW = 0.84 - (0.004×Snaps)	mg	0.002	94
Condition index = 14.7 - (0.2×Snaps)	-	0.094**	94
Snap rate = 59.17 + (2.89×Snaps)	$snaps\ min^{-1}$	0.124**	94
Refractory time = 31.2 - (0.23×Snaps)	min	0.013	96
$V_{O_2 \cdot act}$ = 28.78 + (0.147×Snaps)	$\mu g{-}at \cdot h^{-1} \cdot (std\ ind)^{-1}$	0.007	92
$V_{O_2 \cdot sa}$ = 13.40 + (0.114×Snaps)	$\mu g{-}at \cdot h^{-1} \cdot (std\ ind)^{-1}$	0.004	92
Octopine accum. = 4.6 - (0.06×Snaps)	$\mu mol \cdot (g\ WMUW)^{-1}$	0.043	60
Total ODH act. = 20.4 - (0.05×Snaps)	$\mu mol \cdot min^{-1} \cdot (g\ WMUW)^{-1}$	0.010	89

heterozygotes. This fits the hypothesis of age dependent heterozygote deficiency (presented in Chapter 2), which predicts that with age, the population evolves towards the Hardy-Weinberg equilibrium.

4.3.4 Relationships between heterozygosity and fitness.

Possible relationships between multiple-locus or single-locus heterozygosity and phenotype at a number of organismal levels were tested, i.e. at the growth/behavioural, the physiological and the biochemical level. First I discuss the effects of multiple-locus heterozygosity (Table 4.5).

No correlation between multiple-locus heterozygosity and fitness traits at the ecological/behavioural level (such as AFDW, SHDW, the number of muscle adductions and the snap rate) was observed (Table 4.5). The genetics of growth rate of twenty month old scallops compare similarly to juvenile scallops : low deficiencies in heterozygotes covary with an insignificant correlation between growth and the degree of heterozygosity.

No effect of multiple-locus heterozygosity on weight-specific oxygen uptake rates of standard and active metabolic rate, and scope for activity was observed.

Multiple-locus heterozygosity accounted for 7% of the variance in the accumulation of end product in the phasic part of the adductor muscle; significantly higher concentrations of octopine were measured in heterozygous individuals (Table 4.5; Figure 4.2). The dynamic equilibrium between octopine production and octopine breakdown (or, equivalently, the oxidation of NADH and reduction of NAD^+) which is catalyzed by ODH, was shifted towards the production of octopine in relation to the breakdown of octopine. Total ODH activity was not correlated with multiple-locus heterozygosity.

Two approaches were taken to examine the correlation between the degree of single-locus heterozygosity and phenotype. First, average values of phenotypic traits were compared between homo- and heterozygotes (Table 4.6). I hypothesized that on average homozygotes had a selective disadvantage, even if selected homozygous

Table 4.4 : Allele frequency estimates, D-values, G-test for fit to Hardy-Weinberg proportions (statistical significance and degrees of freedom are given) at 6 polymorphic loci (N = 124).

Locus	Allele	Frequency	D	G-test	P	DF
PGI	87	0.004	0.042	0.512	N.S.	3
	100	0.956				
	115	0.040				
PGD	80	0.008	-0.041	1.576	N.S.	6
	100	0.809				
	130	0.004				
	150	0.179				
AAT	70	0.012	-0.020	5.896	N.S.	6
	80	0.016				
	90	0.468				
	100	0.504				
MPI	87	0.214	-0.152	3.340	N.S.	3
	100	0.782				
	108	0.004				
ODH	100	0.448	-0.038	0.180	N.S.	1
	105	0.552				
PGM	96	0.012	0.062	6.890	N.S.	10
	98	0.093				
	100	0.702				
	102	0.189				
	104	0.004				
$\overline{D}$:			-0.025 (±0.031)			

Table 4.5 : Linear regression of degree of multiple-locus heterozygosity (Het) and phenotypic traits (coefficient of determination, level of significance and sample size are given).

Equation	Unit	r^2	P	N
Shell height = 46.98 + (0.617×Het)	mm	0.009	0.297	123
AFDW = 0.73 + (0.035×Het)	mg	0.011	0.317	97
SHDW = 6.15 + (0.428×Het)	mg	0.018	0.190	97
Number of snaps = 12.98 - (0.15×Het)	-	0.001	0.753	93
Snap rate = 0.19 - (0.015×Het)	$snaps \cdot min^{-1}$	0.034	0.077	93
$\dot{V}_{O_2 \cdot st}$ = 16.54 - (0.308×Het)	$\mu g{-}at \cdot h^{-1} \cdot (std\ ind)^{-1}$	0.007	0.425	96
$\dot{V}_{O_2 \cdot act}$ = 29.12 + (0.611×Het)	$\mu g{-}at \cdot h^{-1} \cdot (std\ ind)^{-1}$	0.005	0.483	94
$\dot{V}_{O_2 \cdot sa}$ = 12.63 + (0.913×Het)	$\mu g{-}at \cdot h^{-1} \cdot (std\ ind)^{-1}$	0.012	0.298	94
Octopine accum. = 3.1 + (0.40×Het)	$\mu mol \cdot (g\ WMUW)^{-1}$	0.068	0.039	63
Total ODH act. = 19.5 + (0.13×Het)	$\mu mol \cdot min^{-1} \cdot (g\ WMUW)^{-1}$	0.003	0.597	93

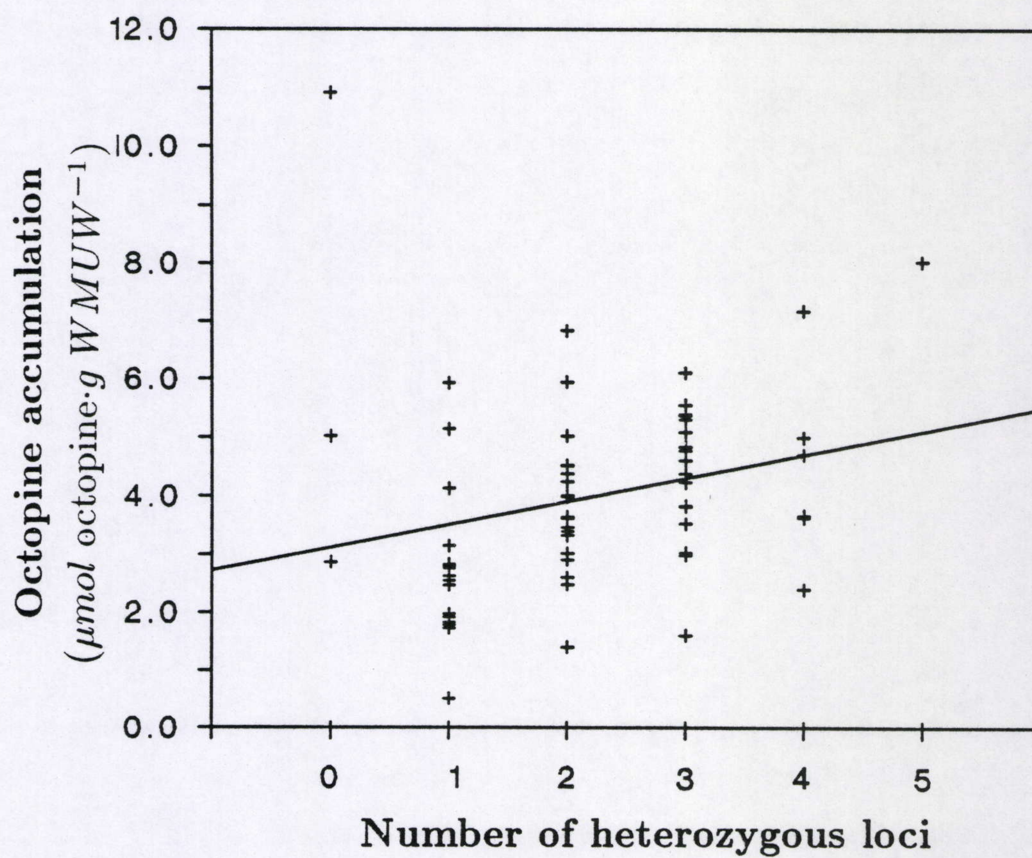


Figure 4.2 : Octopine accumulation and the number of heterozygous loci ($[octopine] = 3.095 + (0.404 \times Het)$); $r^2 = 0.068$, $N = 63$).

genotypes had superior qualities. Second, analysis of variance was used to compare single-locus genotypes with a given phenotypic trait (Table 4.7). The latter approach highlights between genotype differences more than does the comparison between hetero- and homozygotes.

The growth rates of most locus-specific heterozygotes were not significantly different from homozygotes (except at locus PGD where heterozygotes were on average larger than homozygotes). Analysis of variance provided no evidence of locus-specific differences. No locus-specific differences between homo- and heterozygotes nor between the various genotypes were found in regard to the number of valve snaps. The rate of valve snapping did not differ in homo- and heterozygotes, but MPI genotypes were significantly different from each other (Table 4.7). Higher snapping rates covaried with the ^{100}MPI allele.

The physiological variables standard and active respiration rate were not related to locus specific degree of heterozygosity whether one considered homozygotes against heterozygotes or whether genotype was considered separately (ANOVA). Scope for activity was significantly larger in heterozygotes than in homozygotes at locus ODH. The $^{105/105}$ODH genotype showed a smaller difference between oxygen uptake under standard and active conditions (Table 4.7).

I tested also for the locus-specific impact of the variation of octopine accumulation and total ODH activity. Significantly more octopine accumulated in heterozygotes at loci PGD, AAT and PGM and marginally so at 2 other loci (PGI and AAT). The positive correlation between multiple-locus heterozygosity and octopine accumulation is largely attributed to the impact of the loci PGD, AAT and PGM. ANOVA identified a higher concentration of octopine in heterozygotes at locus AAT (genotypes $^{100/70}$AAT and $^{100/90}$AAT) (Table 4.7). Total activity of ODH was larger in homozygotes than in heterozygotes at locus ODH; other loci did not differ in ODH activity between homo- and heterozygotes (Table 4.6). ANOVA did not show that ODH activity among loci was significantly different between genotypes.

Table 4.6 : Average fitness of homo- and heterozygotes at single loci. Locus, shell height (mm), number of valve snaps, snap rate ($snaps\ min^{-1}$), weight-specific oxygen uptake (i.e. standard and active rate, and scope for activity) ($\mu g{-}at \cdot h^{-1} \cdot (std\ ind)^{-1}$), octopine accumulation ($\mu mol\ octopine \cdot (g\ WMUW)^{-1}$), ODH activity ($\mu mol\ octopine \cdot min^{-1} \cdot (g\ WMUW)^{-1}$), significance of t-test and sample size are given (* $P < 0.05$).

Locus		Shell height	Number of snaps	Snap rate	$\dot{V}_{O_2 \cdot st}$	$\dot{V}_{O_2 \cdot act}$	$\dot{V}_{O_2 \cdot sa}$	Octop. accum.	ODH act.
PGI	Hom.	48.02	12.57	0.16	15.65	30.52	14.87	3.9	19.8
	Het.	49.75	16.00	0.13	18.32	30.07	11.75	4.1	19.0
	N	124	94	94	97	94	94	64	93
PGD	Hom.	47.53	12.97	0.16	15.86	30.01	14.21	3.7	19.5
	Het.	50.15	11.97	0.15	15.87	31.51	15.65	4.5	20.1
	N	123*	93	93	96	94	94	63*	93
AAT	Hom.	47.79	13.00	0.17	15.96	30.03	13.99	3.5	19.5
	Het.	48.54	12.60	0.14	15.70	30.91	15.30	4.3	19.9
	N	124	94	94	97	94	94	64*	93
MPI	Hom.	48.14	13.07	0.16	15.81	30.33	14.53	3.8	19.8
	Het.	48.28	12.07	0.15	15.86	30.88	15.02	4.2	19.6
	N	124	94	94	97	94	94	64	93
ODH	Hom.	48.68	12.33	0.16	16.53	28.73	12.26	4.2	20.2
	Het	47.62	13.20	0.15	15.18	32.03	16.79	3.7	19.3
	N	124	94	94	97	94	94*	64	93*
PGM	Hom.	47.62	13.25	0.17	16.10	31.77	15.59	3.6	19.3
	Het.	48.76	12.30	0.15	15.55	29.26	13.79	4.3	20.2
	N	124	94	94	97	94	94	64*	93

Table 4.7 : Analysis of variance of rate of valve snapping (at locus MPI), scope for recovery (at locus ODH) and octopine accumulation (at locus AAT).

Rate of valve snapping ($snaps \cdot min^{-1}$) :

MPI genotypes	Mean	S.E.	N
87/87	0.12	0.03	5
100/87	0.14	0.02	26
100/100	0.17	0.01	62
108/100	0.39	0.00	1

ANOVA : F(3,93) = 3.002; P = 0.04

Scope for activity ($\mu g\!-\!at \cdot (g\ WMUW)^{-1} \cdot (std\ ind)^{-1}$) :

ODH genotypes	Mean	S.E.	N
100/100	14.73	3.00	18
105/100	16.79	1.24	50
105/105	10.55	1.66	26

ANOVA : F(2,93) = 3.656; P = 0.03

Octopine accumulation ($\mu mol \cdot (g\ WMUW)^{-1}$) :

AAT genotypes	Mean	S.E.	N
90/90	3.3	0.4	14
100/70	7.0	0.2	2
100/80	4.0	0.8	3
100/90	4.2	0.2	30
100/100	3.7	0.6	15

ANOVA : F(4,63) = 2.492; P = 0.05

4.4 Discussion.

In this chapter I investigated the connection between heterozygosity at 6 polymorphic loci and indices of fitness at the physiological and biochemical level in the scallop *Placopecten magellanicus*. Multiple-locus heterozygosity and phenotype are correlated at the biochemical level but not at the ecological/behavioural and physiological level. Multiple-locus and single-locus heterozygosity have an effect on the accumulation of octopine in the phasic part of the adductor muscle and to a lesser degree on total activity of ODH. In the previous chapters I concluded that neither growth rate nor metabolic rate were measurably affected by the degree of heterozygosity. The present results on the genetics of valve snapping support this conclusion.

I argue that heterozygosity and growth rate are not correlated in juvenile scallops because pectinids are different from other bivalves through their active life style. Swimming in scallops is a burst event requiring considerable mechanical energy (which is used to force water out of the shell by means of muscle adductions). Energy shunted into mechanical activity becomes unavailable for growth and results in reduced somatic growth (Brett and Groves, 1979). Juvenile scallops spend proportionally more time swimming around than newly settled spat and adults (MacKenzie, 1979; M. Dadswell, Acadia University, pers. comm.); they have the option to actively avoid suboptimal and adverse conditions by moving away from them or to close the valves. Unlike scallops, a "typical" bivalve reacts to adverse situations by closing its shell (e.g. *Mytilus edulis*) or retreating into its burrow (e.g. *Mya arenaria*), which induces environmental anaerobiosis.

The total number of valve snaps per stimulated animal was not different among the various genotypes; the rate variable (snapping rate) was affected by genotype only at locus MPI. A comparable study which looked for a link between the genotypes of ODH and leaping behaviour was published by Baldwin and England (1982). Leaping was induced in the gastropod *Strombus luhuanus* by adding

crushed flesh of *Conus* sp. The authors did not observe any differences between the 6 ODH genotypes in regards to leaping. However, there is evidence in the literature that behaviour may be affected by multiple-locus heterozygosity. The exploratory behaviour, behavioural dominance and aggressiveness of the oldfield mouse *Peromyscus polionotus* is correlated with heterozygosity on the mainland (although it does not correlate in animals collected from beaches located on several islands) (Garten, 1976, 1977). Olive fruit flies *Dacus oleae*, homozygous at locus EstB, are more readily sampled with an air pump from a cage population than heterozygous conspecifics (Tsakas and Krimbas, 1970).

Two strategies are available to optimize the use of assimilated energy in view of the balanced energy equation of Winberg (1971) : either to maximize the amount of food consumed or to minimize the losses (respiration, excretion, feces). Heterozygous *C. virginica* optimize production through reduced respiration costs (Koehn and Shumway, 1982). Similar physiological advantages of heterozygotes over homozygotes have been documented in the coot clam *M. lateralis* (Garton *et al*, 1985), the blue mussel *M. edulis* (Diehl *et al*, 1985, 1986), the rainbow trout *Salmo gairdneri* (Danzmann *et al*, 1987) and the tiger salamander *Ambystoma tigrinum* under normal conditions (Mitton *et al*, 1986). Stressed tiger salamanders showed an inverse (i.e. positive) relationship of oxygen uptake with the degree of heterozygosity (Mitton *et al*, 1986). Another study of oysters (Rodhouse and Gaffney, 1984) and a study of the gastropod *Thais haemastoma* (Garton, 1984) did not find any difference in the weight-specific oxygen consumption rates at different levels of heterozygosity. Rodhouse and Gaffney (1984) suggest that the oxygen uptake rates are not representative because oysters spent variable amounts of time with their shells closed.

I propose separating Mollusca into two functional groups : sedentary species (such as *M. edulis*, *M. lateralis*, *C. virginica*) and motile species (such as *T. haemastoma* and *P. magellanicus*). Passive species have a respiration rate which is negatively correlated with the degree of heterozygosity, while active species are less

likely to show such a correlation. This dichotomy is a logical extension of the observations on the absence of a correlation between heterozygosity and growth in motile species : selection is directed to an optimization of the cost of movement. A gastropod which relies on a strategy of actively searching for prey will make a trade-off between cost of movement and additional success. A scallop which recovers faster from a swimming event will more likely evade the next predator. Heterozygotes of active molluscs carry that competitive edge of being "faster". An alternate explanation of the absence of a correlation between growth and heterozygosity is that the magnitude of the effect of heterozygosity on growth rate cannot be explained by the degree of heterozygosity observed for a locus (Koehn *et al*, 1988). Some or all of the polymorphic loci scored in *T. haemastoma* and *P. magellanicus* might not affect growth rate at all.

Studying genotypic and phenotypic interaction at the ODH locus involves two major assumptions. First, genetic diversity represents phenotypic diversity which must occur specifically as a manifestation of different catalytic properties of enzyme variants. I measured total enzyme activity of ODH and the accumulation of the end-product octopine to quantify such variation. Several papers document the adaptive importance of allelic variation (e.g. Koehn and Hilbish, 1987; Zera, 1987). Second, biochemical diversity presumably has a significant effect on higher levels of biological organisation (Koehn *et al*, 1988). For instance, genetic and catalytic variation at locus ADH in *Drosophila melanogaster* is reflected in geographical clines (Koehn *et al*, 1983). Therefore, valve snapping and oxygen uptake are possibly affected by variation at lower organismal levels.

The kinetic properties of the allozymes of ODH have been studied in several invertebrates. Purified allozymes of ODH of the gastropod *Strombus luhuanus* are indistinguishable in terms of K_m values for substrates, product inhibition by octopine and NAD^+, pH optima and substrate inhibition by pyruvate (Baldwin and England, 1982). Both molecular forms of ODH of the gastropod *Concholepas concholepas* are similar in molecular weight, pH optimum and kinetic properties

(Carvajal and Kessi, 1988). Both allozymes of ODH from *Pecten maximus* are similar in their *in vitro* kinetic properties. The only differences are the charge and the sensitivity to various chemical treatments (Monneuse-Doublet *et al*, 1980). Similarly, muscle tissue from *P. jacobaeus* contains two types of ODH which differ in electrophoretic mobility but not in their physico-chemical and enzymatic properties (Zettlmeissl *et al*, 1984).

In contrast to *S. luhuanus*, *C. concholepas*, *P. maximus* and *P. jacobaeus*, the lower total enzyme activity of ODH from heterozygous *P. magellanicus* suggests kinetic differentiation. The physiological and biochemical properties of ODH in the plumose sea anemone *Metridium senile* are genotype specific. *M. senile* carries two ODH allozymes which vary in frequency between northern and southern California (Walsh, 1981). These populations respond to acclimatory and acute changes in temperature with different oxygen uptake and adenylate charge patterns (Walsh and Somero, 1981). Kinetic properties of the fast allozyme (which dominates in southern California) are very different from the slow allozyme (which dominates in northern California) with respect to substrate saturation, apparent Michaelis constants for various substrates, product inhibition by octopine and optimal activity with respect to pH. The kinetic properties of the slow and fast allozymes resemble ODH specific isozymes in cephalopod brain and muscle respectively. Walsh (1981) concludes that ODH acts analogously to vertebrate lactate dehydrogenase in sea anemones.

I examined the possibility that heterozygosity at several loci would be correlated with ODH activity and octopine accumulation. Octopine accumulation was enhanced (or equivalently octopine breakdown was reduced) by heterozygosity at loci PGD, AAT and PGM. Biochemical pathways, such as glycolysis, are mediated by a suite of enzymes whose individual performance is often higher in homozygotes, but whose performance on the pathway level is dependent on the minimization of overall variation. Heterozygous enzymes in a pathway reduce overall variation because they often have intermediate enzyme characteristics (Koehn *et al*, 1988). Heterozygosity at loci AAT, PGD and PGM (and to a lesser degree PGI and MPI)

facilitates the condensation of pyruvate and arginine. Conversely, a lower accumulation of octopine in heterozygotes at locus ODH than in homozygotes might relate to a higher enzyme activity of heterozygotes at locus ODH. Unfortunately I have no measurements of specific ODH activities. It is noteworthy that heterozygotes at locus ODH have a larger scope for activity. The faster the turnover of octopine, the better the chances to improve the ATP yield during and after muscle activity. Aerobic metabolism is supplemented by anaerobic ATP to sustain energy demand. de Zwaan *et al* (1980) calculated that the aerobic demand during valve snapping is no more than 3% of the anaerobic contribution. However, due to the great difference in ATP yield between aerobic and anaerobic glycogen utilization, this still represents an ATP yield of 30%. In general, any animal which needs to work at high rates for longer than 5 to 20 sec must utilize an endogenously driven backup system (anaerobic glycolysis) to augment the ATP originally produced by phosphagen hydrolysis (Hochachka *et al*, 1983).

In conclusion, growth, valve snapping and oxygen uptake are not linked to the degree of multiple-locus heterozygosity and with a few exceptions to the degree of heterozygosity at single loci. Selected genotypes have an effect on stress responses in *P. magellanicus*. Heterozygotes recuperate faster metabolically at locus ODH and octopine tends to have a faster turnover.

More background data are required before a definitive statement can be made. First, the biochemical characterisation of the 3 common genotypes of ODH in *P. magellanicus* has to include a detailed study of their kinetics, including the Michaelis and catalytic constant, substrate specificity, pH dependence and product inhibition. For comparison, several allozymes have been characterised biochemically and physiologically : (1) the LDH-B allozyme in *Fundulus heteroclitus* has an effect on blood oxygen affinity (DiMichele and Powers, 1984); (2) α-amylase is regulated according to genotype and age in *Drosophila melanogaster*, thus affecting the digestion of carbohydrates and survival (Klarenberg *et al*, 1986), (3) the alcohol dehydrogenase allozymes of *D. melanogaster* have different activities (Koehn *et al*, 1983) and (4)

the ^{94}LAP allozyme controls cell volume regulation suboptimally at lower salinities (Koehn, 1985). Second, the differentiation of allozymes has implications for the energy balance. The "associative overdominance" hypothesis takes into account the potential impact of variation at loci adjacent to the marker enzyme. Chances are that homozygotes for marker enzymes will also be homozygous for adjacent genes, including recessive deleterious genes. Homozygous recessive deleterious genes will code for defective enzymes, precursors, proteins, or other products, and thus lower the fitness of an organism (Zouros and Foltz, 1983).

It is not yet clear what the exact effect is of the various ODH allozymes and other allozymes associated with energy metabolism on the physiology of *P. magellanicus*, but the following could be argued. Valve snapping requires bursts of energy which are critical both in the short term (i.e. one day) and in the long term (e.g. one season). Natural selection occurs in the short term (escaping predators means survival) but also in the long term (e.g. age-specific fertility might be negatively affected by low food rations under crowded conditions). A history of more escapes than average will deplete the ATP pool (energy is "wasted" in activity), but a higher scope for activity provides more opportunities to find prey (e.g. *T. haemastoma* (Garton, 1984)) or to escape predators (e.g. *P. magellanicus*). Therefore fitter scallops do not necessarily grow faster. Chapter 6 introduces a model of the impact of the degree of heterozygosity on metabolism in Mollusca; it incorporates the arguments just mentioned.

Chapter 5.

The non-differential contribution of heterozygosity to growth at individual enzyme loci.

5.1 Introduction.

The search for a causal explanation of the connection between heterozygosity and fitness crystallizes around two theories. Associative overdominance (Zouros and Foltz, 1984) or inbreeding depression (Ledig *et al*, 1983) is not concerned with the direct impact of marker loci but with the effects at the loci to which they are linked. This hypothesis suggests that heterozygosity is a fair indicator of the relative fitness of an organism, regardless of the marker loci scored (Ohta and Kimura, 1970; Zouros *et al*, 1988). Alternatively, the theory of multiplicative overdominance attributes fitness differences to variation of the marker enzymes themselves (Turelli and Ginzburg, 1983; Bush *et al*, 1987). The degree of heterozygosity of the loci scored is related, for example, to growth rate and viability because the allozymes have a specific and direct impact. The biochemical and physiological characteristics of allozymes determine the fitness of an organism. Selective differences among single loci have been studied with modest success (e.g. DiMichele and Powers, 1982; Hilbish and Koehn, 1985) although the vast majority of loci have never been tested for their effects.

Koehn *et al* (1988) present a test of multiplicative overdominance, i.e. that allozyme loci belonging to selected metabolic pathways affect phenotypic traits (such as growth rate) directly. Growth was monitored in a large sample of the coot clam (*Mulinia lateralis*) (1906 individuals) at a relatively large number of loci (15 polymorphic enzymes). The correlation between heterozygosity and growth rate was independently determined at each locus with linear least squares multiple regression. In general, loci linked to protein catabolism and glycolysis contribute most significantly to overall phenotypic variation but the average degree of heterozygosity at each locus is not related to phenotype. The relative importance of glycolytic

and protein catabolic enzymes compared to redox balance, pentose shunt, digestive and Krebs cycle enzymes is tentatively attributed to the involvement of glycolytic enzymes in ATP production (thus affecting the turnover of the protein pool) and to protein catabolic enzymes affecting the cost of whole-body protein turnover. The latter has been related to overall costs of metabolic maintenance and overall fitness (Hawkins *et al*, 1986).

In this chapter, I examine whether functional differentiation of enzymes is a reasonable hypothesis to explain phenotypic variation in growth of the scallop *Placopecten magellanicus*. The conclusion is that a functional classification of allozymes does not improve our understanding of growth variation in pectinids.

5.2 Materials and methods.

I compiled a total of 19 data sets from 5 different population surveys of the scallop *Placopecten magellanicus* which included information on heterozygosity (at minimally 5 loci) and shell height (Table 5.1). Not included were those studies whose sample size was judged too small, such as a study of 280 scallops of various ages collected at 6 sites (K. Fuller, Dalhousie University, pers. comm.). All samples provided data on enzyme polymorphism at 6 loci, except for the collections made by Gartner-Kepkay and Zouros (1985) which included 5 loci. Foltz and Zouros (1984) collected two samples at the same site 3 months apart; the samples did not differ significantly from each other and were pooled to maximize the information content. Subsequently, the sample was divided in age groups. Six polymorphic loci were scored with horizontal starch gel electrophoresis as reported in Chapter 2.

I have divided the complete data set into 3 groups for final analysis according to the number of loci scored and age (which allows the calculation of growth rates). Each genotype was given the value **0** if homozygous or the value **1** if heterozygous. Two statistical methods were used to test the differential contribution of allozymes to growth rate. First, the partial sums of squares (SS) of shell height were examined with multiple regression and the effect of the independent variables (loci) on growth

Table 5.1 : Data sets included in the analysis of enzyme specific contributions to the correlation between growth and heterozygosity. Year of collection, number of loci scored, number of individuals, number of collections, number of sites sampled, knowledge of age of scallops and reference are given.

Year of collect.	# of loci scored	N	# of collect.	# of sites sampled	Age known?	Reference
1982	6	752	2	1	yes	Foltz and Zouros, 1984
1983	5	796	8	8	yes	Gartner-Kepkay and Zouros, 1985
1985-86	6	1471	6	2	yes	Volckaert (Chapter 2)
1986	6	124	6	1	yes	Volckaert (Chapter 4)
1986	6	436	6	2	no	Volckaert and Shumway (In preparation)

rate were evaluated one by one. The significance of each regression was tested with an F-test. The relative contributions of the individual loci to the overall relationship between multiple-locus heterozygosity and growth rate were ranked according to the SS resulting from the inclusion of heterozygosity at each locus as independent effect (the most significant positive correlation was ranked first, the most significant negative one last). Such a test maximizes the information related to locus-specific shell height or growth rate variability. Second, average sizes of homozygotes and heterozygotes were calculated for each locus. The significance of the difference between the size of heterozygotes and homozygotes was evaluated with a t-test.

I calculated in both analyses the average rank per locus of all collections scored at 6 (group I) and 5 loci (group II), and those collections in which age was known (group III). The polymorphic loci of *P. magellanicus* were subdivided in two functional groups according to the classification of Koehn *et al* (1988) : enzymes belonging to the glycolytic and protein catabolic pathways (group A) and enzymes belonging to other pathways (group B) (Table 5.2). The average contribution of the degree of heterozygosity of glycolytic and protein catabolic enzymes to growth variation was compared non-parametrically (Wilcoxon test). The average degree of heterozygosity per locus was compared with the average rankings of the contribution of each locus to growth.

Crassostrea virginica (Zouros *et al*, 1980; Singh, 1982) and *Mytilus edulis* (Koehn and Gaffney, 1984; Diehl and Koehn, 1985; Zouros *et al*, 1988) were not included in this study because all loci scored fell into the same functional group.

5.3 Results.

Ten collections of shell height measurements and electrophoretic data at 6 loci were grouped in a first subset of data (group I; Table 5.3.A). Eight other collections were measured and scored at 5 loci (group II; Table 5.3.B). Seven collections of group I included age specific shell height measurements, which made it possible to calculate growth rate. The pooled sample of Foltz and Zouros (1984) was subdivided

Table 5.2 : Classification of allozymes of *Placopecten magellanicus* according to function (group A : pre-glycolytic, glycolytic and protein catabolic enzymes; group B : pentose shunt enzymes).

Locus	EC number	Function	Group
PGD	1.1.1.44	Pentose shunt	B
ODH	1.5.1.11	Glycolytic	A
AAT	2.6.1.1	Protein catabolic	A
PGM	2.7.5.1	Pre-glycolytic	A
MPI	5.3.1.8	Pre-glycolytic	A
PGI	5.3.1.9	Glycolytic	A

in 7 age specific size classes. A total of 13 age-structured samples were available for further analysis (group III; Table 5.3.C).

Ranking of the loci within each group was similar in the multiple regression and size differential analyses (Table 5.3). Minor differences in the locus rankings per sample did not affect significantly the overall ranking of the loci. The contribution of individual loci to growth was not clear when comparing the average ranks within and between groups I, II and III. Most average ranks of the loci clustered around the expected average of 3.5 (in case of 6 loci) or 3 (in case of 5 loci). The data of the highest quality in evaluating the impact of allozymes on overall growth variation are presented in group III. Those samples were collected on a single date at a single site, belonged to one cohort, and thus experienced the same environmental conditions. Again, growth was not linked to the function of the allozymes considered; all enzymes scored close to the expected average rank of 3.5. The average degree of heterozygosity at a given locus did not correlate with the contribution of that locus to growth variation; heterozygosity did not qualify as predictor of allozyme contribution to growth (Table 5.4).

I tested whether or not enzymes of selected pathways dominated phenotypic variation by subdividing the polymorphic loci of *P. magellanicus* into two functional groups : PGM, MPI, ODH, PGI and AAT (belonging to the pre-glycolytic, glycolytic and protein catabolic enzymatic pathways), and PGD (belonging to the pentose shunt) (Table 5.2). The rankings of the difference in shell height of hetero- and homozygotes of both enzyme groupings were compared non-parametrically and were found to be not significantly different. A different contribution to growth by selected enzymatic pathways in *P. magellanicus* was rejected in all groups (Table 5.4).

5.4 Discussion.

Our understanding of the basis of the correlation between the degree of heterozygosity and phenotype did not improve by incorporating information on

Table 5.3 : Comparison of the contribution of individual loci to growth variation. A non-parametric Wilcoxon test tests whether the rank of PGD (group B) is different from the rank of the enzymes of group A. Rankings of both analyses (multiple regression (analysis 1) and average growth differential between hetero- and homozygotes (analysis 2)) and sample size (N) are given (* $P < 0.05$).

Table 5.3.A : Group I (samples scored at 6 loci)

Analysis 1 :

Sample :	1	2	3	4	5	7	8	9	10	11
Locus :										
PGI	4	2	1	1	3	5	2	5	5	4
PGD[a]	2	1	3	3	6	2*	6	4	3	3
AAT	1	4	5	5	1	1*	3	6	4	6
MPI	3	5	4	4	4	6	5	1*	2	2
ODH	5	6	6	2	5	3	4	3	6	1
PGM	6	3	2	6	2	4	1	2	1	5

Wilcoxon test : $t_s = 0.402$; N.S.

Analysis 2 :

Locus	1	2	3	4	5	7	8	9	10	11
PGI	3	2	1	1	3	4	1	6	5	6
PGD[a]	2	1*	3	5	6	5	6	4	2	3
AAT	1	4	6	4	2	1*	3	5	4	5
MPI	4	5	4	3	4	6	5	1*	3	2
ODH	5	6	5	2	5	3*	4	3	6	1
PGM	6	3	2	6	1	2*	2	2	1	4

Wilcoxon test : $t_s = 0.402$; N.S.

N	752	123	135	149	149	73	212	290	123	224

Table 5.3.B : group II (samples scored at 5 loci)

Analysis 1 :

Sample :	12	13	14	15	16	17	18	19
Locus :								
PGI	2	3	5	1	4	1	4	1
PGD[a]	4	4	3	2	3	2	2	3
AAT	5	1	1*	5	5	4	5	2
MPI	3	2	2	4	1	3	1	5
ODH	1	5	4	3	2	5	3	4

Wilcoxon test : $t_s = 0.276$; N.S.

Analysis 2 :

	12	13	14	15	16	17	18	19
PGI	1	3	5	1	4	3	5	1
PGD[a]	4	4	3	2	3	2	2	3
AAT	5*	2	1*	5	5	4	4	2
MPI	3	1	2	4	1	1	1	5
ODH	2	5	4	3	2	5	3	4

Wilcoxon text : $t_s = 0.276$; N.S.

N	100	100	100	96	100	99	100	100

Table 5.3.C : group III (aged samples scored at 6 loci)

Analysis 1 :

Sample :	1a	1b	1c	1d	1e	1f	1g	2	7	8	9	10	11
Locus :													
PGI	1	3	4	1*	1	6	3	2	5	2	5	5	4
PGD[a]	2	2	1	4	4	5	2	1	2*	6	4	3	3
AAT	3	1	5	3	5	1	5	4	1*	3	6	4	6
MPI	6	5	3	6*	2	2	4	5	6	5	1*	2	2
ODH	4	6*	2	2	3	3	1	6	3	4	3	6	1
PGM	5	4	6*	5	6	4	6	3	4	1	2	1	5

Wilcoxon test : $t_s = 1.164$; N.S.

Analysis 2 :

Locus	1a	1b	1c	1d	1e	1f	1g	2	7	8	9	10	11
PGI	2	3	6	1	1	6	2	2	4	1	6	5	6
PGD[a]	1	2	1	4	4	5	4	1*	5	6	4	2	3
AAT	4	1	4*	3	5	2	5	4	1*	3	5	4	5
MPI	5	5	2	6	2	1	3	5	6	5	1*	3	2
ODH	3	6*	3	2	3	3	1	6	3*	4	3	6	1
PGM	6	4	5*	5	6*	4	6	3	2*	2	2	1	4

Wilcoxon test : ts = 0.619; N.S.

N	32	146	128	183	178	57	16	123	73	212	290	123	224

[a] PGD is classified in functional group B (see Table 5.2).

Note : List of samples included in this table (sample number, age of scallops, source of information and locality are given).

Number	Age	Source	Locality
1	-	Foltz and Zouros (1984)	Halifax (Harrigan Cove)
1a	4 year	idem	idem
1b	5 year	idem	idem
1c	6 year	idem	idem
1d	7 year	idem	idem
1e	8 year	idem	idem
1f	9 year	idem	idem
1g	10 year	idem	idem
2	20 months	Volckaert (1988)	Passamaquoddy Bay
3	-	Volckaert and Shumway (In progress)	idem
4	-	idem	idem
5	-	idem	Gulf of Maine
7	5 months	Volckaert (1988)	Passamaquoddy Bay
8	9 months	idem	idem
9	14 months	idem	idem
10	14 months	idem	idem
11	15 months	idem	idem
12	-	Gartner-Kepkay and Zouros (1985)	Georges Bank (NE)
13	-	idem	Browns Bank
14	-	idem	Georges Bank (N)
15	-	idem	Middle Grounds
16	-	idem	Georges Bank (S)
17	-	idem	Mahone Bay
18	-	idem	Bay of Fundy
19	-	idem	Grand Manan

Table 5.4 : Comparison per locus of the mean rank of the difference in shell height of hetero- and homozygous *Placopecten magellanicus* (analysis 2) and the mean degree of heterozygosity (S.E., standard error; N.S., not statistically significant).

Data sets scored at 6 loci (group I).

Locus	Mean rank	Mean heterozygosity (±S.E.)
PGM	2.9	0.468 (±0.013)
PGI	3.2	0.091 (±0.006)
AAT	3.5	0.507 (±0.017)
MPI	3.7	0.322 (±0.015)
PGD[a]	3.7	0.268 (±0.014)
ODH	4.0	0.466 (±0.021)
	Expected rank : 3.5	

Wilcoxon rank correlation test : $t_s = 0.402$; N.S.

Data sets scored at 5 loci (group II).

Locus	Mean rank	Mean heterozygosity (±S.E.)
MPI	2.3	0.324 (±0.010)
PGD[a]	2.9	0.275 (±0.011)
PGI	2.9	0.096 (±0.005)
ODH	3.5	0.459 (±0.016)
AAT	3.5	0.528 (±0.014)
	Expected rank : 3.0	

Wilcoxon rank correlation test : $t_s = 0.276$; N.S.

Data sets scored of uniform age scored at 6 loci (group III).

Locus	Mean rank	Mean heterozygosity (±S.E.)
PGD[a]	3.2	0.256 (±0.016)
ODH	3.4	0.405 (±0.025)
PGI	3.5	0.079 (±0.005)
AAT	3.5	0.522 (±0.018)
MPI	3.5	0.326 (±0.011)
PGM	3.8	0.477 (±0.017)
	Expected rank : 3.5	

Wilcoxon rank correlation test : $t_s = 0.619$; N.S.

[a] PGD is classified in functional group B (see Table 5.2).

the dichotomy regarding the function of allozymes suggested by Koehn *et al* (1988). The growth rate of *Placopecten magellanicus* (a species with no measurable heterozygosity-dependent growth) was not significantly related to this classification. Differentiating between glycolytic and protein catabolic enzymes (AAT, PGM, PGI, ODH and MPI) and enzymes of the pentose shunt (PGD) did not account for the differential effects of loci on growth. These results differ from the evidence collected from *M. lateralis* (Koehn *et al*, 1988). I conclude that enzyme function is not a general predictor of the contribution of enzyme variation to phenotypic variation in growth. Moreover, the ranking of each locus of *P. magellanicus* varies considerably between samples.

The paper of Koehn *et al* (1988) and my study differ in certain respects. The relatively small number of individuals examined per sample (minimum 124 and maximum 752) may account for the lack of statistically significant partial multiple regressions, but the large number of samples should have allowed detection of an overall trend, if such a trend existed. The smaller number of polymorphic loci scored in *Placopecten magellanicus* does not diminish the fundamentals of the argument because enzymes of both functional groups have been included, despite the fact that only one enzyme is classified in functional group B.

A third difference is related to the enzyme loci. I used a functional classification which remained faithful to the standards of the classification presented in Koehn *et al* (1988). I assigned the loci ODH and AAT, which were not scored in their paper, to the glycolytic pathway (ODH) and as protein catabolic (AAT) (Appendix A). The pyruvate reductase ODH catalyzes the condensation and breakdown of octopine (see Chapter 4). During hypoxia glucose or glycogen are broken down to octopine via the Embden-Meyerhof-Parnas pathway and act as source of ATP (Gäde and Grieshaber, 1986). ODH allozymes are kinetically different in *Metridium senile* (Walsh, 1981) and *P. magellanicus* but not in *Strombus luhuanus* (Baldwin and England, 1981), *Concholepas concholepas* (Carvajal and Kessi, 1988), *Pecten maximus* (Monneuse-Doublet *et al*, 1980) and *P. jacobaeus* (Zettlmeissl *et al*, 1984). AAT

catalyzes the transamination of the amino acid aspartic acid and α-ketoglutaric acid to oxaloacetic acid and the amino acid glutamic acid in the malate-aspartate shuttle system between mitochondrion and cytosol (Hochachka and Somero, 1984). The enzymes PGM and MPI supply carbon skeletons to the glycolysis pathway; PGM catalyzes the conversion of glucose-1-phosphate to glucose-6-phosphate; MPI converts phosphorylated mannose to fructose-6-phosphate. PGI couples glucose-6-phosphate and fructose-6-phosphate in glycolysis and gluconeogenesis (Hochachka and Somero, 1984). PGI alleles in many organisms have different catalytic properties (e.g. Watt, 1977; Hall, 1985). The enzyme 6-PGD converts gluconate-6-phosphate to ribulose-5-phosphate in the pentose shunt (Koehn *et al*, 1985). The flux through the pentose phosphate pathway represents a small fraction of total glucose utilisation in bivalves (Zaba *et al*, 1981).

Although the functions of enzymes and the structure of enzymatic pathways are fairly well known in molluscs, much remains to be learned about the dynamics of enzymes *in vivo*. Consequently, the study of the regulatory role of allozymes - linked to specific pathways - in affecting phenotypic traits (specifically maintenance metabolism in bivalves) remains speculative. Measurements of, among others, protein turnover rates, enzyme concentrations, flux rates and substrate inhibition rates must have priority status. For example, phosphofructokinase catalyzes the first step in glycolysis and as such is an important locus of control (Hochachka and Somero, 1984). PFK control is based upon its interactions with both substrates, fructose-6-phosphate and ATP; fructose-2,6-biphosphate is a potent activator of PFK. The question arises whether PFK allozymes modify catabolism significantly and if so, how this is realised.

Multiplicative overdominance might be an appropriate model to explain the causation of phenotypic and genotypic interactions at a limited number of loci (e.g. Koehn *et al*, 1983), or in a limited number of organisms (Koehn *et al*, 1988; Bush *et al*, 1987), but it is not a general model according to my results. In the final

chapter I present a functional model of the impact of associative overdominance on the phenotype of molluscs.

Chapter 6.

General conclusions.

In this study I have investigated the connection between allozyme genotype and Darwinian fitness at several levels of biological organisation in the scallop *Placopecten magellanicus.* At the start of this project in late 1983 the positive correlation between heterozygosity and growth rate had been documented in several taxa. The number of inconclusive correlations across species, including scallops (Foltz and Zouros, 1984; Beaumont *et al*, 1985), about equalled the number of positive correlations. The general idea then prevalent was that heterozygosity affected growth, but the effect was small (only a few percent of total phenotypic variation) (Koehn and Gaffney, 1984). It was not known whether locus-specific effects or background effects linked to the marker enzyme influenced the phenotype. Variation in oxygen uptake was the only metabolic trait linked to multiple-locus heterozygosity (Koehn and Shumway, 1982).

At present, the population genetics of *P. magellanicus* can be interpreted as follows. One assumes that the large mortality of scallop larvae (more than 90% (C. Couturier, Dalhousie University, pers. comm.)) is the principal cause of the heterozygote deficiency observed in spat (Mallet *et al*, 1985). Selective mortality after settlement reduces this deficiency in heterozygosity of scallop spat to the Hardy-Weinberg equilibrium within a period of less than 12 months. The average degree of heterozygosity increases with age in adults (Foltz and Zouros, 1984). Again this phenomenon might be attributed to selective mortality of the less heterozygous scallops. Growth rate of juvenile and adult scallops does not increase with the number of heterozygous loci. Scope for growth of juvenile scallops is not related to genotypic variation at enzyme loci, but it remains to be proven whether genotypic variation correlates with clearance rate during feeding. Reduced routine metabolic costs in heterozygotes were not found in *P. magellanicus.* However, several traits related to the metabolism of swimming prove to be correlated with heterozygosity : (1) a

higher scope for activity of heterozygotes at locus ODH has been found; (2) octopine accumulation in the adductor muscle is related positively to overall heterozygosity and (3) ODH activity is higher in animals homozygous at locus ODH. Functional differences between marker enzymes classified according to Koehn *et al* (1988) do not allow one to predict the strength of the correlation between heterozygosity and growth. I conclude that the most important connection in the population genetics of *P. magellanicus* is the positive correlation between genotype and traits related to functional anaerobiosis but not between genotype and growth related traits.

I propose the following functional model to explain the concomitant increase in fitness with the degree of heterozygosity in juvenile and adult Mollusca, including in *P. magellanicus* (Figure 6.1). The point of departure is the variable degree of multiple-locus heterozygosity which has been measured often within populations. High degrees of heterozygosity have been correlated with fitness traits (Zouros, 1987). The "associative overdominance" hypothesis assumes that allozymes are in a steady state of linkage disequilibrium with deleterious genes (Zouros and Foltz, 1984) (deduction **A**). The alternative hypothesis of "multiplicative overdominance", which attributes fitness differences observed directly to the loci scored (Smouse, 1986), is considered less appropriate (see below).

The reduced level of homozygosity of recessive deleterious genes in multiply heterozygous individuals translates into a higher efficiency of the enzyme pathway. The higher the degree of heterozygosity, the lower the overall catalytic variation and the higher the enzymatic efficiency (Koehn *et al*, 1988) (**B**). Catalytic properties of heterozygotes are often intermediate to homozygotes; the greater the number of heterozygous enzymes, the smaller will be the variance of the pathway.

The increased efficiency of the enzymatic pathway requires smaller enzyme concentrations in the cytosol, thus reducing the rates of enzyme synthesis to guarantee a similar substrate flux (**C**). Suboptimal environmental conditions jeopardize the efficiency of enzymes; simultaneously, enzymes modulate solute concentrations to buffer the cell environment. Selected alleles compensate better under suboptimal

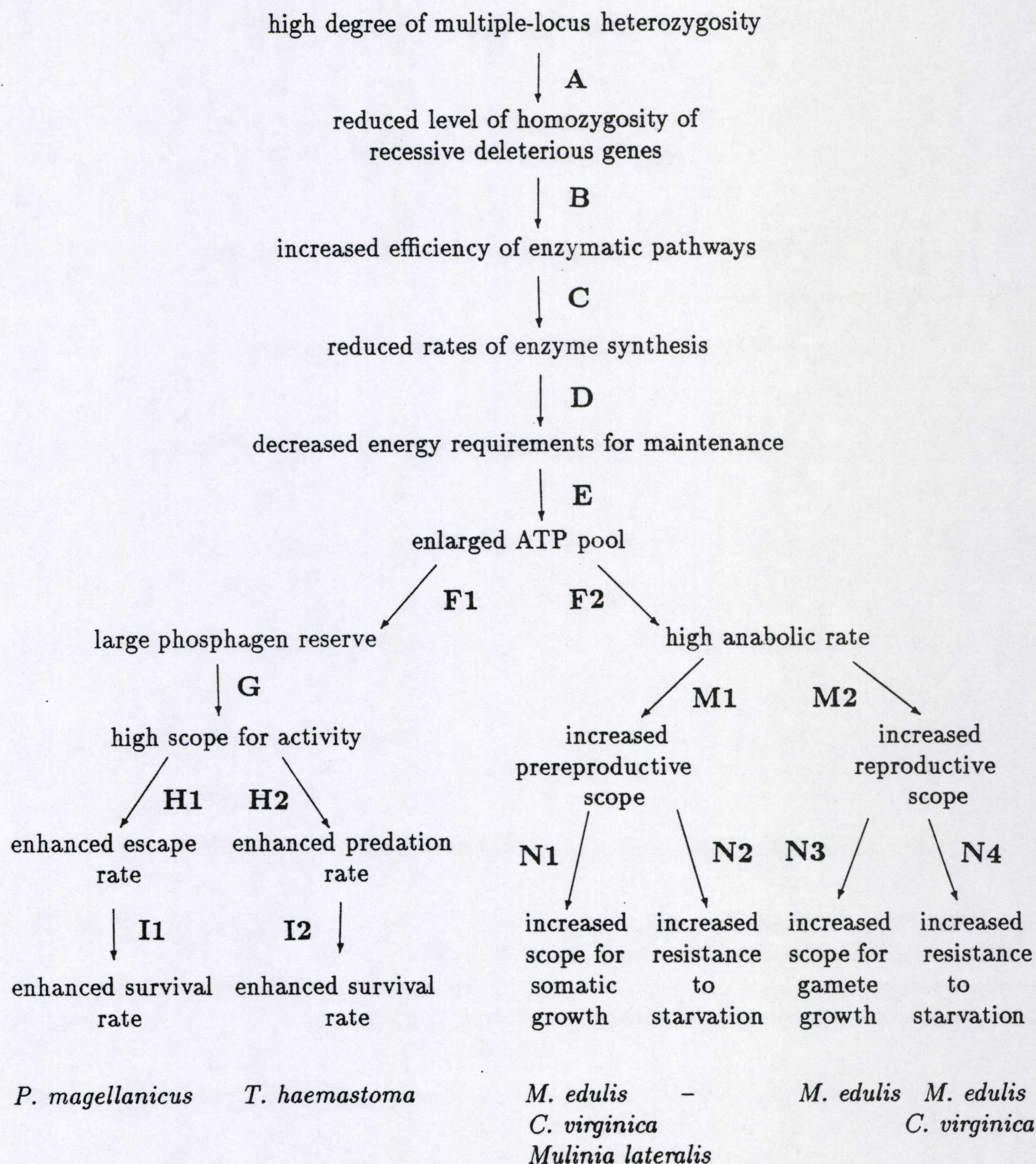


Figure 6.1 : A model of heterozygote superiority of molluscs with special attention to the bivalves *Placopecten magellanicus*, *Mytilus edulis*, *Crassostrea virginica*, *Mulinia lateralis* and the gastropod *Thais haematostoma*.

environmental conditions; for example, hyperosmotic stress in invertebrates is compensated for at the allozymes of GPT and ^{94}LAP (Burton and Feldman, 1983; Koehn, 1985). The greater efficiencies of protein synthesis - especially in heterozygotes - are likely to lower the energy requirements for maintenance (Hawkins *et al*, 1986) (**D**). Protein synthesis is a non-negligible component of metabolism, accounting for about 10% of basal oxygen uptake in man (Waterlow and Jackson, 1981). Energy saved by virtue of a higher metabolic efficiency contributes to the ATP pool of energy not required for the essential metabolic functions such as protein turnover and ion gradient maintenance in membranes (**E**) (Berger, 1976). ATP energy not required for vital functions is allocated differently in sessile molluscs (e.g. clams, mussels and oysters) compared to motile molluscs (e.g. whelks and scallops) (**F**). The surplus ATP of motile species is shunted into growth and build-up of muscle arginine phosphate. If arginine phosphate synthesis is under greater genotypic control than the anabolic process, this does not preclude that anabolic processes are not important; anabolism matters on a rather non-genetically correlated level. Higher phosphagen reserves sustain more locomotory activity (such as sliding and swimming) (**G**), thus allowing higher hunting activity by carnivorous species such as *T. haemastoma* (Garton, 1984) (**H2**) or a greater chance to escape predators and crowded conditions (and thus improve access to food particles) by swimming species such as *P. magellanicus* (**H1**). Both strategies affect survival, a major outcome of selective forces in evolution. No correlation is expected between heterozygosity and shell height; heterozygote deficiency is insignificant.

According to my model, the genetically controlled ATP surplus in sessile organisms translates into an above average anabolic rate of heterozygous individuals (**F2**). Differences in energy budgets between juvenile and adult organisms are such that surplus energy stimulates somatic growth (and resistance to starvation) in juveniles (Koehn and Gaffney, 1984; Garton *et al*, 1984; Koehn *et al*, 1988) (**N1 - N2**) and increased gamete production (and resistance to starvation) in adults (Diehl *et*

al, 1986; Rodhouse and Gaffney, 1984; Rodhouse *et al*, 1986) (**N3** - **N4**). Heterozygote deficiency is large in young animals and tends to decline with time. A similar decline with time occurs with the correlation between multiple-locus heterozygosity and growth.

Several binary divisions ("switches") have been incorporated into the model; distinctions are made between sessile and motile organisms (**F1** - **F2**), carnivorous and filter feeding motile molluscs (**H1** - **H2**), juvenile and adult sessile organisms (**M1** - **M2**) and fed or starved organisms (**N1** - **N2**; **N3** - **N4**). Several categories are unique to either the motile or sessile group (e.g. carnivorous sessile molluscs are rare).

This functional model of heterozygote superiority incorporates as much of the knowledge on the impact of multiple-locus heterozygosity on fitness in molluscs as possible. It is not a "black-and-white" picture of how genotype-specific transfer of energy influences fitness; time will tell which adjustments and what kind of reinterpretation are necessary. One possible amendment of the model is that gamete output (fertility) of scallops might prove to be correlated with heterozygosity. According to the model, only surplus energy shunted into activity in motile invertebrates, is affected by the degree of heterozygosity. It is conceivable that both the ATP required for gametogenesis and for locomotory activity is affected by the enzyme genotype. So far, no experimental evidence is available to prove this. A second amendment relates to the correlation between heterozygosity and clearance rate in the sedentary bivalve *Rangia cuneata* (Holley and Foltz, 1987), suggesting that appetite in filter feeders is influenced by genotype. Since no other information on the genetics of the metabolism of *R. cuneata* is available, it is difficult to incorporate it in the model.

A comparison of the literature with my functional model shows that the majority of the correlations between phenotypic and genotypic variation have focussed on the physiological and general biological level (below deductions **G** and **N**). However, it is exactly there where biochemical processes and genotype interact that

fitness is controlled (see Hawkins *et al*, 1986). Single-locus studies, although they do not really fit in the structure of the model, have followed that route.

My model incorporates the "energetic efficiency" hypothesis (i.e. adult invertebrates divert with age increasing amounts of energy from somatic growth to gamete growth) (Rodhouse *et al*, 1986), the "balanced enzyme pathway" hypothesis (Koehn *et al*, 1988), the hypothesis of greater catalytic efficiency of enzymes in heterozygotes (Berger, 1976) and the "associative overdominance" hypothesis. The last has been preferred to the "multiplicative overdominance" model because of the lack of a link between enzyme function and the strength of the correlation of heterozygosity and growth in scallops (see Chapter 5), because of evidence from analytical models (e.g. Zouros *et al*, 1988), from empirical studies (Mallet *et al*, 1986), and because of the possible impact of chromosomal defects on phenotype (Thiriot-Quiévreux, 1986). Associative overdominance explains the decline of the correlation between heterozygosity and growth with age, and the roughly equal amount of phenotypic variation explained in various studies by heterozygosity (Zouros, 1987). Growth rate might be affected by aneuploidy (i.e. differences from an integral multiple of the haploid number of chromosomes); faster growers show no chromosomal defects. Aneuploidy has a direct impact on homozygosity : the electrophoretic phenotype will resemble the homozygous phenotype. It might be more appropriate to rename the correlation of phenotype with the degree of heterozygosity, the correlation of phenotype with the degree of homozygosity.

In conclusion, the model incorporates most of the observations that relate allozyme heterozygosity to phenotypic traits in molluscs. Although I have focussed my argument on the phylum Mollusca, because it is the single best studied and most suitable taxon for this kind of studies, there are good reasons to generalize the model to other invertebrate and vertebrate species after making some modifications.

Appendix A.

Figure A.1 : Scheme of the Embden-Meyerhof-Parnas pathway and Krebs cycle of *Placopecten magellanicus*, including the pentose shunt and the allozymes PGM, PGI, MPI, ODH, PGD and AAT.

The Embden-Meyerhof-Parnas pathway and Krebs cycle of *Placopecten magellanicus*.

Embden-Meyerhof-Parnas pathway

glycogen

glucose-1-P

PGM

glucose

glucose-6-P

D-glucolatone-6-P

PGI

mannose-6-P

fructose-1,6-PP

D-gluconate-6-P

MPI

PGD

D-glyceraldehyde-3-P

D-ribulose-5-P

1,3 diphosphoglycerate

arginine phosphate

phosphoenolpyruvate

arginine

pyruvate

octopine

ODH

Krebs cycle

oxaloacetate

acetyl-CoA

AAT

malate

citrate

fumarate

isocitrate

succinate

α-ketoglutarate

succinyl-CoA

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