# Tissue variability in the infaunal bivalve <u>Axinopsida</u> <u>serricata</u> (Lucinacea: Thyasiridae) exposed to a marine mine-tailings discharge; and associated population effects.

Douglas Arthur Bright
Bachelor of Science, University of Victoria, 1984
Master of Science, University of Victoria, 1987

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTORATE OF PHILOSOPHY
in the Department
of Biology

ACCEPTED
FACULTY OF GRADUATE STUDIES

DATE _199} - 03 - 04_ DEAN

We accept this thesis as conforming
to the required standard

Dr. D.V. Ellis

Dr. A.R. Fontaine

Dr. V.J. Tunnicliffe

Dr. A. McAuley

Dr. E. Vander Flier-Keller

Dr. N. F. Bourne

© Doug A. Bright, 1991
UNIVERSITY OF VICTORIA
January, 1991

Supervisor: Dr. D.V Ellis

ABSTRACT

Axinopsida serricata (Bivalvia) is abundant in coastal waters of British Columbia subjected to natural and anthropogenic disturbance. To investigate the monitoring potential of histological lesions, field populations were sampled in Holberg Inlet and Quatsino Sound, British Columbia, from benthic habitats affected by the submarine discharge of copper-mine tailings, and from a reference site in Mill Bay, Saanich Inlet. Based on a quantitative analysis of the digestive gland, ctenidia, kidney, gonad and stomach, the relationship between histological variation and site, size, season, sex and parasitism was explored. The relationship between occurrence of histological lesions in this species and further ecological consequences of mine-tailings discharge was also explored by comparing population characteristics of clams living in deposited tailings with clams from the reference site.

Between-sample differences were observed in the structure of digestive tubule digestive cells, digestive ducts, ctenidial frontal cells, laterofrontal cells, and abfrontal mucocytes, kidney concretions, and stomach epithelial cells. The pattern of differences in tissue structure between samples reflected proximity of the collection site to the mine-tailings discharge and seasonally-dependent reproductive activity. Simultaneous examination of six of the tissue variables (using a principal components analysis) showed that clams collected from three stations in Lower Holberg Inlet which were in closer proximity to the tailings discharge pipe were distinguishable from clams collected from the reference site, upper Holberg Inlet, and Quatsino Sound. Tissue structural variability in *A. serricata* was not influenced by sex, or ectoparasitism by a flagellate. Tissue variables were not causally related to clam size (and thus of age and duration of exposure). In spite of the notorious natural plasticity of molluscan tissues, the variability can be partitioned to provide a very effective interpretation of exposure to stressors.

Based on an increased abundance in degraded habitats, *A. serricata*, and the superfamily Lucinacea in general, have been described as r-selected or opportunistic species. An investigation of life-history traits showed that *A. serricata* has a maximum longevity of five years or longer, exhibits sporadic growth primarily in the

summer months, and is an iteroparous, gonochoristic broadcast spawner with gamete release occurring primarily in November. The observed life span of the clam and presence of ova which are very large (maximum diameter is approximately 100 μm) and yolk-rich for a broadcast spawner are somewhat at odds with the contention that *A. serricata* is an r-selected species.

Tissue variations which occurred in the digestive tubules and ctenidia with increased incidence and severity closer to the tailings discharge pipe are similar to histopathological effects in molluscs as described by others. However, there is no evidence that tissue lesions in *A. serricata* negatively affect fecundity, growth, or abundance. The sub-population sampled closest to the discharge pipe is in a state of decline, but this is due to the absence of recruitment since 1986, rather than increased mortality in the established population.

The apparent decoupling of tissue-level and population-level effects may be due to a time lag in manifestation of decreased fitness at the population level, selection of stress-tolerant individuals in response to the stressor, a strategy of neglect of somatic maintenance and repair, or some other mechanism. It is possible that *A. serricata* and other small Thyasirids have an evolutionary history which provides pre-adaptation to environmental stressors.

Dr. D.V. Ellis

Dr. A.R. Fontaine

Dr. V.J. Tunnicliffe

Dr. A. McAuley

Dr. E. Vander Flier-Keller

Dr. N.F. Bourne

TABLE OF CONTENTS

3. A quantitative analysis of tissue variability in A. serricata associated with site, size, season, sex, and parasitism.

Methods

Results

4. Changes in population attributes associated with changes in tissue structure: I. A baseline study of the ecology of A. serricata in Mill Bay, Saanich Inlet.

Methods

Results

5. Changes in population attributes associated with changes in tissue structure: II: Between-site variation in fecundity, growth, and abundance related to the spatial distribution of tissue structural alterations.

Methods

LIST OF TABLES

LIST OF FIGURES

Acknowledgements

Clams and sea, scientists and me
you put up with the lot
And gave me roots, a place to grow,
a family, and your thought
This product of our sleepless nights lies complete
for you to see
It would be my present to you, my love
If it weren't your gift to me.

(with apologies to A.A. Milne)

1. Introduction

Molluscan histopathology in perspective; and study objectives.

The ability to predict, detect, and respond to environmental change arising from human activities is of increasing interest to our society. Several researchers have proposed that examination of deleterious changes in the cells and tissues of natural populations of fish and invertebrates may be an effective method of early detection of human-induced environmental perturbations (Auffret, 1988; Lowe, 1988; Hinton and Couch, 1984; Sindermann, 1980; Dethlefsen, 1978; Yevich and Barszcz, 1976). This thesis is a study of tissue structural alterations and associated population effects in marine infaunal bivalves, *Axinopsida serricata* (Carpenter, 1864)(Lucinacea: Thyasiridae), exposed to a marine discharge of copper mine tailings. This is also the first major account of the tissue structure and population ecology of this genus and species.

Abnormalities, or lesions, in mollusc tissues have been described by several authors (e.g. Sunila, 1987; Yevich and Yevich, 1985; Lowe *et al.*, 1981; Fries and Tripp, 1977; Gardner *et al.*, 1975). However, the usefulness of histopathology in routine environmental monitoring using marine invertebrates is still doubtful because the etiology of specific changes in tissue structure has not been investigated in detail. Some attempts have been made using laboratory manipulations to study the relationship between exposure concentration of different contaminants and molluscan tissue structure. For example, Calabrese *et al.* (1984) showed that exposure of mussels (*Mytilus edulis*) to 5 μg/g Cu for 18 months resulted in consistent histopathological changes in the digestive diverticula, stomach, and posterior adductor muscles. However, fewer mussels exhibited lesions when exposed to a copper concentration of 10 μg/g.

Within a single organism, different tissue changes may suggest different etiologies. Auffret (1988) studied histological changes in mussels collected from field populations along a gradient of industrial contamination (including heavy metals, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls) and in mussels exposed in mesocosms to different concentrations of diesel oil and copper. The results suggested that different pathological changes were associated with

chronic versus acute exposure. There were no linear relationships between the incidence of nineteen different pathological conditions and the concentration of contaminant exposure.

For any given stressor[1], a complete understanding of the etiology of tissue responses may require an understanding of biochemical mechanisms of contaminant uptake, sequestration, detoxification, and excretion, the site of toxic action, biochemical and physiological perturbations associated with immediate physical damage, and homeostatic response mechanisms. Some molluscan histological changes may represent a generalized stress response (for example, changes in digestive cell height: Bright and Ellis, 1989) while others are contaminant-specific responses.

Organisms from field populations may be simultaneously exposed to complex combinations of physical, chemical and biological stressors, with additive and non-additive effects. Therefore, the immediate cause of changes in tissue structure may be difficult to determine. Nevertheless, if an examination of tissue structure can provide an indication of integrated response to complex environmental changes at particular sites, then there may be some advantage to using histopathology in environmental monitoring. However, impact-related tissue changes may be confused with variations in molluscan tissue structure associated with natural processes. These potentially include seasonally-dependent physiological processes such as feeding and reproduction; size- and age-dependent differences in metabolism, senescence, and duration of exposure; sex-related differences; site to site differences in food availability; tidal responses; and parasitism. Variation between individuals in the bioaccumulation of metals in molluscs occurs in relation to size (Fischer, 1983; Julshamn, 1981; Boyden, 1977), season (Boyden and Phillips, 1981), sex, and diverse environmental factors (Engel et al., 1981; Jackim et al., 1977). Presently, there do not appear to be any published investigations of the relationship

---

1. The terms "stress" and "stressor" have been used throughout the thesis, and are therefore defined here. Selye (1957) defined a stressor as an agent which causes stress in an organism. I have also used stressor to mean the external factor or factors which elicit a response in the organism. Stress is defined here as a specific suite of interrelated hormonal, physiological, and structural responses to any of a diverse assemblage of agents which perturb the functioning of an organism.

between perturbation-induced tissue variability and other potential influences except for parasitism.

In theory, altered form implies altered function. By definition, terminology employed in studies of contaminant effects on tissue structure in fish and invertebrates- i.e., "idiopathic lesions" (Malins et al., 1982; Brand, 1990), "lesions" (Sunila, 1987; Malins et al., 1988), "indicators of pathology" (Lowe, 1988), "cytopathological effects" (Bodammer, 1979), "histopathological effects" (Sunila, 1985; Yevich and Yevich, 1985)- have presupposed suffering or morbid changes for affected individuals. Sunila (1987) suggested that, for environmental monitoring, tissue alterations in resident populations should have ecological significance; tissue lesions should have adverse effects on growth, reproduction, or survival of individuals and populations. Few studies have attempted to demonstrate functional impairment and associated consequences related to changes in tissue structure for organisms and populations. If exposure conditions are similar for all members of a local population and genotypic differences in susceptibility are not highly variable, then the occurrence of lesions or histopathological effects arising from chronic exposure should be reflected at the population level. A search of published literature failed to reveal any previous attempts to bridge the gap between documented changes in tissue structure of fish or invertebrates and population effects in field populations.

The objectives of this study are (1) to provide a description of normal and abnormal tissue structure in a marine infaunal bivalve, *Axinopsida serricata*, from an undisturbed reference habitat, and from areas exposed to environmental perturbation, (2) to investigate the extent to which variation in tissue structure is associated with proximity to a point-source discharge of mine-tailings as opposed to natural effects of size, seasonal variation, sex, or incidence of an ectoparasitic flagellate, and (3) to determine the extent of congruence between site-to-site differences in the incidence and severity of tissue structural alterations and differences in population characteristics (fecundity, growth, abundance). No attempt is made in this study to elucidate mechanistic models for induction of histological change by specific stressors.

The organization of this thesis is as follows. The introduction continues with three sections which provide background information on the use of *Axinopsida serricata* as a bioindicator, the sites employed in this study, and a review of quantitative studies of histopathological change in other molluscs. Chapter 2 includes baseline descriptions of the histology of *A. serricata* collected from a reference station (Mill Bay, Saanich Inlet) and further descriptions of abnormalities in clams influenced by mine-tailings deposition (Holberg Inlet, Quatsino Sound) or by the residual effects of now discontinued copper smelting activities (Granby Bay, Observatory Inlet)(Figure 1). Also included in Chapter 2 is a description of field methods employed and locations of sampling stations. These sites were used not only for qualitative descriptions of tissue structure, but also for the observation of quantitative histological variation and associated population-level effects. Chapter 3 explores factors controlling the variability of tissue structure (site, size, season, sex, parasitism). In Chapter 4, a baseline study of the ecology of *A. serricata* from Mill Bay, Saanich Inlet is provided. Chapter 5 examines the strength of covariation between tissue-level and population-level effects of mine-tailings discharge. A general discussion of the relationship between histological and whole-organism effects of stress is provided in Chapter 6.

Figure 1: Sampling sites for the study of tissue structure in the marine
bivalve, *Axinopsida serricata* in British Columbia, Canada.

55°N

Granby Bay

USA
CANADA

B R I T I S H

C O L U M B I A

50°N

Rupert / Holberg Inlets

N

Saanich Inlet

100 50   0   50 100 150 200 250

Km

125°W   120°W
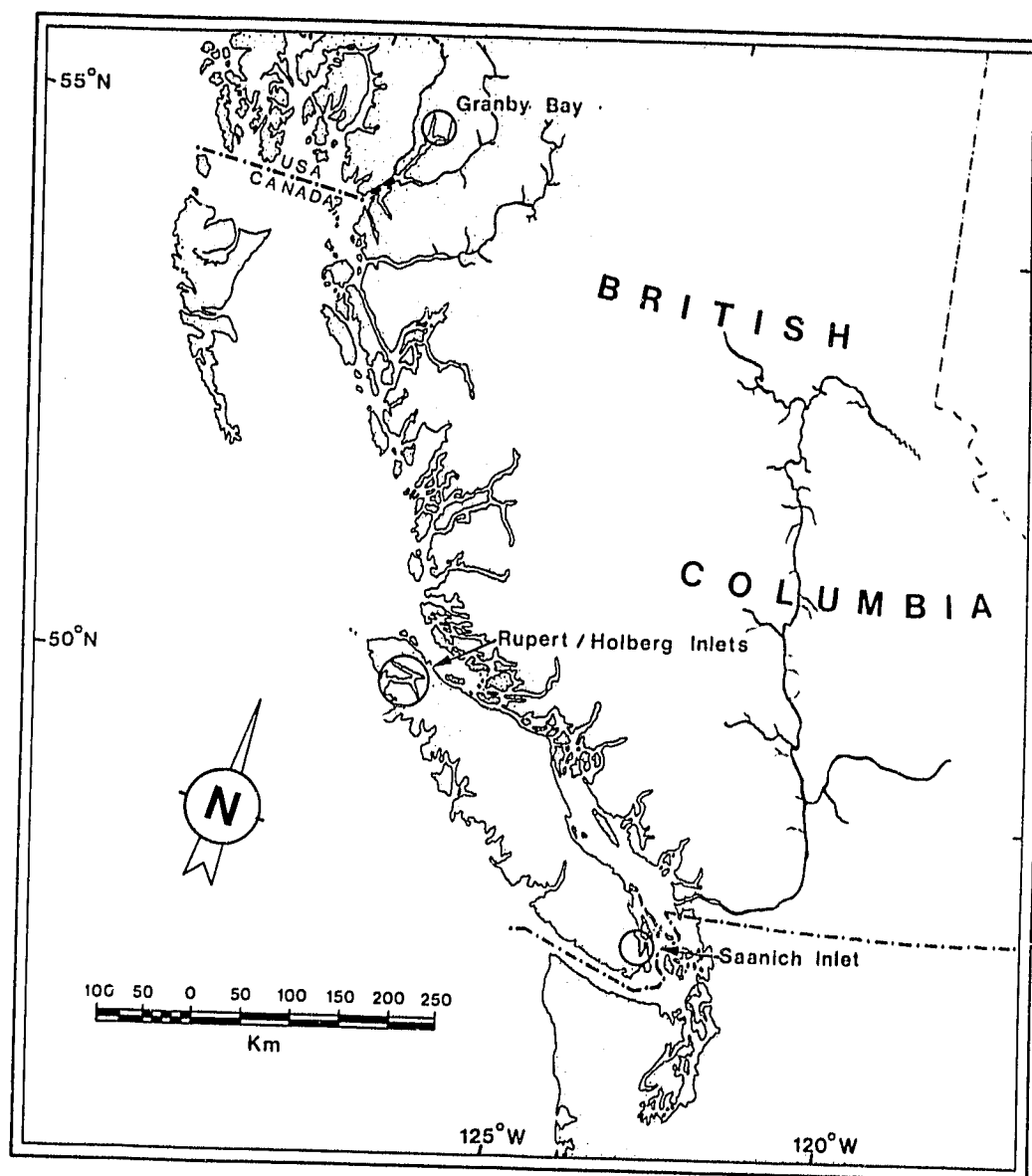
The use of _Axinopsida serricata_ as a bioindicator.

Mussels, _Mytilus_ spp., have been studied extensively as sentinel organisms for marine pollution as part of an international collaborative effort (Cossa, 1988; Hietanen _et al._, 1988; Goldberg, 1980). Concentrations of bioaccumulated contaminants, physiological changes, and histopathological effects in mussels provide an indication of the distributions of contaminants and environmental health. However, _Mytilus_ spp. are hard-substrate epifauna living in the intertidal zone or shallow subtidal zone. The geochemical cycling of contaminants often involves deposition, precipitation, and adsorption onto sedimented particles such that only a limited portion of contaminants introduced to the sea are biologically available within the overlying water column. Consequently, soft-bottom infauna may be exposed to higher concentrations of contaminants than hard-substrate epifauna because of the potential for contaminant remobilization from sediment associated with diagenesis and bioturbation (Aller, 1982).

In this study, effects of marine mine-tailings discharge were hypothesized to be most severe on the seabed. Therefore, a marine infaunal species was needed as a bioindicator.

Axinopsida serricata (Carpenter, 1864) was chosen for histopathological analysis because it is the only infaunal bivalve consistently abundant in deposited copper-mine tailings at an accessible site (Rupert and Holberg Inlets; Figure 1) and other sites in British Columbia where mining wastes have been deposited in the sea (Ellis and Hoover, in press). _A. serricata_ is a member of the family Thyasiridae within the superfamily Lucinacea. Several researchers have noted that the superfamily as a whole appears well adapted to living under adverse conditions (Allen, 1958; Reid and Brand, 1986). _A. serricata_ does not appear to be an exception to the generalization, and has been described as an r-selected species (Reid and Brand, 1986).

A. serricata is a numerically dominant bivalve in many near-shore silt-clay benthic communities of the northeast Pacific. The species, along with _A. viridis_ (considered by many researchers to be a synonym of _A. serricata_: Abbott, 1974) and _Thyasira_ sp., was considered by Thorson (1957) within his framework of parallel level-bottom communities to be a major component of a discrete, somewhat impoverished community (foraminiferan community) inhabiting deeper muddy

sediments in Arctic and boreal seas. Lie and Kisker (1970) described a community along the continental shelf of Washington State, U.S.A. in which the bivalves *A. serricata*, *Adontorhina cyclia* and *Macoma carlottensis* co-occur as numerical dominants.

In British Columbia, *A. serricata* is abundant in benthic environments exposed to natural and anthropogenic disturbance. Elevated abundances occur along the sides of a fjord (Saanich Inlet: Conlan, 1977; personal observation) which undergoes seasonal anoxia in the bottom waters, and in organic- and sulfide-rich sediments along the periphery of fiber blankets produced by wood waste deposition by a pulp mill (Crofton: personal observation). Abundances are also high within rapidly accumulating tailings deposits in Rupert Inlet (Ellis and Hoover, in press) containing extremely low concentrations of total organic carbon (<1%- Pedersen, 1985). Transient occurrences of dense populations were noted in previously-deposited mine tailings beds in Alice Arm (Brinkhurst *et al.*, 1987), off Anaconda Britannia Mine in Howe Sound (Bright and Ellis, 1989; Ellis and Hoover, 1990) and in Tasu Sound, Queen Charlotte Islands, previous site of Wesfrob Mine (personal observation). *A. serricata* is the numerically dominant bivalve in a mixed copper-slag sediment off Anyox, Granby Bay (personal observation), in Alberni Inlet at a dredge-spoil dump-site (Levings *et al.*, 1985), and in areas of Burrard Inlet subjected to a wide range of anthropogenic inputs, including industrial ship traffic and a raw sewage overflow discharge (Burd and Brinkhurst, 1990).

A. serricata may be less sensitive to environmental perturbation than some other bivalves, based on its distribution in stressful environments. Nevertheless, *A. serricata* has merit as a bioindicator for several reasons. *A. serricata* has a more cosmopolitan distribution than many of the infaunal bivalves. Furthermore, *A. serricata* is more likely to persist in an area subjected to anthropogenic disturbance; this permits use of the same bioindicator to evaluate environmental impact over time, e.g. in the analysis of environmental recovery. Finally, *A. serricata* is a small clam (maximum shell length is approximately 5 mm.) relative to other infaunal bivalves. For histopathology, this makes it easier to examine tissue structure in the entire clam rather than in isolated tissues.

A description of the study areas: Rupert and Holberg Inlets, Granby Bay, and Mill Bay.

Island Copper Mine, Utah Mines Ltd., is located on the northern shore of Rupert Inlet. Rupert Inlet and Holberg Inlet merge an' are connected at their confluence to Quatsino Sound proper, a large inlet on northern Vancouver Island, British Columbia (Figure 2).

Island Copper Mine (I.C.M.) is an open-pit mine and concentrator operation extracting copper and molybdenum and minor amounts of gold, silver, and rhenium from an ore body containing chalcopyrite and molybdenite. After separation of the metal-containing ore from metal-poor surrounding waste rock, crushing of the ore, and separation of copper and molybdenum concentrates in flotation cells, residual tailings are discharged to a settling pond. The thickened tailings are then mixed with seawater and discharged subtidally via a submarine outfall at a depth of approximately 50 m. The tailings are composed primarily of quartz, feldspar, biotite, and chlorite (Pedersen, 1985). Pyrites are present in concentrations of 2-4%. Copper and molybdenum are present in approximate concentrations of 700 ppm. and 40 ppm. respectively.

I.C.M commenced operation in 1971 and intends to cease mining/extraction activities in 1996. Knowledge of the operational-phase impacts of marine mine-tailings disposal, produced from an in-house monitoring program, as well as from external sources is perhaps unsurpassed for an industrial discharge of this type (see I.C.M., 1987, 1988, 1989; Pedersen and Losher, 1988; Ellis, 1987, 1989; Fleming et al., 1983; Jones and Ellis, 1976). Deposited tailings occur along the trough of Rupert and Holberg Inlets and form a blanket which varies in depth from approximately 50 meters adjacent to the discharge site to less than one centimeter near the head of Holberg Inlet. Chemical traces of tailings, i.e. elevated solid-phase concentrations of copper and zinc, are detectable in both deep and shallow areas in virtually all of Rupert and Holberg Inlets as well as in Quatsino Sound. Hay (1981) and Drinkwater and Osborn (1975) have investigated factors controlling the deposition and redistribution of mine tailings.

In a review conducted for the federal and provincial Ministries of Environment, Waldichuk and Buchanan (1980) concluded that the main impacts of the mining

operation were (1) a smothering of benthic fauna and alteration of habitat caused by tailings discharge, and (2) destruction of habitat associated with the dumping of waste-rock along the northeast shoreline of Rupert Inlet. Increased turbidity caused by the re-suspension of tailings by tidal currents may have caused indirect effects through a greater areal distribution of tailings, although direct effects on primary or secondary productivity have not occurred.

The soft-bottom benthic community tends to be highly depauperate along the trough of Rupert Inlet; there has been a partially asymptotic trend toward the discharge site in the reduction along the trough of species richness. A handful of invertebrate species persist in areas of heavy tailings deposition and are considered to be early colonizers, or opportunists (Ellis, 1989). *Axinopsida serricata* is considered to be one of these (I.C.M., 1986).

If the known effects on the benthic community have occurred as a result of physical smothering and/or modification of the physical structure of the sediment, then it might be expected following the cessation of mine-tailings discharge that the benthic community would rapidly undergo succession toward an assemblage similar to that occurring prior to the onset of discharge. Taylor (1986), using artificial substrates, demonstrated some ability of deposited mine-tailings to recolonize. Although tailings and control substrates were colonized by similar assemblages, there was a slower rate of colonization in the tailings. In twelve months, the assemblages in the substrates had not reached equilibrium.

Figure 2: The location of collection sites for the clam, *Axinopsida serricata*, in Rupert Inlet, Holberg Inlet and Quatsino Sound, British Columbia.

Island Copper

Pit

Outfall

R1

Holberg Inlet

Rupert Inlet

Marble R.

A H5

H4

H3

H2

H1

0 —— 5
Km

QS

Quatsino Sound

50° 35'

50° 30'

127° 50'

127° 30'

12
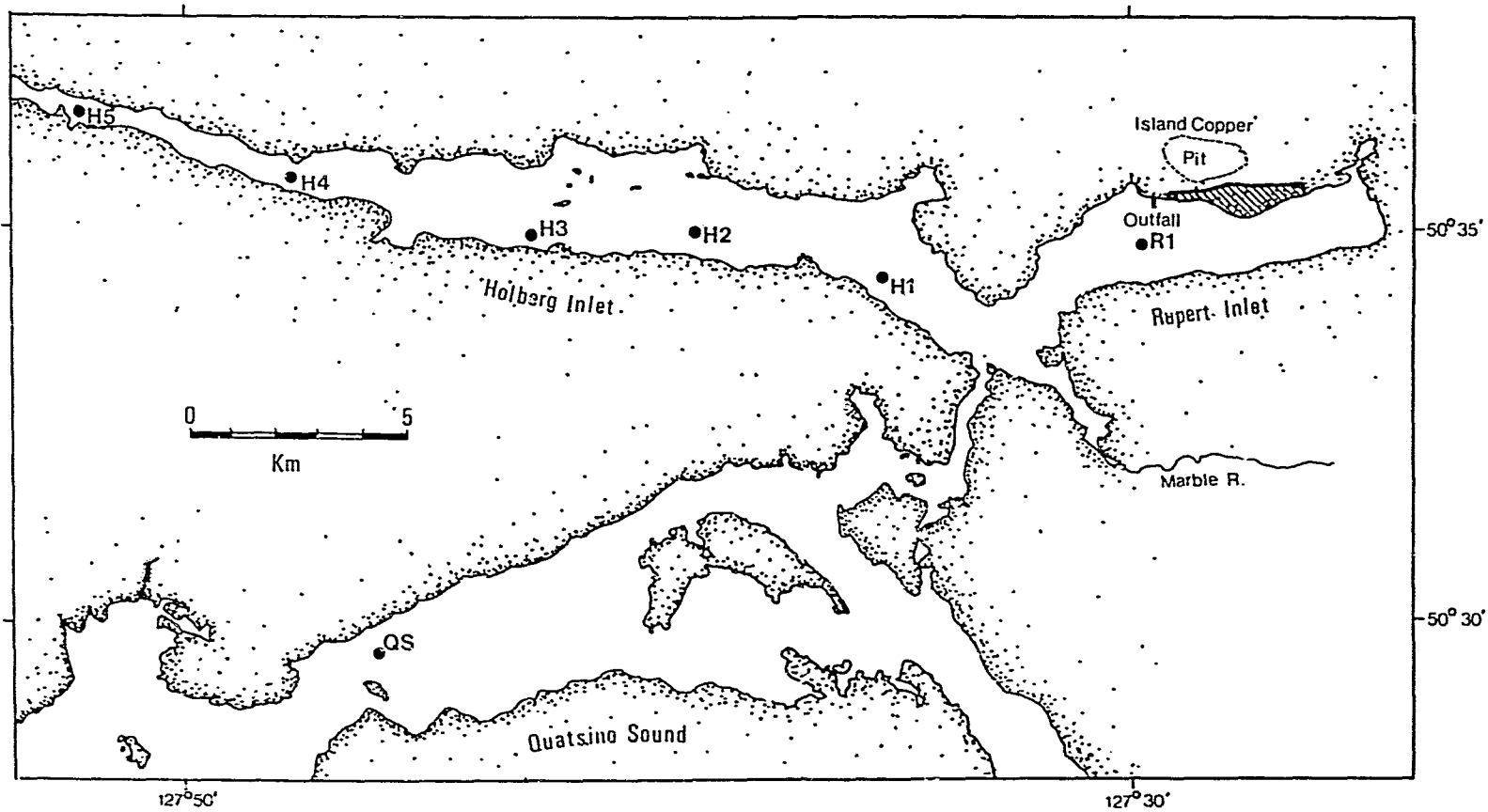
If the effects on benthos are at least partially attributable to mobilization of metals to sediment interstitial waters during diagenesis, then there might be long-term inhibition to recovery. Hoff *et al.* (1982), using a closed column that contained sediment and seawater, demonstrated a potential for the oxidative release of metals (copper and zinc) from I.C.M. tailings. However, there has been no clear temporal trend of metal bioaccumulation in fish or invertebrates collected from Rupert and Holberg (I.C.M., 1988). Although moderate levels of bioaccumulation of some metals occasionally have been found for some species, the pattern is inconsistent and does not generally support a model of net metal flux from the deposited tailings. Metal and nutrient cycling associated with early diagenesis in deposited tailings were investigated by Pedersen (1983, 1985). Copper and molybdenum are enriched in near-surface interstitial water of deposited tailings beds, although the rate of flux is sufficiently low that metal concentrations in the overlying, transient water are not elevated. On the other hand, *A. serricata* like many of the infauna occurs in intimate association with the near-surface sediment, and may be exposed to deleterious levels of heavy metals.

Anyox, located on Granby Bay, Observatory Inlet (Figure 1), is a deserted area which from 1904 to 1939 was the site of a copper mine, smelter, and associated town. Coarse metalliferous slag, a byproduct of the smelting process, was discharged onto the shoreline and into the waters of Granby Bay and probably continues to contribute high levels of some metal species to the interstitial and overlying water. Littlepage (1978) suggested, based on limited data, that Anyox was a point-source for solid-phase sediment levels of copper and mercury. Cadmium was elevated over background levels both near Anyox and in Alice Arm. Separate studies are available for Alice Arm and Hastings Arm, Observatory Inlet, which document biological effects on the benthic community of the discharge of molybdenum mine-tailings to the head of Alice Arm (Kathman *et al.*, 1983, 1984; Brinkhurst *et al.*, 1987). Although there is no published information on the extent of metal remobilization or biological effects in Granby Bay, unpublished data on metals in the sediments and water column as well as on continued acid drainage from the original mine and ore storage areas have been collected by the Environmental Protection Service (D. Goyette, pers. com.).

The literature indicates that metal remobilization is more likely to contribute to bioaccumulation and to affect benthic community structure in Granby Bay than in Rupert/Holberg Inlet. *Axinopsida serricata* is found in relatively high densities along the submerged toe of the slag heap in a mixture of slag and natural sediment. Granby Bay was therefore included in the study as a potential worst-case scenario for the field exposure of clams to metals within the sediment.

Mill Bay in Saanich Inlet (Figure 1, Figure 3) was chosen as a reference site for the investigation of normal histology based on the availability of clams, *A. serricata*. Preliminary investigations revealed that clam abundance was depth-dependent. Since Saanich In'et is a seasonally anoxic fjord (Pickard, 1963), a station was established at a depth of 90 m., above the depth at which anoxia is encountered.

There are few available observations of the benthic biology or sediment geochemistry of Mill Bay specifically, but Saanich Inlet has been studied extensively (Richards, 1965; Nissenbaum and Swain, 1976; Presley *et al.*, 1972; Berrang and Grill, 1974) as a model fjord which undergoes seasonal anoxia of near-bottom waters due to a limitation of renewal by denser, oceanic waters imposed by a shallow sill at the mouth. Ellis and Conlan (1979) reported effects on the benthic community of wood waste associated with log booming in a near-shore area of Saanich Inlet just south of Mill Bay. *A. serricata* was present in peripheral areas with little wood waste deposition (Conlan, 1977). However, the sandy sediment and associated benthos at Conlan's study site are not directly comparable with the silt-clay environment occurring in Mill Bay.

Figure 3: Location of reference station in Mill Bay, Saanich Inlet used for collection of the clam, *A. serricata*. Bottom profile (inset) is included to show the relationship between the collection site (SI) and trough of the inlet which experiences seasonal anoxia. The abundance in Mill Bay of *A. serricata* was depth dependent, with no clams found below a depth of 120 m. and reduced abundances on the slope above 90 m. depth.

123°30'

48°
40'

SAANICH INLET

SI

Mill Bay

Depth (m.)

0

100

200

SI

0   1   2

Distance (km.)
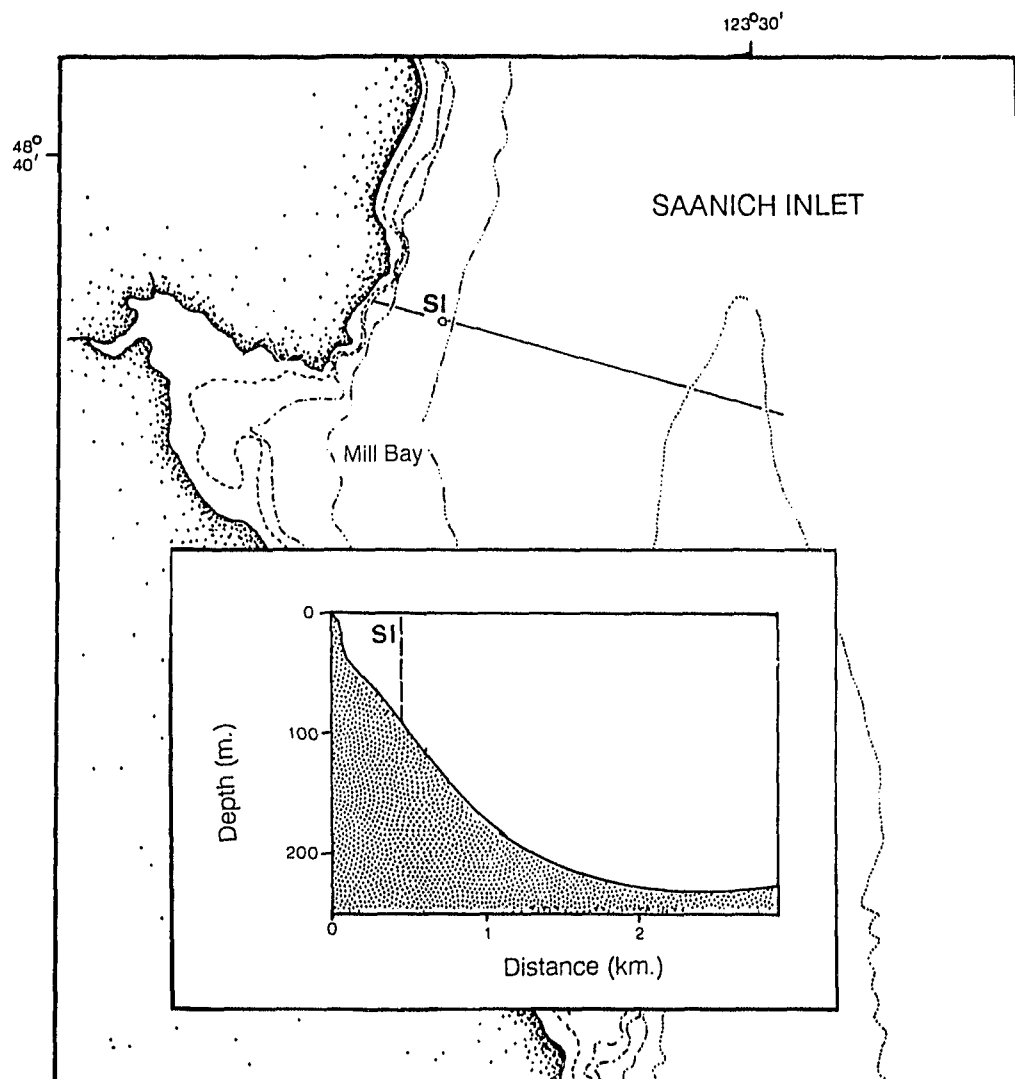
A review of quantitative studies of molluscan histopathology.

Histological, cytological, and cytochemical responses of marine animals to environmental perturbation offer promise as tools in environmental monitoring. The recent desire of researchers to understand these chemical/structural changes in considerably greater detail, both in terms of their etiology and further significance, has prompted a shift in emphasis from purely descriptive to quantitative methods. Techniques used to quantify cytological/histological structure are diverse; one such technique is stereology, the statistical reconstruction of three dimensional structure (for example, volume ratios) from two dimensional histological or ultrastructural sections (Weibel, 1979).

Of all marine invertebrate phyla, most quantitative studies of histological lesions are directed at the mollusca. This is probably due to the strong interest in molluscs as bioindicators and a concomitant wealth of background information relative to other phyla. Within the mollusca, research on histological change has been directed primarily at two tissues: the digestive diverticula, due to prior qualitative observations of its plasticity as well as sensitivity to environmental changes, and on the gonad and associated nutrient cells because of implications for overall energetics and reproductive potential.

Morton (1970), Langton and Gabbott (1974), Langton (1975), Robinson and Langton (1980), and Robinson (1983) described normal phasic changes in columnar digestive cells of the digestive diverticula associated with feeding and/or tidal cycle. Lowe et al. (1981) first demonstrated quantitative changes in digestive tubule structure of *Mytilus edulis* associated with contaminant exposure (30 μg l^{-1} water accommodated fraction of crude oil) in the laboratory. Quantitative changes included a 'thinning' of the digestive tubule epithelium associated with a reduction in height of digestive cells, desynchronization of adjacent digestive cells and tubules, and morphometric changes in secondary lysosomes. There were statistically significant differences between exposed and control mussels in digestive cell structure; number of lysosomes decreased on exposure and lysosomal surface area to volume ratio increased.

Axiak et al. (1988) observed similar effects on digestive cell height (as measured only in holding phase tubules) in the bivalve *Venus verrucosa* exposed in the

laboratory to petroleum hydrocarbons. Thinning of the digestive tubule epithelium was also quantitatively assessed by Vega *et al.* (1989) in *Littorina littorea* (Gastropoda) exposed in the laboratory to cadmium, and by Marigomez *et al.* (1986) in the slug *Arion ater* exposed to copper.

Lowe *et al.* (1981), Moore and Clark (1982), and Lowe (1988) demonstrated stereological and cytochemical changes in digestive cell secondary lysosomes associated with histological changes. After exposure to crude-oil derived hydrocarbons, secondary lysosomes were pathologically enlarged. Alteration of lysosomal structure is also associated with reduced membrane stability as assayed by the measurement of latency of B-*N*-Acetylhexosaminidase (Widdows *et al.*, 1982; Moore, 1980).

The cellular lysosomal system is implicated in cellular and protein turnover through hydrolysis and catabolism of macromolecules, as well as in the detoxification through compartmentalization of, in particular, heavy metals (Moore, 1980). The response of lysosomes within digestive cells of *Littorina littorea* was investigated using morphometric techniques after laboratory exposure to cadmium (Marigomez *et al.*, 1989) or 1-napthol (Cajarville *et al.*, 1989). These studies provide a preliminary model which relates contaminant exposure to lysosomal activity and to histological change.

Metal exposure decreased the surface to volume ratio in secondary lysosomes (Marigomez *et al.*, 1989) indicating a fusion of secondary lysosomes, which is probably related to membrane destabilization associated with metal uptake. George *et al.* (1982) demonstrated that trace metals within lysosomes stimulate lipid peroxidation and the formation of lipofuschin. In contrast, exposure to hydrocarbons (Cajarville *et al.*, 1989; Lowe *et al.*, 1981) results in a dose-dependent increase in surface area to volume ratio as well as lysosome volume. Lowe *et al.* (1981) suggested that these enlarged lysosomes are autolysosomes, which probably form through dilation or swelling (Cajarville *et al.*, 1989). In either case, the net result would appear to be an increased destabilization of the lysosomal membrane, leading to leakage of hydrolytic enzymes into the cell cytoplasm. This may be related to an increased rate of autolysis, vacuolation and cell fragmentation leading to digestive tubule desquamation.

However, the model fails to adequately address intermediate stages of cell aging prior to autolysis and reduction in height. No clear distinction has yet been made between processes (both normal and pathological) which lead directly to destruction of the cell and those which precede autolysis and cause swelling rather than shrinkage of digestive cells through phagocytosis or pinocytosis, as well as through production of materials for export from the cell.

Morphometric changes in the reproductive follicles of molluscs were studied both in the laboratory and in the field. The most widely used approach for assessing effects on reproductive tissues involves stereological estimates of the volume fraction occupied by gametes, nutrient storage cells, and surrounding connective tissue (Bayne *et al.*, 1978; Maung Myint and Tylor, 1982; Lowe and Pipe, 1986, 1987).

Sunila (1986a) used morphometric techniques in a laboratory investigation of the effects of copper or cadmium on the gill of *M. edulis*. Areas relative to the total filament area in transverse paraffin sections were calculated for the branchial vein, ostium endothelial cells, frontal cells, lateral cells, and abfrontal cells. Exposure to copper caused oedema of the endothelial cells as well as atrophy of the endothelial cells and abfrontal cells one year after exposure. The main effect of cadmium was a consistent dilation of the branchial veins.

In a separate study, Sunila (1987) measured the density of granules in kidneys (indicative of metal uptake) by measuring light absorbance of kidney cells in paraffin sections employing a photometer attached to a microscope. Densitometric techniques can be used in conjunction with studies of cytochemical localization. For example, Homer and Pierce (1989) demonstrated a dose-dependent reduction in the density of catalase-containing peroxisomes in rat liver cells following injection with copper. It is difficult to employ such techniques without the use of a computer-aided image analysis system.

Perhaps one of the simplest methods for scoring histological sections is to record the incidence of putative lesions, for example presence or absence of granulocytomas. Sunila (1987) employed this approach for mussels (*M. edulis*) collected from the Gulf of Finland. Auffret (1988) recorded the incidence of mussel lesions from the field populations as well as in a mesocosm study. Incidence-type

observations are quick and allow observation of a wide range of possible histological changes in more than one tissue.

There are two major disadvantages to incidence-type data: (1) pathological alteration conceivably results in a change in number of structural components rather than in an all-or-none destruction or creation of structure. The uncertainty associated with dichotomizing a pathological alteration which has an underlying continuous distribution is often large.   (2) Incidence data follow a binomial distribution. An appropriate statistical method for assessing the presence of between-site differences in putative lesions based on incidence data is contingency analysis. Unfortunately, many of the more powerful parametric techniques used for exploring the relationship between several independent and dependent variables are inappropriate for this type of data (although see the discussion provided by Green, 1979 (p. 74)).

A closely related quantitative technique is the estimation of the percentages of two or more alternate structural forms. Seiler and Morse (1988) observed differences in the relative percentage of granulocytes and agranulocyte within the circulating blood of soft-shell clams (*Mya arenaria*) collected from a polluted and clean reference site in Massachusetts, U.S.A. The percentage of haemocytes identified as granulocytes in the oysters *Crassostrea gigas* and *C. virginica* increased after exposure to copper and zinc (Ruddell and Rains, 1975). Observations of this nature follow an underlying Poisson distribution and, as for incidence data, place constraints on the statistical methods employed for analysis. There are appropriate non-parametric univariate tests for such data, but no satisfactory non-parametric multivariate tests presently exist.

A brief mention is made here of studies employing the quantitative assessment of cytogenetic change associated with contaminant exposure. Studies of the cytogenetic alteration of invertebrate populations are based on techniques adapted from vertebrate research on mutation and carcinogenesis. The range of changes encountered (i.e. changes in quantity or rearrangement of DNA) is considerably narrower than that of phenotypic changes encountered in most studies of invertebrate histological lesions. Subsequently, the measures employed have

generally been screened as both sensitive to and dependent on concentration of those pollutants which specifically increase the rate of mutation.

Scarpato *et al.* (1990) and Majone *et al.* (1987) studied the induction and persistence of micronuclei in the gill tissue of *Mytilus galloprovincialis* exposed to xenobiotics. The frequency of sister-chromatid exchanges induced in the laboratory by various mutagens was investigated in *Mytilus edulis* by Jones and Harrison (1987), in *M. galloprovincialis* by Brunetti *et al.* (1986), and in the polychaete *Neanthes arenoceodentata* by Pesch and Pesch (1980).

Quantitative methods should enable a better understanding of structural differences between organisms experiencing environmental differences. Statistical procedures may be used to distinguish between normal and pathological variability. Knowledge about this variability could then be used to generate hypotheses about and ultimately to predict functional impairment which is likely to lead to further ecological consequences. The potential of this approach is suggested by the following example.

Differences in the distribution patterns of nuclear DNA as condensed or decondensed may be functionally significant, although according to Sahota *et al.* (1985) the "patterns are too complex for analysis by visual examination to provide discrete, yet simple relationships between these patterns and cell functioning." Sahota *et al.* (1985) employed computer-aided digital analysis to investigate chromatin distribution patterns within feulgen-stained nuclei of follicular epithelial cells of the Douglas fir beetle *Dendroctonus pseudotsugae*. Twenty five different measures of chromatin density and distribution were obtained for nuclei of follicular epithelial cells from insects undergoing reproductive differentiation or manipulated to remain in a non-differentiating state (control). Insect groups at different stages of differentiation could be accurately distinguished from each other and the control using a discriminant functions analysis. Sahota *et al.* (1985) suggested that similar measures on field populations might accurately predict the potential for and timing of outbreaks of this commercially important pest.

2. The anatomy and histology of Axinopsida serricata: normal tissue structure and possible disturbance-induced structural alteration.

Methods

Field collections.

Clams (*Axinopsida serricata*) were collected from Mill Bay, Saanich Inlet, in sediment considered to be minimally contaminated by human activity, and from seabeds containing mine-tailings (Holberg Inlet, Quatsino Sound) or metalliferous slag from a copper smelter (Granby Bay, Observatory Inlet)(Figures 1 - 3 in Chapter 1). Detailed descriptions of the study sites are provided in Chapter 1. The locations, dates, depths and sample sizes for the collections are shown in Table 1 below. A total of 101 clams were examined.

A. serricata were collected either in November, 1987, when males and females are reproductively ripe or spawning, or in April-May, 1989, when most adult clams are spent and gonadal tissue is either recovering or indifferent (Bright, personal observation).

Five stations were established in Holberg Inlet (H1 to H5) along the trough of the inlet at increasing distance from the point-source discharge of mine-tailings (Figure 2, page 11, Chapter 1). An additional station (QS) was established in outer Quatsino Sound.

There are several possible environmental consequences of mine-tailings discharge (increased turbidity, smothering, dilution of sediment organic content, chemical effects, et cetera) which might influence *A. serricata*; however, there was *a priori* no way of determining which of these were important as stressors. Therefore, distance from the discharge pipe was assumed to be a reasonable surrogate of environmental changes associated with mine-tailings discharge.

TABLE 1: Summary of field collections for histopathology of *A. serricata*.

Date (m/y)	Location	Latitude (°N.)	Longitude (°W.)	Depth (m.)	Distance from discharge (km)	Sample Size
11/'87	Mill Bay, Saanich Inlet (SI)	48°39.3'	123°31.95'	90	na	9
05/'89	as above			90	na	10
11/'87	Granby Bay, Observatory Inlet (GB)	55°24.6'	129°48.6'	82	na	10
	Holberg Inlet:					
11/'87	H1	50°34.6'	127°35.3'	129	7.7	10
04/'89		as above		130	"	10
11/'87	H2	50°35.3'	127°39.3'	108	12.1	10
04/'89		as above		138	"	10
11/'87	H3	50°35.35'	127°43.25'	87	16.0	4
04/'89		as above		89	"	4
11/'87	H4	50°36.1'	127°47.6'	73	21.8	10
11/'87	H5	50°37.2'	127°51.9'	38	26.8	10
11/'87	Quatsino Sound (QS)	50°29.8'	127°46.1'	195	22.1	·r

na: not applicable.

Clams were collected in Mill Bay using the University of Victoria research vessel the M.S.S.V. John Strickland with a 0.1 m² Van Veen grab, and in Holberg Inlet, Quatsino Sound, and Granby Bay using the Federal Department of Fisheries and Oceans vessel C.S.S. John P. Tully with a 0.1 m² Smith-McIntyre grab. All grab samples were screened through a 0.5 mm stainless-steel mesh. *A. serricata* specimens were immediately recovered, opened with the tip of a sharp scalpel inserted from the dorsal side between the hinge teeth, and then fixed whole in Lillie's phosphate-buffered 10% formalin (pH = 7.2). Where more than 10 clams were recovered from a grab, a subset of clams was selected with sizes of clams chosen to represent the range and proportions of sizes within the entire sample.

This allowed the analysis of tissue structure for all sizes (and presumably ages) of clams present within a population.

Histological examination.

The clams were held in the fixative for up to 4 months until embedded in paraffin (Paraplast) using conventional histological techniques. The clams were serially sectioned *in toto* (excluding the mantle and distal portion of the foot) at a thickness of 4-6 μm. Slides were stained with Harris' iron haematoxylin and eosin.

Some specimens were fixed in Millonig's PO_4-buffered 2.5% glutaraldehyde for observation under the scanning electron microscope. These clams were not post-fixed in osmium tetroxide since one of the original goals was to perform x-ray microanalysis on the specimens.

The normal tissue structure of *A. serricata* was described using all clams collected from Mill Bay, Saanich Inlet, during both November and April. Originally, clams collected at intervals throughout the year over a three-year period were embedded with the intent of quantitatively examining seasonal changes in tissue structure; however, this was not possible due to time constraints.

Three to four clams were initially examined from each of the lower Holberg Inlet stations and Granby Bay and compared to clams from Mill Bay to assess departures from normal tissue structure. During and following a quantitative examination of various tissue structures (Chapter 3) but prior to data compilation and analysis, further qualitative observations were made using the entire set of samples (101 A. serricata from 8 sampling stations and 2 seasons).

Results

External morphology.

In living *A. serricata*, the edges of the outer shell at the anterior and posterior extremity have a small-sized, intense brown stain, which may be composed of iron oxides. The presence and intensity of the stain varies between sites, with the staining strongest in upper Holberg Inlet (H4, H5). Similar staining has been noted on the shells of other Thyasirids including *Leptaxinus ferruginosus* and *Kellyella*

miliaris (Nicolaidou *et al*, 1989). Accumulation of external rust-colored deposits is most obvious in the Protobranch bivalve *Acila castrensis* which occurs with *Axinopsida* in many of the local soft-bottom communities.

The external anatomy of *A. serricata* (Figure 4a) is typical of Thyasirids, as described by Allen (1958) and Bernard (1972). Although Thyrasirids are considered to be members of the Eulamellibranchia, certain features of the mantle cavity are reminiscent of the Protobranchia. These include the absence of inhalant and exhalant siphons, passage of the exhalant ventilatory current anteriorly rather than posteriorly, and a relative lack of mantle fusion along the ventral surface. This should, in theory, leave the mantle cavity more exposed to the surrounding environment than in other families of Eulamellibranchia.

One morphological feature unique to the Thyasiridae is the extension of the visceral mass into numerous club-like extensions, or "arborescent tufts" (Allen, 1958), which contain the digestive diverticula as well as gonad, depending on the season. Extension of the digestive diverticula outward in arborescent tufts does not occur in the other two families of the superfamily Lucinacea; i.e., the Lucinidae and the Ungulinidae. Bernard (1972) noted that a similar structure is observed in the Septibranch bivalves, *Myonera*, *Cuspidaria* and *Poromya*, and may be an adaptation to macrophagy.

Three folds are distinguishable on the mantle edge (Figure 4b): an outer unciliated fold, an inner heavily ciliated fold, and a middle fold containing discrete ciliary bundles with a probable sensory-related function (Figure 4c). The foot epithelium, ctenidia, and visceral mass are extensively ciliated as is typical of the mollusca. However, the ciliation of the arborescent tufts is limited to their distal surfaces, suggesting a possible localized specialization of function. The inner surface of the mantle proper lacks ciliation with the exception of the mantle folds.

Figure 4: Features of the external anatomy of an adult *A. serricata*.

(A) Scanning electron micrograph of the soft body morphology with the soft parts removed from but perched on left valve. The left mantle remains attached to the shell; the right mantle was loosened and now covers the posterior of the body. Ct: ctenidium; DD: digestive diverticula; Ft: foot; M: Mantle. Scale bar = 1 mm.

(B) Light micrograph of a section of the mantle at an anterior point of fusion of the inner folds of the mantle. Inn: inner fold; Mid: middle fold; Per: periostracum; Out: outer fold. Scale bar = 100 μm.

(C) Higher power S.E.M. of one of many ciliary bundles on the middle lip of the mantle edge. Scale bar = 5 μm.

A

B

C
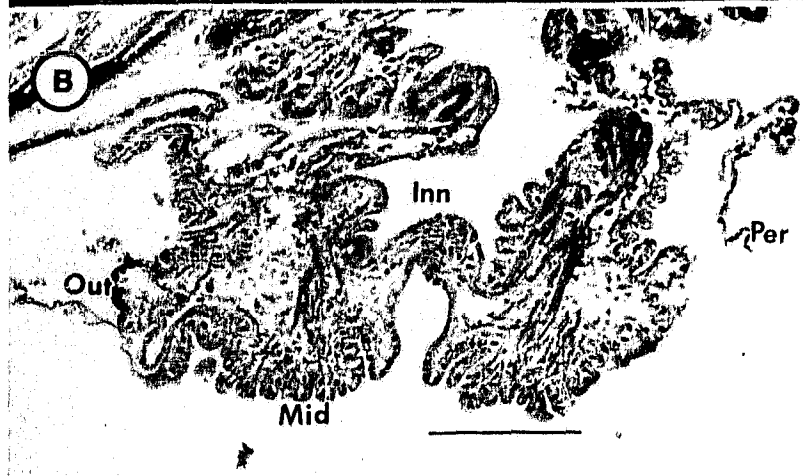
Histology of the ctenidia.

The histology and ultrastructure of the ctenidia of clams within the superfamily Lucinacea have been described in detail by Reid and Brand (1986) with particular reference to the presence of membrane-bound bacteriocytes containing sulfide-oxidizing chemoautotrophic bacteria. *Axinopsida* differs from the Lucinids and the few larger Thyasirids previou y studied in that no endosymbiotic bacteria have been found (Reid and Brand, 1986; E.C. Southward, pers.com.; personal observation).

The frontal surface of ctenidial filaments is sufficiently similar to that of other bivalves (Figure 5 a,b) that it will not be described here in detail. The frontal surface of the filament is composed of four to six frontal ciliated cells. Laterofrontal ciliated cells do not exhibit the specialization or the long length of cilia seen in *Mytilus edulis* (Sunila, 1986a) or *Macoma carlottensis* (Bright, 1987).

Both Lucinids and Thyasirids exhibit elaboration of the subfilamentar tissue which is, for the most part, accompanied by fusion of the abfrontal surfaces of the ascending and descending ctenidial filaments (Figure 5b,c). However, there are major differences in the architecture of the subfilamentar tissue between *A. serricata* and endosymbiont-containing lucinids typified by *Parvilucina tenuisculpta* (Reid and Brand, 1986). Unlike *Parvilucina*, in *Axinopsida* the abfrontal surfaces of the ascending and descending filaments of both the inner and outer demibranch are fused only near their dorsal point of attachment, the ventral extremity of the gill curtain, and along the first several filaments from the anterior or posterior end of the ctenidia. This arrangement creates a pouch or envelope which is surrounded entirely by gill filaments (Figure 5 a-c).

At frequent intervals along the ctenidial filaments, the abfrontal surface facing the ctenidial pouch has much enlarged, granular basiphilic cells (Figure 5b,c) which may secrete large amounts of mucus to the ciliated frontal surface of the gill. These cells are called "ctenidial mucocytes" following the terminology of Axiak *et al* (1988) employed for the bivalve *Venus verrucosa*.

Figure 5: Histology of the ctenidia of *A. serricata*. All scale bars = 100 μm.

(A) Transverse section through the ascending and descending filaments of the inner left demibranch. dig: digestive diverticula.

(B) Higher magnification of transverse section through ctenidial filaments illustrating frontal ciliated cells (fc), laterofrontal ciliated cells (lfc), lateral cells (lc), abfrontal cells (ac) on the ascending filaments and mucocytes (m).

(C) Longitudinal section along the length of a single filament showing the extent of fusion of abfrontal surfaces and the resulting ctenidial pouch. Arrow: chitinous support rod; fc: frontal cell; lc: lateral cell; m: mucocyte.
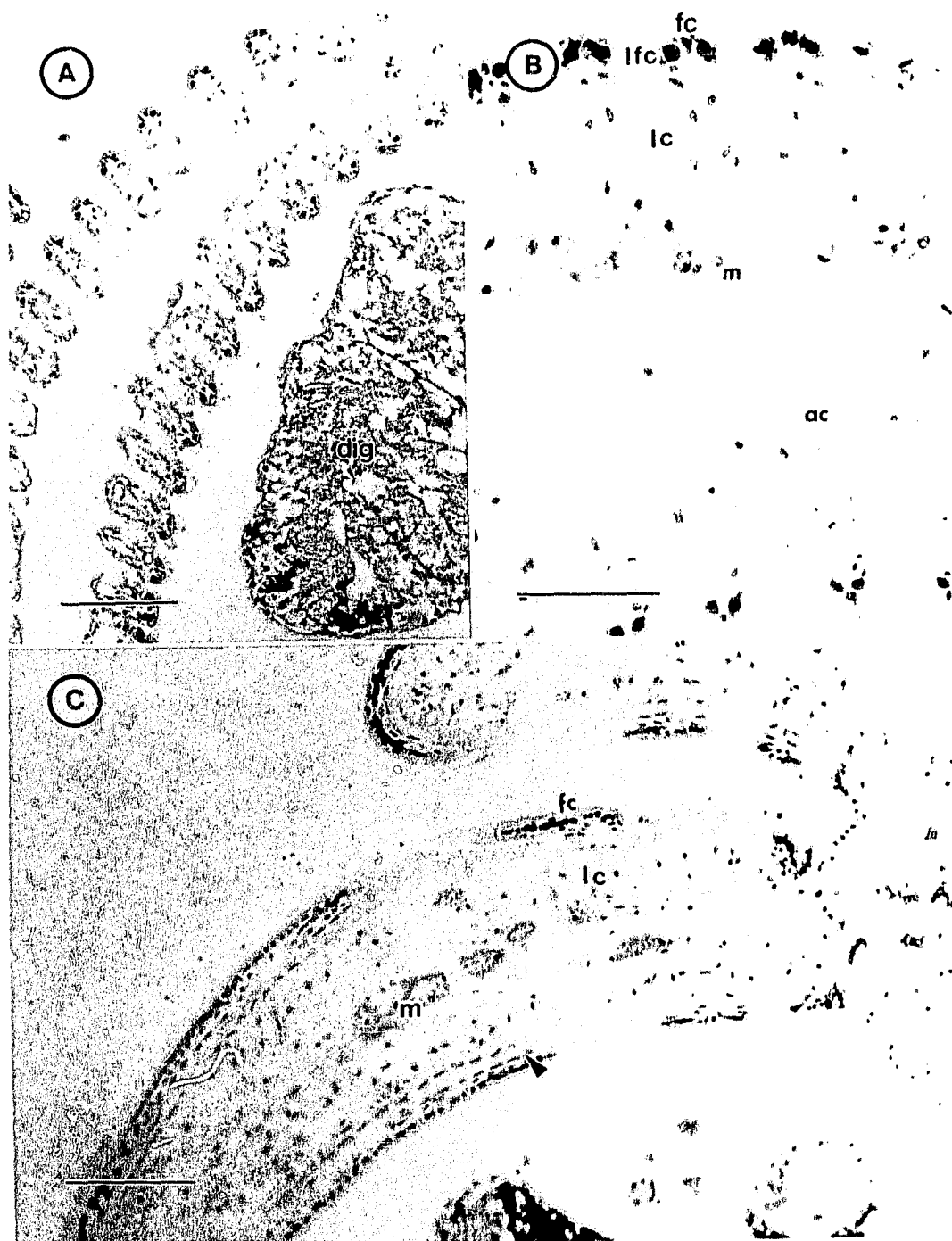
Features of the digestive tract.

Allen (1958) described the digestive tract within the families Ungulinidae, Lucinidae, and Thyasiridae of the superfamily Lucinacea. For all three families, there is a simplification of the stomach with a progressive loss of sorting areas. Simplification is most pronounced within the Lucinidae and least pronounced within the Ungulinidae. The digestive diverticula in the Ungulinidae and, to a lesser extent, in the Lucinidae is similar to that of other bivalves (Owen, 1955). Paired digestive ducts from the stomach ramify laterally in a somewhat regular manner to end 'n blind-ended digestive tubules. The transition between ducts and tubules is dis. ete in histological sections.

However, Thyasirids are unique in having the digestive diverticula expanded outward into arborescent tufts. Differences from other members of the Lucinacea are also noted in the tissue structure. Allen (1958) noted that the Thyasirids do not have ducts which are clearly distinguishable from tubules. In *A. serricata*, while the transition from blind-ended digestive tubules to digestive ducts (and eventually to stomach epithelium around the duct openings) is not visually discrete, there is considerable specialization of tissue structure along the course of the digestive diverticula (Figure 6). There is also considerable difference within and between individuals in the tissue structure at any given location along the diverticula.

The digestive diverticula of *A. serricata* were generally divisible into digestive tubules proper at the distal extremity (Figures 6 a-e), and the proximal digestive ducts (Figures 6 f-h) which occupied the majority of diverticular volume. Within the digestive tubules proper (at the outer tips of the arborescent tufts), two principle cell types were distinguishable as have been described for other molluscs (Owen, 1955; Pal, 1971, 1972): digestive tubule digestive cells and digestive tubule basiphil cells (or crypt cells).

Figure 6: Histology of the digestive diverticula. All scale bars = 100 µm.

(A - E) Cross-sections through distally located digestive tubules hypothetically arranged in order of decreasing integrity. Tubules are composed of darkly staining pyramidal basiphil cells (B) and cuboidal to columnar digestive cells (D). Phasic and/or stress-related activity result in increased height of digestive cells accompanied by appearance of large basal vacuoles (arrows) which often contained light brown inclusions. Tubules in an advanced stage of breakdown contain digestive cells which are highly vacuolated, fragmented, and ultimately necrotic.

(F - H) Sections through the more proximally located digestive ducts (PDD) showing relationship to digestive tubules proper (DT) and variation in the size and abundance of large spherical vesicles in the otherwise large spongiform cells.
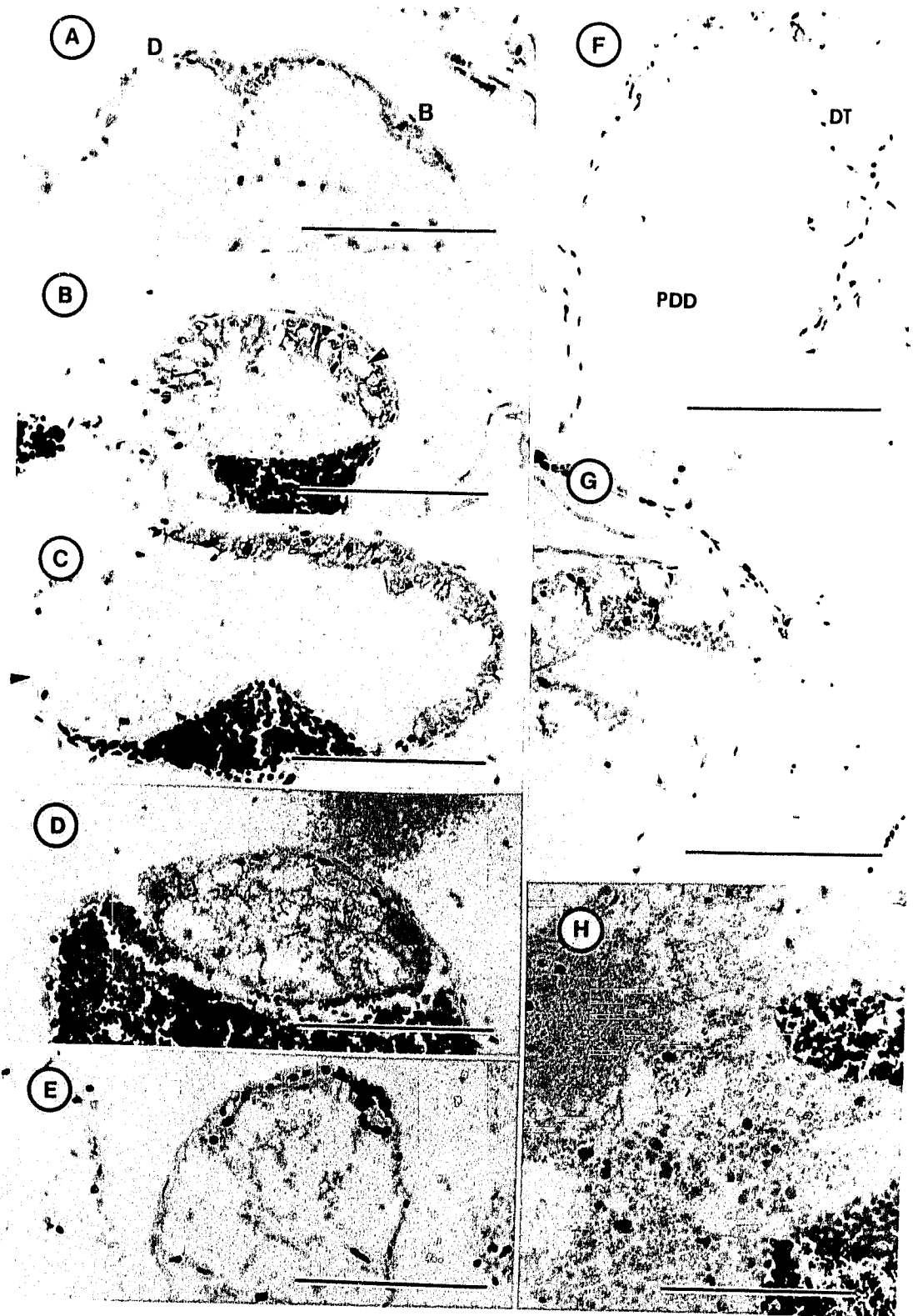
Digestive tubules of the Mollusca undergo phasic changes in structure (Langton, 1975), with the height of the digestive cells varying according to feeding activity, and/or tidal cycle (Morton, 1970). In contrast to studies on most other molluscan taxa, the digestive tubules in *A. serricata* could not be arbitrarily separated into holding, absorptive, fragmenting and resorptive phase (Langton, 1975). The range of variation in tubules of *A. serricata* collected from all sites is illustrated in Figure 6a-e, arranged in a sequence which is thought to represent progressive deterioration. Digestive cells varied in different tubules from being cuboidal (Figure 6a) to swollen. In Figure 6b, large vacuoles adjacent to the basal lamina can be seen in most of the digestive cells. Many of the vacuoles contain large inclusions. The most likely interpretation of the digestive cell structure is that the basal vacuole represents an autolysosome or residual body which is associated with increased cell lysis and ultimately to fragmentation and breakdown (Figure 6 c-e). The degree of fragmentation illustrated in Figure 6d and nearly complete tubule necrosis illustrated in 6e were only rarely encountered in tubules of clams from the reference site, and may be beyond the normal range of physiological variation.

Digestive ducts have been described in larger bivalve taxa (reviewed by Owen, 1955), but intraspecific structural variation has not been documented. In *A. serricata*, the proximal digestive ducts from different individuals exhibit considerable structural alteration. In any given individual, only one cell-type can be distinguished. The cells are very large (approximate height = 20-35 μm), not obviously ciliated, and range in structure from being relatively empty, spongiform cells (Figure 6f) to being swollen and densely packed with large spherical to ovoid vesicles (Figure 6h). In some clams (Figure 6g), the vesicles are localized at the cell apices adjacent to the lumen, but in densely packed cells, vesicles occur throughout the cells and other structures including nuclei and cell membranes are often obscured. In a few cases, the proximal digestive ducts appeared to be completely necrotic with few if any cell membranes remaining intact.

The stomach contents of *A. serricata*, as observed in paraffin sections, typically consisted of small amounts of biogenic debris, including diatom frustules, intermixed with smaller amounts of sediment. This material was confined to the stomach and was rarely observed in the ducts and tubules of the digestive diverticula.

The intestine of *A. serricata* lacks the coiled elongation of other deposit-feeding clams such as *Macoma carlottensis* (Bright, 1987). The entire intestine consists of an S-shaped loop which initially passes from the stomach anteriorly and dorsally through the pericardium. The terminal loop is reflected posterodorsally and runs along the extreme dorsal surface of the visceral mass. Compacted faecal matter is found consistently only in the terminal region.

Given that the distribution of nuclear chromatin in any cell may have functional significance (Sahota *et al.*, 1985), one further characteristic within the alimentary tract of *A. serricata* is worth mentioning. Epithelial cells of the intestine and portion of the stomach posterior to the opening of the digestive tubules could be distinguished from all other cells in the clam's body based on nuclear morphology alone. The peculiar arrangement of nuclear DNA is illustrated in Figure 7a in a portion of the style sac. Dense basiphilic material which is probably condensed chromatin is arranged around an acidophilic sphere in broad rings. There were no obvious gross departures in any of the clams examined from this arrangement, and it is unclear based on the absence of pre-mitotic or mitotic nuclei whether cell renewal was occurring at any appreciable rate.

The kidney/pericardial complex.

The arrangement of organs within the pericardium and structure of the kidney are illustrated in Figure 7 b-f. The posterior loop of the intestine passes through a thin-walled ventricle within the pericardium. Triangular auricles emerge from the ventricle and end at the outer dorsoventral wall of the kidney. The wall of the auricles is composed of spheroidal cells which are not tightly opposed and sit on a basement membrane: these are probably podocytes. The overall arrangement of the pericardium is essentially the same as described by Pirie and George (1979) in *Mytilus edulis*, and by Bright (1987) in *Macoma carlottensis*.

The structure of the kidney is illustrated in Figure 7 c-f in different clams. The kidney is located ventral to the pericardium and adjacent to the inner surface of the ctenidia for much of their length.

In many clams, the nephrocytes within the kidney occur around the periphery of the paired, connected kidneys (Figure 7c). In these clams, individual nephrocytes are spherical to cuboidal, relatively transparent and have small, basally-located nuclei. In other clams, nephrocytes are swollen and extend farther into the kidney lumen. These nephrocytes usually contain translucent purple (basiphilic) and brown material, and have a more irregular shape.

Large extracellular nephroliths were observed in clams from most collection sites (Figure 7 e, f), and were most prominent in clams collected in November from lower Holberg Inlet (stations H1, H2). The nephroliths were typically dark purple to dark brown in haematoxylin/eosin stained sections and were composed of concentric layers. In several clams, large agglomerative masses of concentric nephroliths were observed. Clams from some sites contained kidneys with membrane-bound basiphilic material and a decreased size of the kidney lumen, as indicated in Figure 7d. Accumulation of material in the nephrocytes, mineralization and peroxidation of lipid membranes (into successive layers), and nephrocyte breakdown may be intermediate steps in the formation of nephroliths.

Figure 7: Features cf the stomach epithelium, pericardial complex and kidney. Scale bar = 100 μm unless otherwise indicated.

(A) Distinct nuclear morphology exhibited by large portions of the epithelium in the stomach and intestine. Based on the basiphilic staining, the chromatin appears to be highly concentrated in a uniform ring around the periphery of the germinal vesicle. L: stomach lumen. Scale bar = 50 μm.

(B) Structure of the pericardial complex. Podocytes can be seen on the wall of the auricle. Au: auricle; int: intestine; K: kidney; V: ventricle.

(C - F) Histology of the kidney in sagittal section from four clams arranged according to apparent progressive production and retention of mineralized concentric granules, or 'nephroliths'.

Specializations of the foot and sensory structures.

The foot in *Axinopsida serricata* is long and vermiform (Figure 4a) with a large central haemocoel surrounded by muscle bundles. The histology of the foot is similar to that described in other Thyasirids by Bernard (1972), and by Allen (1958). *A. serricata* differs from *Thyasira flexuosa* in having considerably less specialization of the surface epithelium near the tip of the foot. Mucus-producing cells near the tip are numerous, but, in contrast to *T. flexuosa*, the cells are arranged irregularly directly underneath the highly folded epithelium.

The general size and shape of the cerebropleural, visceral and pedal ganglia are illustrated in Figures 8a, 8b, 8c respectively. Note the relatively large size compared to the overall body size. Paired statocysts, associated with the pedal ganglion, are illustrated in Figures 8c and 8d. Bernard (1972) did not find any statocysts in four species of *Thyasira* examined, but this may have been an oversight related to the small size of statocysts and potential for loss during sectioning. Although Barnes (1980) noted that statocysts are a common feature of the Bivalvia, there are few published descriptions of statocyst functional morphology or mineralization. Johnstone (1899, in Hickman, 1967) described the statocyst/sensory pit in *Cardium* sp.

Figure 8: Histology of the cerebropleural (A), visceral (B), and pedal ganglion (D) as well as statocysts associated with the pedal ganglion (C,D). The well developed nervous and sensory structures suggests a refined ability to respond to environmental cues. eso: oesophagus; fm: foot retractor muscle; PG: pedal ganglion; arrows: dark brown statocysts. Scale bars = 100 μm for A, B; 50 μm for C, D.
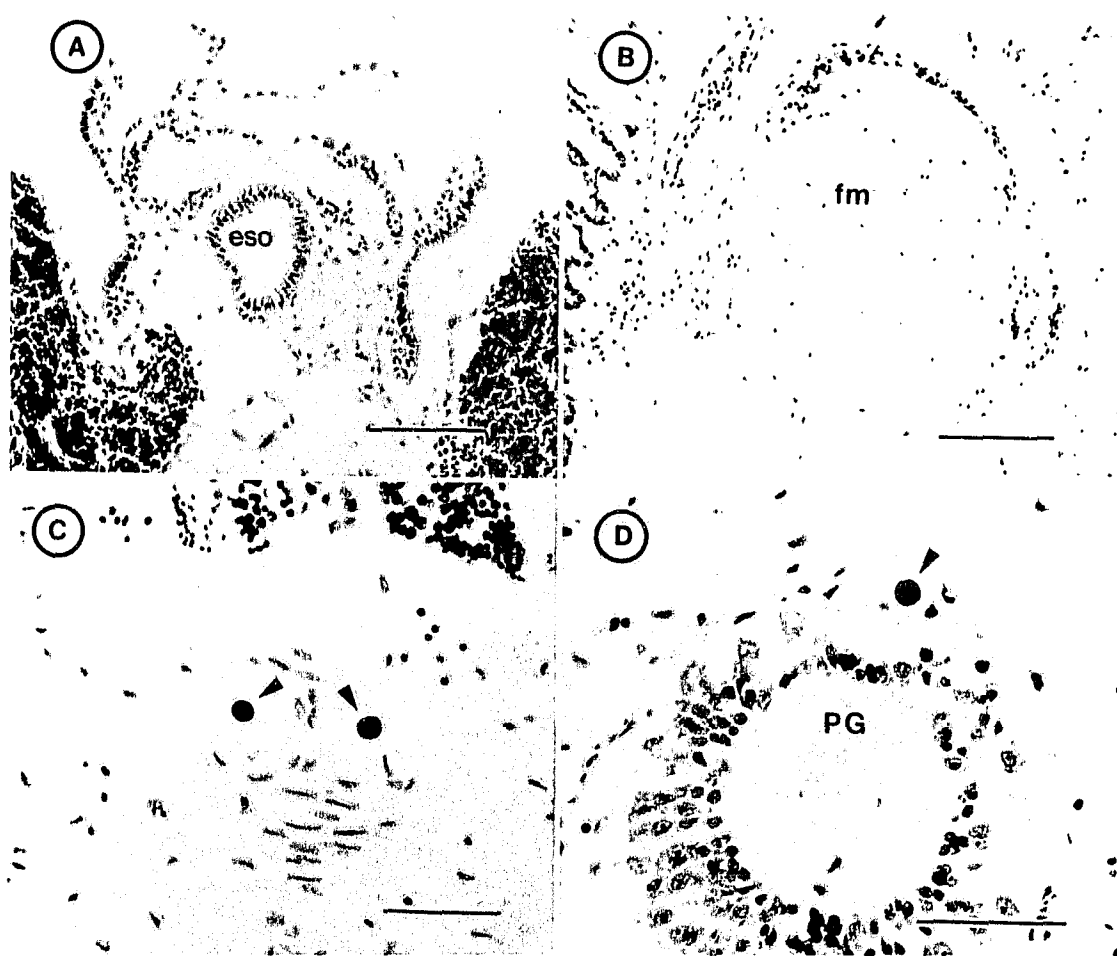
Departures from normal tissue structure: I. Infectious diseases.

A number of structural features are interpreted as being beyond the normal range of physiological functioning, and are possibly reflective of a pathological condition. However, the relationship between environmental disturbance and some of these tissue changes could n oe ascertained due to the'r low overall incidence and/or suspected infectious etiology. Those conditions that were suspected to have an infectious etiology include proliferation of flagellates in the ctenidial pouch in a majority of the 101 clams examined, cellular proliferative disorders in two individuals, and two cases of what was suspected to be a sporozoan infection (see below).

Possible cellular proliferative disorders were noted within the reproductive follicles of two clams, both sampled from Saanich Inlet during the spring of 1989 (Figure 9 a-d). The disorder illustrated in Figure 9a was comprised of a dense proliferation of cells in all follicular tissue. Individual cells (Figure 9 c,d) tended to have a large nuclear-to-cytoplasmic volume ratio. The nuclear chromatin of individual cells showed varying degrees of condensation. A large number of cells having the appearance of granular haemocytes could be identified using phase contrast microscopy (Figure 9c). The degree of cell proliferation was not as severe in the second clam (Figure 9b).

Both conditions described above may have arisen through neoplastic transformation of an unknown cell type, through massive infiltration of haemocytes into post-spawn follicles (see, for example, invasion by granular haemocytes of post-spawn follicles in mussels as illustrated by Sunila, 1987), or through some other route. For the less affected clam (Figure 9b), it is possible that the cells were infiltrating haemocytes associated with the recovery of follicles. These cells had a much lower nuclear to cytoplasmic volume ratio than in the more severely affected clam (Figure 9a), or in other published cases of sarcomatous neoplasms (Peters, 1988). A number of inclusions within the follicles were thought to be spermatozoa undergoing pyknosis.

Figure 9: Tissue variation in *A. serricata* possibly related to an infectious etiology. Scale bar = 100 μm unless otherwise indicated.

(A) A possible proliferative disorder within the reproductive follicles of a clam from Mill Bay, Saanich Inlet. A large number of detached cells are limited in distribution to the follicles. dig: digestive diverticula; F: follicle.

(B) Similar condition to the above in another clam from Mill Bay but less severe. Cells may represent invading haemocytes associated with post-spawn recovery or may represent a proliferative disorder. Arrow points to small basiphilic inclusions in follicles that are most likely pyknotic sperm nuclei.

(C, D) Higher magnification of proliferative disorder shown in (A) above under phase-contrast and normal illumination respectively. Dotted lines in (C) delineate edge of one of many granular haemocytes which co-occured with the abnormal cells. Abnormal cells exhibited a high nuclear to cytoplasmic ratio (a thin halo of cytoplasm is barely visible around some of the nuclei) and nuclei which were either condensed or decondensed. Scale bars = 10 μm.

(E) Inclusion between gill filaments that were possibly sporozoan sporangia. Scale bar = 50 μm.

(F) Similar inclusions to those illustrated in (E) in the pericardial cavity of another clam from the same location. In both cases, clams that were collected in the fall contained no gametes in the reproductive follicles and were considered to be castrated. int: intestine; per: pericardium.

$\int$
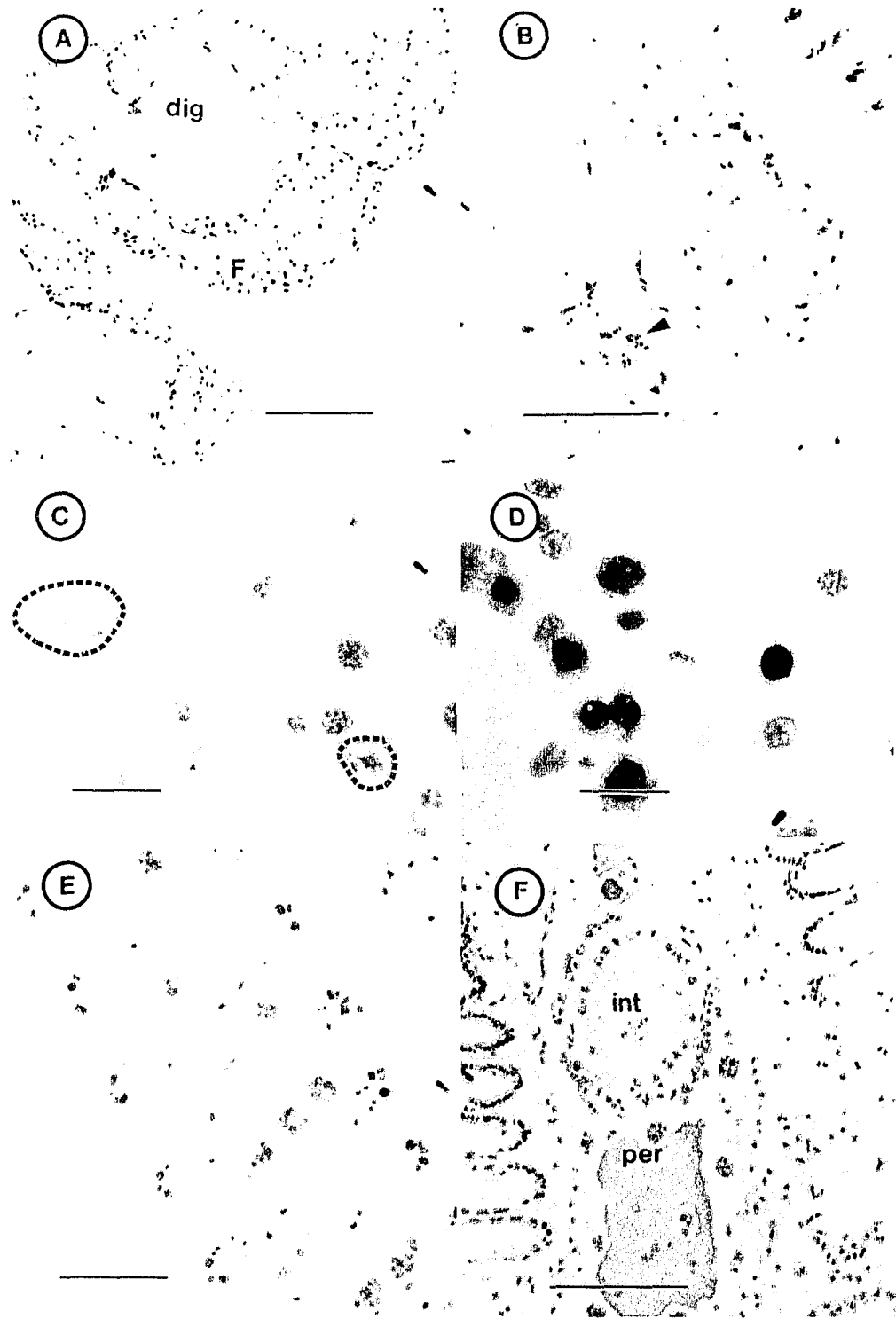
In the more severely affected clam, an etiology related to infiltration of untransformed haemocytes is less likely. 27 of 101 clams were in a post-spawn or recovering condition at the time of fixation. Haemocyte densities were uniformly low except in these two cases, although residual unspawned ova or spermatozoa and a few haemocytes were often seen. There is a possibility that massive haemocyte infiltration occurs as a transient stage of short duration relative to the total post-spawn recovery time and is thus rarely seen. Another possibility is that the abnormal cells represented a massive haemocyte response to an undetected pathogen.

Particles resembling sporozoan sporangia were detected in two separate clams both sampled from Saanich Inlet in November, 1987. In one case, the particles were seen associated with the external surfaces of the outer ctenidial demibranch adjacent the lateral cells (Figure 9e). In the other case, the particles were seen within the pericardium surrounding the heart (Figure 9f). In the later case, a basiphilic-staining exudate not seen in other clams was observed throughout the pericardial cavity. In both clams, the gonad was undeveloped and these clams are interpreted as being castrated. In spite of parasitic castration, no obvious relationship with other documented lesions was observed.

Of 101 specimens examined, 58 clams contained protozoans within the space between the abfrontal surfaces of ascending and descending filaments, or ctenidial pouch (Figure 10a). The flagellar apparatus of the protzoans was observed at a higher magnification (Figure 10 b,c) in paraffin sections. The flagellates occurred in extremely high densities in several of the clams (Figure 10d) and some disruption of structure of filament abfrontal surfaces was observed in extreme cases, suggesting that the relationship was a parasitic rather than commensal one. Only in Granby Bay were all specimens free of infestation by flagellates.

Figure 10: Protozoans in the ctenidia of *A. serricata*.

(A) A severe infection of the ctenidial pouch by a flagellate parasite. The deleterious nature of the infection in clams severely infected is suggested by disruption of adjacent abfrontal surfaces of the gill filaments. Scale bars = 100 μm.

(B, C) Higher magnification of flagellates inhabiting the ctenidial pouch. A flagellum can be seen which was interpreted to be a single flagellum with an accompanying undulating membrane or a fused complex of several flagella. A single nucleus and several nucleoli can be distinguished. Scale bars = 25 μm.
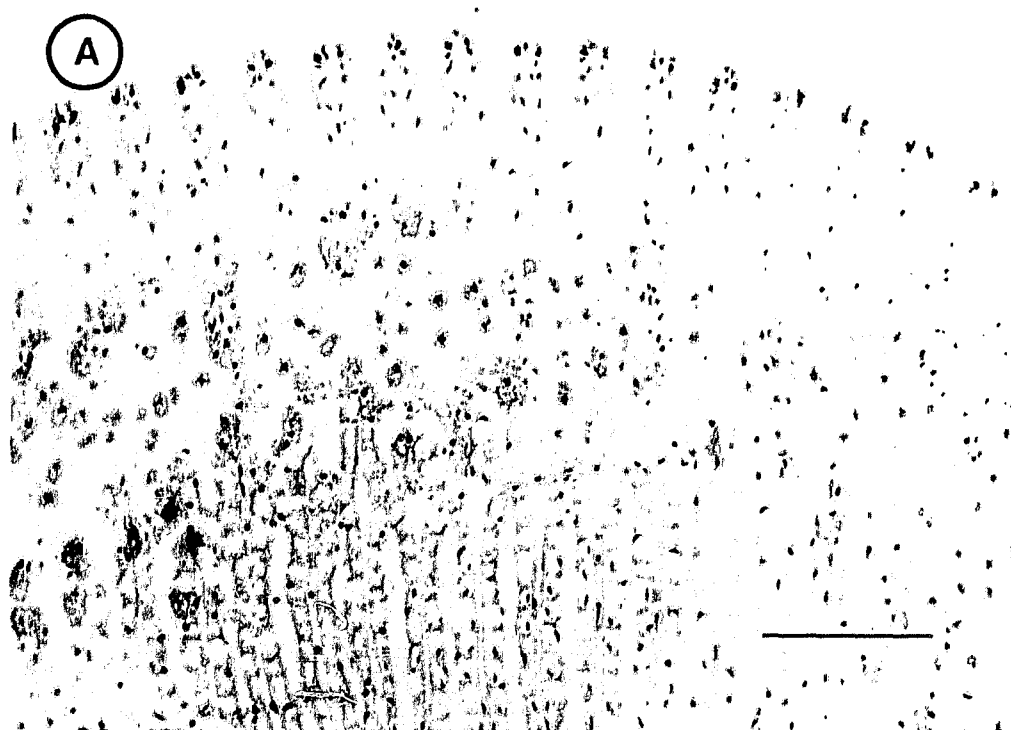
Figure 11 (a,b) illustrates a hyperplastic growth of the kidney which extended dorsolaterally in one clam collected from outer Quatsino Sound. Ceroidosis, or accumulation of granular material, was observed along the periphery of individual nephrocytes (Figure 11b).

Departures from normal tissue structure: II. Structural features associated with environmental disturbance.

Possible abnormal variation in the tissue structure of *A. serricata* occurred in specimens from all sampling stations including those from Saanich Inlet, albeit with differing incidence or severity. The tissue structure observed within Saanich Inlet clams was used as a reference for identifying deviations associated with environmental disturbance. Stages in the deterioration of individual tissues were reconstructed through an examination of clams from all sites except Saanich Inlet; in the initial qualitative description of abnormal variation, specific site-to-site differences in tissue structure were largely ignored. A more detailed account of between-site and between-season differences in tissue structure is provided in the next chapter.

Three interrelated tissue abnormalities were observed in the digestive tubules of *A. serricata*: digestive cell vacuolation, increased fragmentation of digestive cells, and basiphil cell vacuolation (Figure 6 c-e). Affected digestive cells and basiphil cells contained a single large vacuole close to the basal lamina, possibly a residual body. In clams with a large portion of digestive cells which were fragmented, intact digestive cells were taller and the apical cell membrane /as less distinct. Vacuolation, swelling, and fragmentation of digestive cells may be intermediate stages in autolysis.

The proximal digestive ducts of different clams differed in the density of large spherical vesicles (Figure 6 f-h). Large vesicles were more conspicuous in clams from lower Holberg Inlet (station H1 and H2) than in Saanich Inlet clams, but there was a large amount of within-site variation at all stations, including Saanich Inlet.

The ctenidium was a major site of tissue deterioration (Figure 12). Possible disturbance-induced structural changes included metaplasia of abfrontal ctenidial

mucocytes, oedema of lateral cells and sloughing of frontal ciliated cells. Two clams had large numbers of agranular haemocytes within the ctenidial blood sinus or branchial vessel (Figure 12c).

Abnormal mucocytes (Figure 12 a,b) were distinguished from normal mucocytes by a larger size in histological sections (metaplasia) and more intense staining by haematoxylin. Oedema of lateral cells (Figure 12b) was associated with constriction of the branchial vessel and a decreased interfilament gap. Sloughing of frontal ciliated cells was usually limited to a small portion of the ctenidial surface (Figure 12 b). Loss of frontal ciliated cells, oedema of lateral cells, and metaplasia of mucocytes often co-occurred within the same individual.

Figure 11: (A,B) Hyperplasia of the dorsolateral surface of the kidney. Individual nephrocytes contained abnormal peripheral accumulations of fine, dark brown granules (arrow: Figure 11B). Scale bars = 100 μm and 50 μm for A and B respectively.
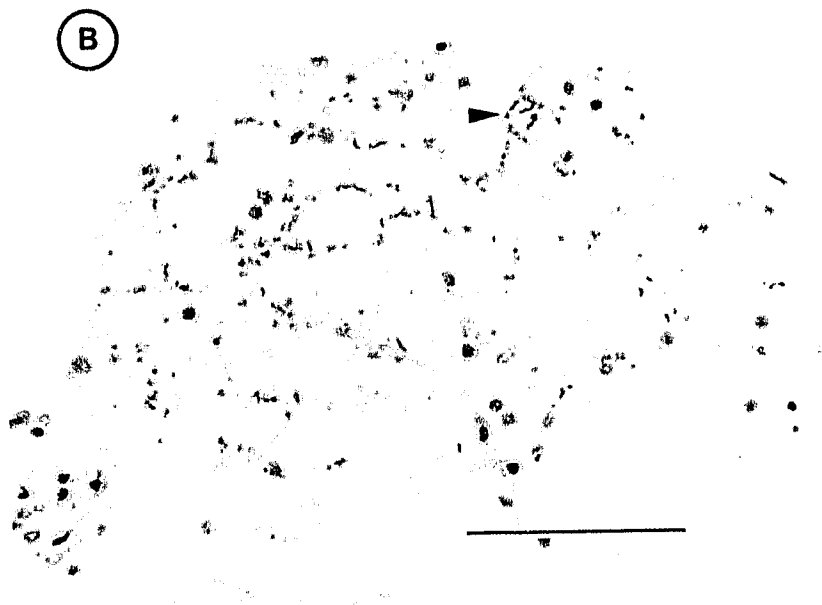
Figure 12: Abnormal tissue structure of the ctenidia of *A. serricata*. Scale bars = 100 μm unless otherwise indicated.

(A) Metaplasia and hyperactivity of ctenidial mucocytes (m) along the abfrontal surface. K: kidney.

(B) Severe oedema of ctenidial lateral cells. In this clam, hyperactivity of the mucocytes and limited disruption of frontal ciliated cells were also observed. lc: lateral cell; m: ctenidial mucocyte.

(C) Occlusion of branchial vessel of several ctenidial filaments by accumulations of haemocytes (arrow). Scale bar = 50 μm.

The posterior foot retractor muscle in several *A. serricata* from all stations appeared to be rarefied; i.e., transverse to oblique sections dorsal to the pedal ganglion contained less area composed of stained, solid muscle bundles and more area as empty space or connective tissue. Rarefaction may have occurred as a result of atrophy of individual muscle bundles. Within individual clams, there was variability in the relative proportion of space taken up by muscle bundles dorso-ventrally along the retractor muscle. In addition, there was no clear indication of between-population or between-season differences in the extent of muscle atrophy.

Cells similar in appearance to agranular haemocytes (or hyalinocytes) were observed in low densities within the posterior loop of the intestine (Figure 13a). These cells, with a relatively condensed nucleus, high nuclear to cytoplasmic volume ratio and dense cytoplasm were observed in 33 of the 98 clams examined from all sites. One of several possible interpretations of this observation is that the cells represented haemocytes undergoing diapedesis (i.e. active migration out of the body), to export wastes and toxins from the body. If this is a reasonable interpretation, then increased stress, especially that induced by contaminants, could result in an increased rate of diapedesis and increased passage of cells through the intestine. Observations of the presence or absence of cells within the posterior intestine indicated that this condition had a high incidence only in the reference population (Saanich Inlet) in the spring of 1989 (see Chapter 3), when stress due to reproductive activity was probably at a minimum. Therefore, this condition probably does not represent diapedesis.

Several female clams collected from seasonally mature populations (i.e., in November, 1987) had oocytes which appeared to be undergoing lysis prior to any major spawning event. Normal oocytes (Figure 13b) are characterized by a yolk-rich cortex and large germinal vesicle containing several inclusions (nucleus, nucleolus, and others). The number and types of inclusions within the germinal vesicle of different oocytes varied. No pattern of inter-individual differences in the structure of the germinal vesicle could be found. Breakdown of oocytes was

Figure 13: Putative lesions in the clam *A. serricata* associated with environmental perturbation.

(A) Intact cells (arrows) within the posterior intestine which may be haemocytes undergoing diapedesis. Scale bar = 50 μm.

(B, C) Normal and abnormal ova respectively which may be undergoing premature breakdown. Note inclusions in germinal vesicle of normal ova (B). Abnormal ova have a greater irregularity of shape, rarification of structures within the germinal vesicle, and are surrounded by an exudate between individual ova. pdt: proximal digestive tubule. Scale bars = 50 and 100 μm for (B) and (C) respectively.

(D) Necrosis of follicular material (i.e. castration) which may have been caused by a sporidian infection in a clam from Mill Bay, Saanich Inlet.

(E, F) Dense accumulations of fine brown granules within columnar epithelial cells of the mid-stomach which were probably metal-accumulating tertiary lysosomes. L: lumen; nu: nucleus. Scale bars = 50 μm.

pdt

L

F

L

nu
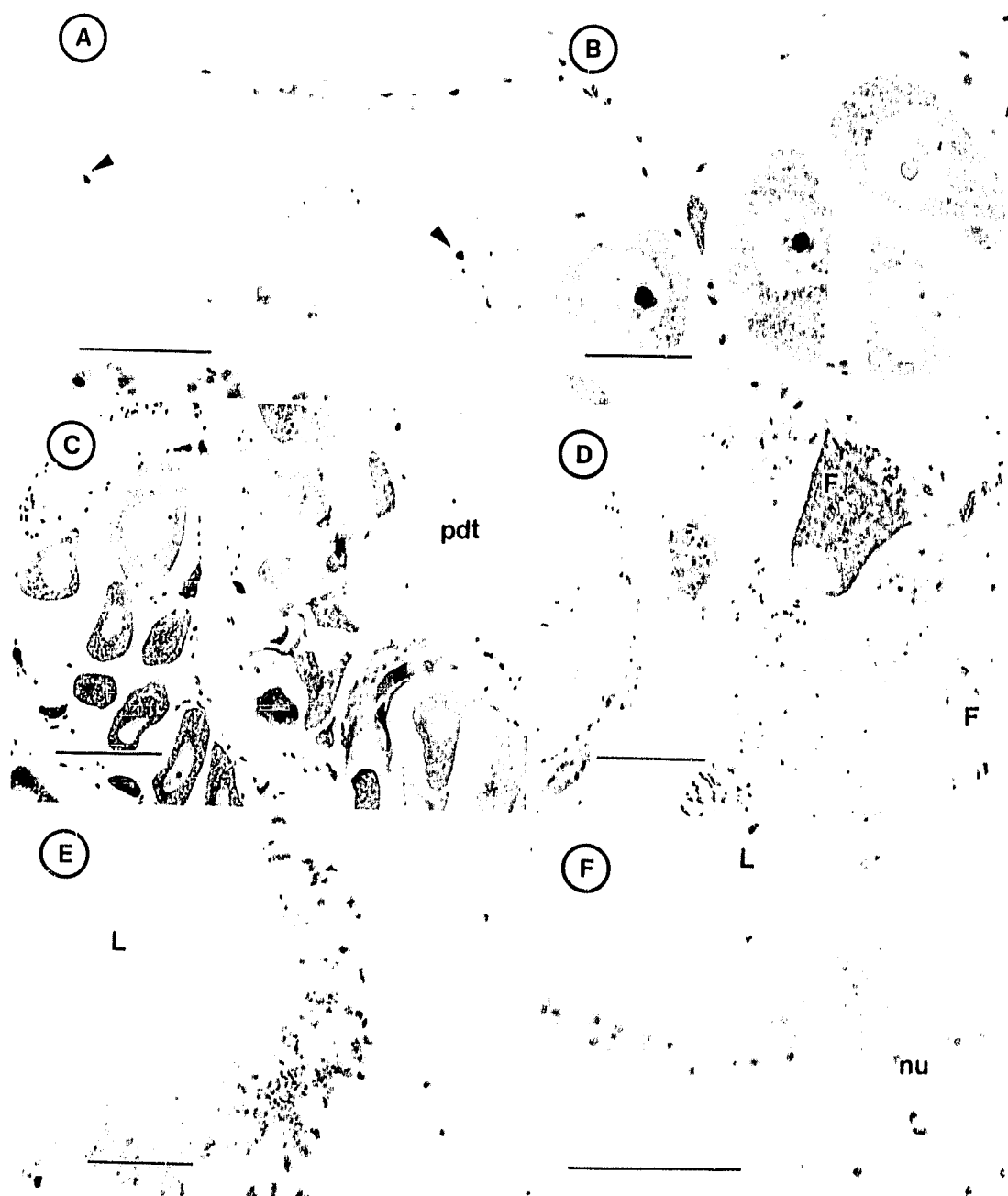
characterized by a greater translucency of the germinal vesicle, loss of germinal vesicle inclusions, aggregation of yolk granules in the cortex, greater irregularity of the outer profile of the oocytes, appearance of basiphilic exudate between oocytes, and lysis. Given that the oocytes of *A. serricata* are relatively large and yolk-laden compared to many other broadcast spawners (see Chapter 4), some of the breakdown of ova observed may have been an artifact resulting from poor penetration of the fixative. Sunila (1984, 1987) observed in *M. edulis* the infiltration of haemocytes into follicles which were experiencing premature breakdown. The breakdown of oocytes in *A. serricata* was not accompanied by a noticeable haemocyte response (Figure 13b), which further suggests that the condition was an artifact of poor fixation.

One clam of indeterminate sex had reproductive follicles which contained only basiphilic-staining, lysed material (Figure 12d). The etiology of this lesion was most likely related to a pathogen; the condition was found in the same clam containing probable sporozoan sporangia between the ctenidial filaments (page 45).

Large numbers of light- to dark-brown tertiary lysosomes were observed within cells in a portion of the stomach epithelium anterior to the style sac and close but not directly adjacent to the opening of the digestive ducts (Figure 13 e,f). The tertiary lysosomes were observed in 50 of 98 clams examined (the appropriate area of the stomach could not be unambiguously identified in sections of the other three specimens), and occurred with greater consistency in clams from lower Holberg Inlet (H1 - H3) collected during the spring.

Discussion

There are several structural features of *Axinopsida serricata* which are atypical in comparison with larger, often commercially valuable Eulamellibranchs. One of these is the external anatomy which, by virtue of the absence of siphons and relative lack of mantle fusion, suggests that *A. serricata* has a limited ability to isolate the mantle chamber from the surrounding environment..

In Thyasirids, the outward externalization of the visceral mass as visceral tufts containing digestive diverticula is unusual, and there are presently no adequate theories pertaining to functional significance. Bernard (1972) speculated that the arborescent tufts which house atypically large digestive ducts are modifications associated with macrophagy. However, observations on the contents of stomachs and digestive diverticulae in *A. serricata* do not suggest a macrophagous feeding habit.

If Thyasirids are deposit-feeders as suggested by Allen (1958), then the efficient transport and sorting of food and sediment within the mantle cavity would be advantageous. The extension of the visceral mass in arborescent tufts which are only partially ciliated creates a convoluted surface which may not be amenable to the transport of large quantities of sediment. In addition, the short intestine and relatively simple structure of the frontal surface of the ctenidia suggest a mode of nutrition other than deposit feeding.

One possible explanation for the atypical structure of small Thyasirids, as typified by *A. serricata*, is that the morphology and histology reflect adaptations to the uptake of dissolved organic matter (D.O.M.) from sediment interstitial water. The arborescent tufts would provide increased surface area for D.O.M. uptake. This is discussed in greater detail in Chapter 6. Structural variability in the proximal digestive ducts has not been described previously. It is possible that the digestive ducts contribute to the storage of nutrients and energy, possibly associated with uptake of D.O.M. through the arborescent tufts.

Several histological features described in this study may represent variation beyond the normal range of structure and function in unstressed clams. These

include variation in the digestive tubules, proximal digestive ducts, ctenidia, the kidney, stomach epithelial cells, the foot retractor muscle, and unspawned oocytes.

Changes in tissue structure could potentially be adaptive (e.g. compartmentalization and detoxification of metals; Simkiss and Mason, 1983) or maladaptive (e.g. atrophy of gonadal tissue prior to spawning; Sunila, 1984, 1987). In this study, two histological features are considered to be adaptive responses to the environment rather than pathological responses: stomach tertiary lysosomes and nephroliths.

Tertiary lysosomes observed in a portion of the stomach epithelium were similar in appearance to those described by Mason and Nott (1980) in the gastropod *Littorina littorea*, by Reynolds (1990) in the oesophagus of the scaphopod *Dentalium rectius*, and by Bright and Ellis (1989) in the oesophagus of the bivalve *Macoma carlottensis*.

Incorporation of various metals in mussel tertiary lysosomes (reviewed by George and Viarengo, 1983) is one mechanism of detoxification. In *A. serricata*, the occurrence of the lysosome-containing cells in the stomach epithelium suggests that the alimentary tract may be an important route of metal uptake.

Another possibility is that lysosomes in the stomach epithelium are directly associated with the metabolic storage and control of essential metals, for example, copper, zinc, iron, and molybdenum. In *Littorina littorea* (Mason *et al.*, 1984), the metal composition of stomach tertiary lysosomes in animals from clean and polluted sites was very similar (iron with traces of calcium and sulphur). In snails from the polluted site, copper was occasionally detected; however, the relative absence of between-site differences in metal composition suggests that lysosomes within the stomach epithelium, unlike lysosomes in the digestive tubule basiphil cells, nephrocytes, and ctenidial cells, are not influenced by environmental metal contamination. *Axinopsida serricata* exhibited between-site differences in the presence of tertiary lysosomes within the stomach epithelium (see Chapter 3). A study of between-site differences in metal composition of these lysosomes would be helpful in determining the extent of environmental mediation of lysosomal structure for this species.

Mineralized nephroliths can also be considered as adaptive features. The large concentrically arranged nephroliths in *A. serricata* are very similar in apearance to iron-containing granules described by Reid and Brand (1989) in the bivalve *Pinna bicolor*. Metals in bivalves are generally sequestered in the kidney (Simkiss and Mason, 1983), associated with intracellular, membrane-bound vacuoles or vesicles. However, the kidney concretions in *A. serricata* are mostly extracellular.

Degenerative features of the digestive diverticula (vacuolation, fragmentation and lysis of digestive cells and vacuolation of basiphil cells) of *A. serricata* from lower Holberg Inlet and Granby Bay are considered to be pathological. Vacuolation, thinning, and necrosis of digestive tubules in bivalves has been described as a pathological response to contaminant exposure (Auffret, 1988; Sunila, 1986b, 1987; Yevich and Yevich, 1985; Calabrese *et al.*, 1984; Fries and Tripp, 1977). Lowe *et al.* (1981) interpreted thinning of digestive tubules as a result of accelerated catabolism of cellular components by lysosome-mediated autolysis. On the other hand, less severe alterations of tissue structure in the digestive diverticula might have adaptive value in re-mobilizing energy and materials for other physiological functions such as gamete production. Between-season digestive tubule variability observed in *A. serricata* collected from Mill Bay may occur as part of the normal reproductive process.

Oedema of ctenidial lateral cells and sloughing of frontal ciliated cells are probably of little adaptive value and are considered to be pathological. Atrophy of the foot retractor muscle of individual *A. serricata* possibly occurs only after the depletion of other more readily available energy reserves has occurred, and may interfere with burrowing and burrow ventilation activities. The breakdown of oocytes before spawning occurs is also considered here to be maladaptive, or pathological.

No relationship between conditions with a suspected infectious etiology and other tissue effects could be discerned through qualitative examination of *A. serricata*. Two conditions with a possible infectious etiology were limited to a single sampling location: two cases of possible cellular proliferative disorders and two cases of possible sporozoan infections were limited in their distribution to Mill Bay, Saanich Inlet from spring or fall collections respectively. A flagellate occurring in

the ctenidia had a more widespread distribution but was absent from Granby Bay, a site originally chosen as a disturbed area. Therefore, these conditions are likely of little use as bioindicators of environmental disturbance. Allen (1958) described a number of both ecto- and endoparasitic protozoa within the Lucinacea, but did not observe any specifically within the Thyasiridae.

In *A. serricata*, extracellular mineralized granules in the kidney and conspicuous tertiary lysosomes in the stomach epithelium might be adaptive responses to habitat. The external anatomy appears to be poorly adapted for isolation of the clam from its surroundings. In sediments undergoing early diagenesis, the pore-water contains fluctuating concentrations of metal sulfides and dissolved heavy metals (Rhoads and Boyer, 1982). Nephroliths and stomach tertiary lysosomes in *A. serricata* might detoxify dissolved heavy metals within the clam.

Conditions inferred to be pathological in *A. serricata* were vacuolation of digestive tubule digestive and basiphil cells; swelling, fragmentation, and lysis of digestive cells; sloughing of ctenidial frontal cells; oedema of ctenidial lateral cells; and hyperactivity of abfrontal ctenidial mucocytes. The atrophy of foot retractor muscles, lysis of oocytes, presence of hyalinocyte-like cells in the posterior intestine, and occlusion of the ctenidial branchial vessel by accumulated haemocytes might represent pathological change. However, the evidence for abnormality of the later four conditions is less clear.

Reproductive status could affect both adaptive and pathological tissue variation in *A. serricata*. Reproduction alters energy expenditure for growth and maintenance repair (Kirkwood, 1987; Bayne, 1985). Chapter 3 formally explores tissue effects associated with reproductive stress in *A. serricata*.

3. A quantitative analysis of tissue variability in A. serricata associated with site, size, season, sex and parasitism.

Methods

Evaluation of sediment properties.

Site-to-site differences in sediment characteristics, such as grain size and the amount of organic matter, could potentially mask disturbance-induced between-site variation in tissue structure. Sediment particle size distribution is often related to food availability, the depth at which sediments become anoxic, burrowing and mobility of infauna, *et cetera*. Therefore, sediment particle size and percent organic matter were measured at stations established for the collection of *A. serricata* (Figure 1 in Chapter 1). When benthic samples were obtained from Mill Bay or Holberg Inlet, the Van Veen or Smith McIntyre grabs were subsampled for sediment using a 5.4 cm. diameter plastic tube pushed vertically into the grab. The sediment characteristics of a single grab sample taken in November, 1987 from the trough of Rupert Inlet near the end of I.C.M.'s mine-tailings discharge pipe were also assessed. The benthic substrate near the discharge pipe is composed almost entirely of deposited mine-tailings. All sediment samples were immediately frozen and kept frozen until the particle size distribution and percent organic matter were determined.

A particle size distribution was constructed for each sediment sample using the rapid method outlined by Buchanan (1984). This involved dispersing a known mass of previously air-dried sediment and dry seiving (particle size > 63 μm) or carrying out settling/pipette analysis (< 63 μm) in order to size-fractionate the sample.

An estimate of the organic matter present in each of the sediments was obtained by observing the weight-loss of previously air-dried sediment on ignition at 450° C for 14 - 16 h. This method may over-estimate organic-matter content by a further partial volatilization of carbonates, but it represents a reasonable compromise between problems associated with various methods (Byers et al., 1978).

Quantitative histological methods.

Quantitative differences in tissue structure between *A. serricata* individuals sampled from different locations and in different seasons were examined in specimens collected and prepared as in Chapter 2 (pages 22, 23). Slides of specimens from all locales and from both fall and spring (Table 1, page 23) were randomly reassigned a number from 1 to 101 and the original label covered over. Thus, specimens were scored 'blind', without prior knowledge of the original collection date or location.

Thirteen variables were recorded for each specimen, which were intended to reflect histological changes occurring in the digestive gland, gonad, kidneys, ctenidia, stomach and musculature. Two further variables were devised to assess the stage of reproduction in each clam and indicate the body size of the clam. A more detailed description of each variable is provided below:

MWVM: Maximum width of the visceral mass. The maximum distance across the visceral mass in transverse or sections of prepared slides was used as an index of individual clam size. This was considered to be a measure which could be used to distinguish clams in their first year of growth from those two to three years old and from very old, potentially senescent individuals.

SRC: Stage of reproductive cycle. Since the stage of the reproductive cycle in individuals and populations was considered to be theoretically important in modifying tissue structure, an index was used to assess differences in reproductive activity. The SRC was scored with values from -2 to 3 as follows: -2: spent; -1: recovering; 0: indifferent; 1: developing; 2: reproductively mature; 3: spawning. The SRC was devised such that clams collected in the fall (November, 1987) and clams collected in the spring (April-May, 1989) would tend to fall at opposite ends of the scale.

DTI: Digestive tubule index. This index was used to assess the condition of the digestive tubules immediately adjacent to the tips of the arborescent tufts (as opposed to tissue near the tuft bases). Measurement of DTI was confined to the tubules near the tips of the arborescent tufts because the digestive diverticulum exhibits continuous histological variation from the stomach openings to the digestive

tubules, which might further complicate inter-individual comparison. An index from 5 to 0 was used over the range in structure from being in excellent condition to being completely necrotic respectively. For each clam, the DTI was assigned after examining sections throughout the visceral mass.

DCH: Height of digestive cells. DCH was measured across digestive cells (the distance between the tubule basal lamina and the tubule lumen perpendicular to the basal lamina) but not across pyramidal basiphil cells. Only those cells in the portion of the digestive tubule which were adjacent to the distal end of the arborescent tufts were selected. Measurements were taken with an ocular micrometer at 400x magnification on randomly selected tubules within randomly selected sections. Up to sixteen tubules per clam were counted. Where the apical cell membrane of the digestive cells (facing the tubule lumen) could not be distinguished due to breakdown (blebbing or lysis), the DCH could not be reliably measured. Such tubules were excluded from calculation of DCH, but the proportion of tubules encountered in each clam which were completely fragmented and could not be scored for DCH was recorded. The proportion of tubules which were not fragmented was used as a tubule fragmentation index, or **FTI**.

PDD: Proximal digestive duct density is the percent of graticule points (out of a possible 90) at 400x magnification falling on stained versus unstained areas of the digestive diverticula, exclusive of the more distally located digestive tubules proper. The percent area was assumed to be directly proportional to the percent volume in unsectioned tissue. The PDD is thus a measure of percent volume density of tissue, primarily of large vesicles. This index arose from large perceived differences in the type and number of spherical vesicles and density of tissue occurring in digestive ducts leading to the tubules (Chapter 2, page 33, 34). Although the functional significance of large between-individual differences in duct structure is unknown, the variation encountered was thought to be important with possible relevance to energy stores, stress, reproductive cycle, and/or senescence.

NVR: Nephrolith Volume Ratio. The relative volume of mineralized granules and large agglomerative masses within the kidney, collectively termed nephroliths, was measured by counting at 250x magnification those points in a 9 x 10 eyepiece graticule which fell on nephroliths versus other structures. For each clam, counts

were made on five randomly selected sections which passed through the kidney lumen. The relative volume of nephroliths was then estimated as the percent of point counts falling on nephroliths.

MAI: Muscular Atrophy Index: Possible atrophy of muscle bundles within the foot retractor muscle was measured by estimating the percent volume of the muscle occupied by muscle fibers versus empty space, connective tissue, or haemocytes. Five randomly selected sections were counted from an area of the foot retractor between the dorsal point of attachment and the pedal ganglion/statocyst complex. The percent area of muscle bundles was measured at 400x magnification using point counts on an ocular grid, and area was considered to be directly proportional to volume.

Several histological conditions were recorded as incidence data, i.e. as present (1) or absent (0) for each clam:

IC: Intestinal Cells: Some clams had intact cells within the lumen of the posterior intestine (Chapter 2), which might be undergoing diapedesis. Those specimens which did not have a sufficient area of intestinal lumen represented due to incomplete sectioning were not scored.

S.E.L.: Stomach Epithelial Lysosomes: Serial sections of the entire stomach were examined in order to ascertain the presence or absence of light to dark brown tertiary lysosomes in columnar epithelial cells.

OVA: Premature Breakdown of ova: Female clams having abnormal oocytes (Chapter 2) but which had not commenced spawning were recorded as experiencing a premature breakdown of ova.

CTEN: Disruption of Ctenidial Filaments: Changes to the lateral and/or frontal surfaces including loss of frontal and laterofrontal ciliated cells, and alteration of filament architecture were scored as a disruption of ctenidial filaments.

MUCOHYP: Metaplasia and Hyperactivity of Ctenidial Mucocytes: Clams were scored positive for alterations of ctenidial mucocytes if mucocytes located on gill filament abfrontal surfaces were consistently prominent by virtue of swelling and greater intensity of basiphilic staining.

OEDEMA: Clams containing ctenidial filaments with oedema of the lateral cells were noted.

FLAG: Ectoparasitic Flagellates: As noted in Chapter 2 (page 45, 46), a majority of clams were found to contain flagellates within the ctenidial pouch. Those clams in which flagellates were present were noted.

Data analysis.

Data analysis was carried out using Systat 4.0^{TM}. Summary statistics were generated, and each continuous variable was independently assessed for between-site, between-seasor heteroscedasticity (unequal variances). Where violations of normality or homoscedasticity were detected, an appropriate transformation (logarithmic, arcsin, or square root) was applied.

For MWVM, DTI, DCH, PDD, FTI, NVR, and MAI, separate one-way analyses of variance (ANOVAs) were run for each variable with each collection (site x season) considered as independent units. A Tukey-Kramer post-hoc test was applied to detect differences between individual groups. Unlike other post-hoc tests, the Tukey-Kramer test does not unduly inflate the actual type I error in employing a harmonic mean to compensate for unequal sample size (Wilkinson, 1988).

Incidence data for IC, SEL, OVA, CTEN, OEDEMA, MUCOHYP, and FLAG were tabulated as percent frequency of occurrence for each station and date. An overall distributional difference in the incidence of each lesion was assessed using a log-likelihood estimate of chi-square in a heterogeneity chi-square analysis (Zar, 1984).

The relationship within individuals between different lesions was examined by tabulating the raw Pearson Product Moment correlations. The statistical significance of the Pearson r was evaluated for each correlation ($\alpha = 0.05$) employing the Bonferroni-adjusted probability in order to avoid inflation of the overall type I error.

The effect of size (MWVM) on severity of tissue alterations was also examined using the Pearson correlation and associated Bonferroni-adjusted probability.

The relationship between individual clams and between sub-populations from different locations and seasons based on the overall tissue structure was assessed with a Principal Components Analysis with a varimax rotation. The number of factors retained was determined based on a scree-test. Individual clams were compared using their positions (component scores) on pairwise plots of the first three principal axes.

Results

A comparison of sediment characteristics between tailings-affected and natural sites.

A between-site comparison of sediment particle size is provided in Figure 14. Station R1, directly off the tailings discharge pipe (Figure 2, page 12), was a depauperate area containing no molluscs and composed of silt-sized mine-tailings. Station H1 was similar to R1 in sediment features, suggesting a strong tailings influence. Sediments from the head of Holberg Arm (H4, H5) and outer Quatsino Sound (QS) contained a higher percentage of sand. Sediment collected from the reference site (SI) in Mill Bay, Saanich Inlet (Figure 3, page 16), had a particle size distribution very similar to sediment from stations H2 and H3, i.e. finer grained clay-silt.

Between-site differences in sediment organic matter reflected between-site differences in grain size (Figure 15). The influence of tailings on R1 and H1 was reflected in the dilution of sediment organic matter by rapidly accumulating mine-tailings. Higher values of percent volatile matter at stations H2 and H3 suggest a greater influence of natural sedimentation. The reference site, SI, had a similar amount of sediment organic matter as the Holberg Inlet stations exclusive of H1.

Figure 14: The relative composition, as sand, silt or clay, of natural or tailings-influenced sediment. The locations of sediment sampling stations correspond to those used for collection of *A. serricata* (Chapter 1, page 11), with exception of Rupert Inlet station R1. Station R1 was located directly off I.C.M.s tailings-discharge pipe, and the sediment was composed almost entirely of silt-sized deposited mine-tailings. The sediment composition of the sampling station in Holberg Inlet closest to the discharge pipe (H1) was similar to that of station R1. Holberg Inlet stations H2 and H3, and the reference station SI, had a relatively larger clay component, indicative of natural sedimentation.
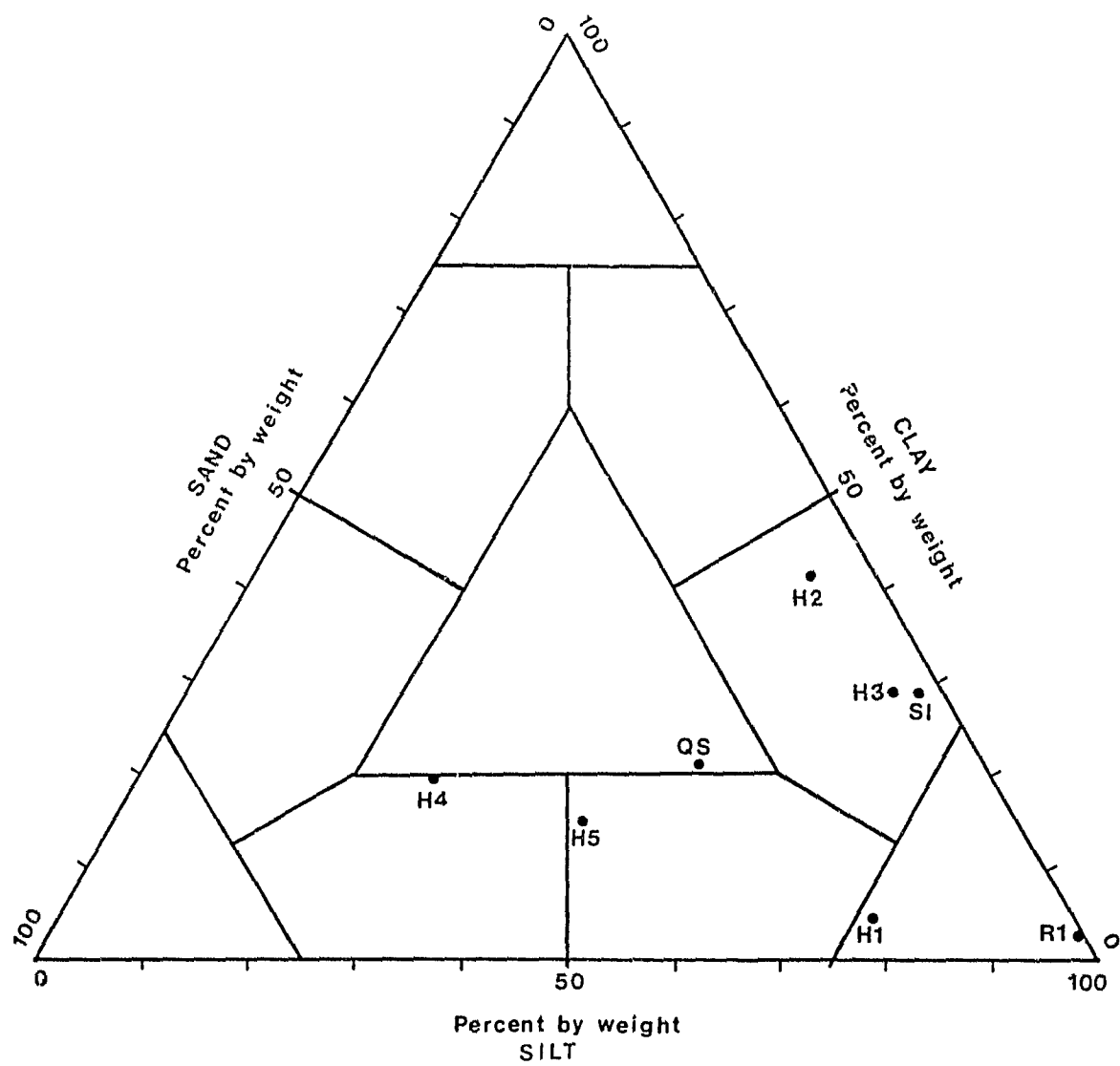
Figure 15: The sediment organic content of natural and tailings-influenced sediment. The locations of sediment sampling stations correspond to those used for collection of *A. serricata* (Chapter 1, page 11), with exception of Rupert Inlet station R1, which was largely deposited tailings. Dilution of sediment organic matter by rapidly accumulating tailings is observed in the station closest to the discharge pipe which was used for collection of clams (H1), but not at Holberg Inlet stations further away (H2-H5).

Percent weight loss on ashing
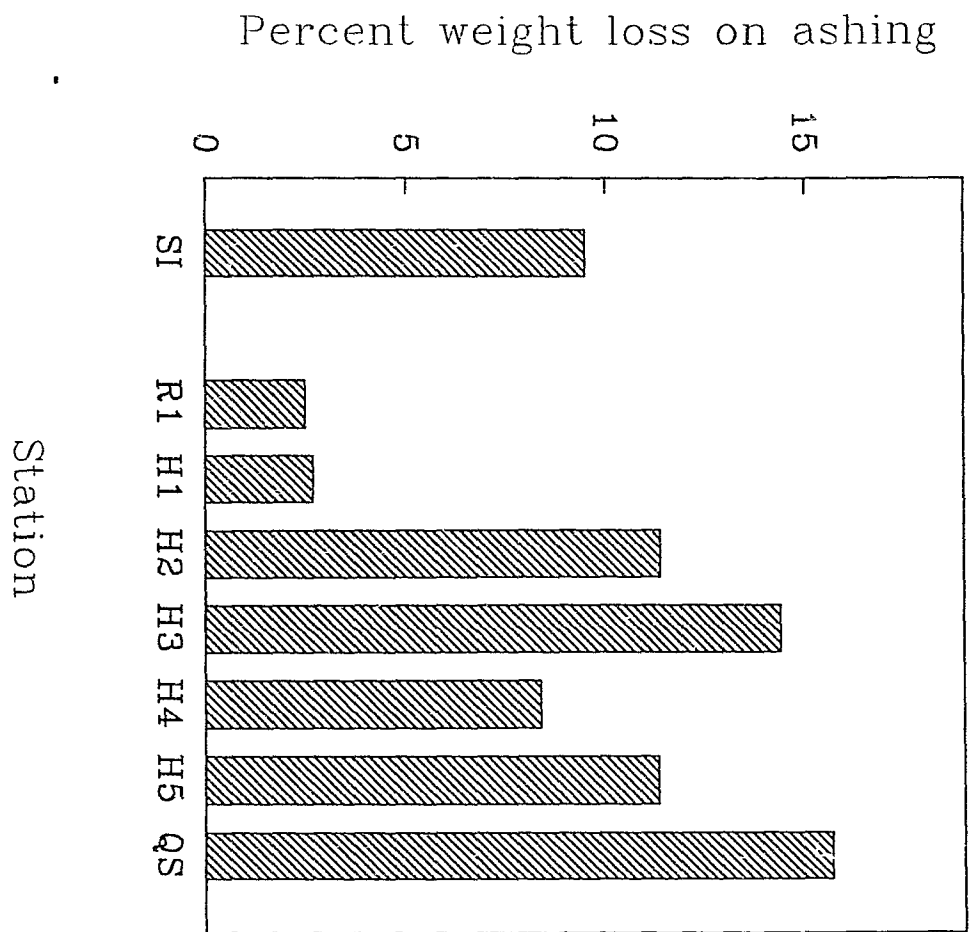
Between-site and between-season variation in individual tissue features of A. serricata.

The means and associated variation of clam size and six tissue conditions in specimens of *A. serricata* are provided in Table 2. Before the data were subjected to separate one-way analyses of variance (ANOVAs), each variable was tested for between-sample homogeneity of variance. The tissue conditions fragmented tubule index (FTI), proximal digestive duct density (PDD), nephrolith volume ratio (NVR) and muscle atrophy index (MAI) showed between-sample differences in the amount of variation (Table 3: Box's F).

A log-transformation was applied to NVR and PDD to correct the departure from homogeneity of variance. A logarithmic, squareroot, or arcsin transformation did not correct the unequal variances of FTI and MAI, and these variables were left untransformed.

Clams collected from lower Holberg Inlet (H1, H2) in 1987 were significantly larger than *A. serricata* from other collections except for those from Granby Bay (Table 2), as indicated by the maximum width of the visceral mass (MWVM).

The tissue structure of *A. serricata* also differed significantly across samples for the digestive tubules (scores using DTI, DCH and FTI), digestive ducts (log-transformed PDD scores) and kidney concretions (log-transformed NVR scores), as indicated in Table 3. The measure of muscle wasting, MAI, was not significantly different between samples.

Table 2: Group means for measures of tissue structural alterations and post-hoc analysis of statistically significant differences between groups. Station codes are as indicated in Table 2, page 23. S: Spring, F: Fall. Bars represent group similarities at $\alpha = 0.05$.

Variable[1]

Station/Season (Fall or Spring)
Mean
(standard deviation)

MWVM

QS/F	H3/S	H5/F	SI/S	H2/S	H4/F	SI/F	H1/S	H3/F	GB/F	H2/F	H1/F
1.33	1.35	1.40	1.42	1.44	1.45	1.47	1.48	1.53	1.72	2.03	2.08
(0.15)	(0.17)	(0.23)	(0.18)	(0.20)	(0.18)	(0.36)	(0.29)	(0.36)	(0.16)	(0.39)	(0.25)

DTI

SI/F	H4/F	SI/S	QS/F	H5/F	H3/F	GB/F	H1/F	H1/S	H2/F	H2/S	H3/S
2.67	2.40	2.10	2.00	1.80	1.50	1.20	1.20	0.90	0.90	0.70	0.25
(1.23)	(1.27)	(0.74)	(1.41)	(0.63)	(0.58)	(0.63)	(0.63)	(0.88)	(0.57)	(0.68)	(0.50)

DCH

H3/S	H2/S	H4/F	SI/F	QS/F	GB/F	H5/F	H1/S	SI/S	H2/F	H3/F	H1/F
30.0	32.9	33.0	34.7	37.0	37.9	40.5	40.8	44.3	46.0	47.0	52.0
(8.41)	(9.39)	(8.37)	(5.22)	(6.38)	(11.0)	(9.77)	(6.18)	(3.97)	(8.74)	(15.7)	(9.02)

FTI

QS/F	SI/F	H4/F	H5/F	SI/S	H1/S	GB/F	H3/S	H2/S	H3/F	H1/F	H2/F
0.91	0.90	0.83	0.77	0.76	0.75	0.72	0.71	0.64	0.61	0.53	0.27
(0.12)	(0.11)	(0.19)	(0.26)	(0.21)	(0.13)	(0.21)	(0.34)	(0.27)	(0.38)	(0.19)	(0.12)

Table 2 (continued):

-ln(PDD)

H2/F	HI/F	H5/F	GB/F	SI/S	QS/F	J13/F	SI/F	II1/S	II3/S	II4/I	II2-S
1.00	1.03	1.43	1.46	1.47	1.70	1.76	1.80	1.80	1.86	1.97	2.11
(0.33)	(0.23)	(0.41)	(0.29)	(0.36)	(0.37)	(0.65)	(0.44)	(0.31)	(0.20)	(0.31)	(0.58)

-ln(NVR+.001)

H3/S	H2/F	HI/F	II3/F	GB/F	SI/F	HI/S	II2/S	II4/F	QS/I	SI/S	II5/I
3.77	3.82	3.85	4.12	4.71	4.74	4.98	5.03	5.10	5.12	5.??	6.15
(1.23)	(1.80)	(1.79)	(1.26)	(1.37)	(1.38)	(1.47)	(1.73)	(1.85)	(0.24)	(1.52)	(0.??)

MAI (ns)

H5/F	H3/F	SI/F	GB/F	H2/S	II1/F	SI/F	II2/F	II1/S	II4/F	II3/S	QS/I
0.52	0.53	0.54	0.56	0.60	0.61	0.62	0.64	0.66	0.66	0.70	0.70
(0.09)	(0.11)	(0.08)	(0.11)	(0.06)	(0.05)	(0.05)	(0.04)	(0.06)	(0.01)	(0.03)	(0.02)

ns: not significantly different

1: Legend- MWVM: maximum width of visceral mass; DTI: digestive tubule index; DCH: height of digestive cells; PDD: proximal digestive duct density; NVR: nephrolith volume ratio; MAI: muscle atrophy index.

Table 3: Quantitative analyses of tissue structural alterations in clams, *A. serricata*, collected from Saanich Inlet, Holberg Inlet, Quatsino Sound, and Granby Bay, British Columbia: one-way ANOVA. N = total number of clams examined.

Variable[1]	N	Box's F $F_{(approx)}$	Prob.	ANOVA F	Prob
MWVM	101	1.57	0.099	8.76	0.000
DTI	101	1.55	0.105	6.03	0.000
DCH	101	1.46	0.138	4.82	0.000
FTI	101	1.82	0.045	6.33	0.000
PDD	100	1.91	0.033	12.76	0.000
ln(PDD)	"	1.31	0.214	10.30	0.000
NVR	94	9.69	0.000	1.96	0.043
ln(NVR+.001)	"	1.43	0.153	2.58	0.007
MAI	100	2.05	0.021**	1.32	0.228

**: Test indicated a statistically significant departure from homogeneity of variance, but variance could not be stabilized by applying a log, square-root or arcsin transformation. The results of the ANOVA should therefore be interpreted with caution.

1: Legend- MWVM: maximum width of visceral mass; DTI: digestive tubule index; DCH: height of digestive cells; PDD: proximal digestive duct density; NVR: nephrolith volume ratio; MAI: muscle atrophy index.

Table 2 also shows the pattern of between-sample differences for each variable indicated by a Tukey-Kramer post-hoc test. In spite of the significance of overall difference between samples for all variables except MAI, the interpretation of significant between-sample differences was complicated by the large number of 'overlapping tails'. However, the ordering of variable means and the pattern of group similarities were instructive in interpretting the overall between-sample difference.

The mean of the digestive tubule index (DTI) was lower in clams collected from Holberg Inlet stations H1, and H2 than in clams from upper Holberg Inlet (H4, H5), Quatsino Sound, and Saanich Inlet. The DTI in clams from station H3, and GB was intermediate between these two groups. For those stations sampled both in the fall and the spring, DTI was consistently higher in the spring.

Digestive cell height increased with decreasing distance from the discharge pipe in reproductively active clams (sampled in the fall). The height of digestive cells is seasonally dependent with reproductively quiescent clams having a greater height of digestive cells than those actively involved in gametogenesis. This pattern was reversed in Saanich Inlet clams, but there was not a significant difference between seasons.

A. serricata collected from lower Holberg Inlet had fewer unfragmented digestive tubules (as indicted by FTI) than those from Saanich Inlet, Quatsino Sound or upper Holberg Inlet. Clams collected in the fall were invariably in poorer condition based on the FTI than their counterparts collected in the spring.

The density of large vesicles in the proximal digestive ducts, as indicated by PDD, was significantly greater in reproductively active (fall-sampled) clams from stations H1 and H2 than in other collections. A spatial component to between-collection differences was not obvious, whereas a seasonal component was.

Nephroliths occupied a greater proportion of the kidney volume in clams from stations H1, H2, and H3 than in clams from stations H4, H5, QS or SI. There also appeared to be a seasonal pattern to NVR, with reproductively active clams having a higher relative volume of nephroliths than reproductively quiescent clams.

Seasonally-dependent alteration of tissue structure appeared to be enhanced by mine tailings discharge for two variables: DCH and PDD. For both variables, fall collections and summer collections from H1 to H3 fell at opposite ends of the spectrum of sample means; the change in means between seasons was of considerably greater magnitude than for clams collected from the reference site.

The incidence of seven different tissue conditions for each collection is listed in Table 4. Overall between-sample differences in incidence were statistically significant for all variables except for the premature breakdown of ova (Table 5).

The incidence of cells within the intestinal lumen (IC) was significantly higher than the overall incidence (calculated from all *A. serricata* combined) in clams from Saanich Inlet sampled in the spring. Cells in the intestine were observed in only a small percentage of clams from lower Holberg Inlet or Granby Bay; this suggests that the etiology of IC was unrelated to environmental disturbance.

The incidence of ctenidial breakdown (CTEN) and oedema of ctenidial lateral cells (OEDEMA) were significantly lower in reproductively quiescent *A. serricata* from Saanich ·..let, and significantly higher in Granby Bay and lower Holberg Inlet (H1) clams which were reproductively active. For both conditions, the incidence in spring collections was lower than in fall collections.

All *A. serricata* collected from station H1 during both seasons exhibited hyperplasia of ctenidial mucocytes (MUCOHYP). All *A. serricata* from H1 also contained flagellates (FLAG) within the ctenidial envelope. However, the incidence of MUCOHYP was also high (90%) in Granby Bay clams, where no flagellates were observed.

The incidence of conspicuous tertiary lysosomes in the stomach epithelium (SEL) was significantly lower in reproductively quiescent Saanich Inlet clams and significantly higher in reproductively quiescent clams from station H1.

Within the lower Holberg stations, trends in the incidence data parallel trends in the spatial arrangements of stations relative to tne mine-tailings discharge, both in spring and fall, for the variables CTEN, OEDEMA, MUCOHYP, and FLAG (i.e. H1 > H2 > H3). The incidence of stomach epithelial lysosomes, SEL, also appears

to be related to the proximity to mine-tailings discharge in spring collections from lower Holberg Inlet. The pattern of spatial trends in upper Holberg stations is unrevealing and may represent error variability, or 'noise'.

Table 4: Relative frequency of occurrence across stations of various tissue conditions in the clam *Axinopsida serricata* collected from Saanich Inlet, Holberg Inlet, Quatsino Sound, and Granby Bay, British Columbia. F: fall sampling; S: spring sampling. N = number of replicates.

Variable[5]					% Freq. of Occurrence by Station							
N =	SI/F 9	SI/S 10	QS/F 4	H5/F 10	H4/F 10	H3/F 4	H2/F 10	H1/F 10	H3/S 4	H2/S 10	H1/S 10	GB/F 10
IC	33	90[4]	0	20	60	25	22	44	33	22	10	20
CTEN	44	0[2]	25	40	70	0	67	80[2]	0	33	40	100[4]
OEDEMA	11	0[1]	0	10	10	0	22	70[3]	0	11	30	90[1]
MUCOHYP	44	30[1]	50	50	40	25	67	100[3]	75	60	100[3]	90
FLAG	67	30	25	40	60	25	56	100[4]	75	90	100[4]	0[1]
SEL	50	10[3]	25	30	80	75	50	44	25	80	90[2]	11
OVA	33	na	67	20	33	67	50	33	~	na	na	75
(no. females)	(3)		(3)	(5)	(3)	(3)	(4)	(6)				(8)

1: indicates stations with a frequency of occurrence of that lesion which is significantly different from the overall frequency for all stations at $p < 0.05$.
2: significantly different at $p < 0.01$.
3: significantly different at $p < 0.005$.
4: significantly different at $p < 0.001$.
5: Variables legend- IC: intestinal cells; CTEN: -ctenidial breakdown; OEDEMA: oedema of ctenidial lateral cells; MUCOHYP: hyperplasia of ctenidial mucocytes; FLAG: ectoparasitic flagellates in ctenidial envelope; SEL: conspicuous tertiary lysosomes in the stomach epithelium; OVA: premature breakdown of oocytes.

Table 5: An analysis of site differences in the incidence of tissue conditions in the clam, *Axinopsida serricata* (employing a log-likelihood estimate of a heterogeneity chi-square. DF = 11).

Variable [1]	Σ G ($\approx$ Σ chi-sq.)	Probability
IC	25.55	$0.01 > p > 0.005$
CTEN	38.21	$p < 0.001$
OEDEMA	46.14	$p < 0.001$
MUCOHYP	34.05	$p < 0.001$
FLAG	53.37	$p < 0.001$
SEL	27.28	$0.01 > p > 0.005$
OVA	6.02	$0.90 > p > 0.75$

1: Variables legend- IC: intestinal cells; CTEN: -ctenidial breakdown; OEDEMA: oedema of ctenidial lateral cells; MUCOHYP: hyperplasia of ctenidial mucocytes; FLAG: ectoparasitic flagellates in ctenidial envelope; SEL: conspicuous tertiary lysosomes in the stomach epithelium; OVA: premature breakdown of oocytes.

Reproductively quiescent *A. serricata* from Saanich Inlet had a significantly lower overall incidence of ctenidial fragmentation, oedema of lateral cells, hyperplasia of mucocytes and tertiary lysosomes in the stomach epithelium.

The effect of size on tissue variability.

The increased size of clams from lower Holberg Inlet stations H1 and H2 could possibly account for the between-site differences in tissue structure. Table 6 shows the Pearson correlation and associated Bonferroni-adjusted probability for clam size and variables for tissue structural alteration.

When *A. serricata* from all sites and both seasons were examined, those variables which were correlated with body size included digestive cell height, fragmented tubule index, proximal digestive duct density, and oedema of ctenidial lateral cells. However, the distance from I.C.M.s tailings discharge pipe, mean size of *A. serricata*, and several histological conditions co-vary within the lower Holberg Inlet stations (H1-H3: Table 2). The data were therefore analyzed as two subsets in which there were no statistically significant between-site differences in body size (clams collected in the fall from stations H1 and H2, and all other clams (see MWVM, Table 2)). Within these two subsets, there were no statistically significant correlations between MWVM and any other variable (Table 6).

Table 6: Evaluation of the correlations between clam size, as measured by the maximum width of the visceral mass, and other tissue variables. Only those correlations which are significantly different from zero, using a Bonferroni-adjusted probability, are listed. The total number of *A. serricata* in each group is indicated in brackets.

Variable	Correlations with clam size (MWVM)		
	Group or subset of clams analyzed		
	All (101)	H1/F, H2/F (20)	Others (81)
DTI	n.s.	n.s.	n.s.
DCH	0.38	"	"
FTI	-0.40	"	"
PDD	0.44	"	"
NVR	0.40	"	"
MAI	n.s.	"	"
IC	"	"	"
CTEN	"	"	"
MUCOHYP	"	"	"
OEDEMA	0.37	"	"
FLAG	n.s.	"	"
SEL	"	"	"
OVA	"	"	"

n.s.: not significantly different from zero.

The effect of sex on tissue variability.

Male and female *A. serricata* might invest different amounts of energy in gametogenesis. Since this would affect the amount of energy available for maintenance and repair processes, possible sex-related differences in tissue responses were investigated. Out of 67 *A. serricata* sampled from Saanich Inlet, Holberg Inlet, Quatsino Sound and Granby Bay during the fall, 30 were identified as males, 35 as females, and 2 as indifferent (unsexable). An independent sample t-test was performed on all tissue variables (except OVA) to compare means for males and females. No statistically-significant sex-related differences were observed (for each of 12 variables, $0.21 < p < 0.94$). Furthermore, no sex-related differences were observed if reproductively-active (fall) and reproductively-quiescent (spring) samples were combined (for each of 12 variables, $0.21 < p < 0.99$).

Tissue effects related to the incidence of parasitism.

Chapter 2 (page 42-47) summarizes those conditions which may have an infectious etiology. The incidence of most of these conditions was too low to examine possible secondary effects in other tissues. However, there was a high overall incidence of ectoparasitic flagellates in the ctenidial pouch (58 of 10? specimens). The incidence of flagellate ectoparasites in the ctenidia was not correlated with any other tissue variable based on Bonferroni-adjusted probabilities (Table 7).

The incidence of flagellate ectoparasites was marginally correlated with the digestive tubule index and mucocyte hyperactivity (based on non-adjusted probabilities). However, these correlations may have been unduly influenced by a 100% incidence of infection at station H1, closest to the discharge; the low DTI and high incidence of mucocyte hyperactivity may have been related primarily to mine-tailings exposure and only secondarily to infection by the flagellate. If clams from near the outfall (station H1) are excluded from the analysis, the correlation between incidence of flagellates and hyperactivity of mucocytes is not significantly different from zero (n = 80, r = 0.08, P(unadjusted) = 0.49). Similarly, the correlation between incidence of flagellates and digestive tubule index is not significantly different from zero when station H1 is removed from the analysis (n = 80, r = -0.21, p(unadjusted) = 0.06).

Co-variation between different tissue structural alterations.

Different tissue effects should be correlated if they respond to the same stressor. The correlations between different tissue variables is provided in Table 7. For those conditions considered to be pathological (Chapter 2, page 61), different measures of deleterious change in digestive tubules (DTI, DCH, FTI) were partially intercorrelated; FTI was significantly correlated with DCH and DTI, but DCH and DTI were not correlated. In addition, ctenidial degenerative changes (CTEN, OEDEMA, MUCOHYP) were partially intercorrelated. Deleterious changes in the digestive tubules were only marginally correlated with some of those in the ctenidia.

Table 7: Matrix of raw Pearson correlations and associated probabilities for tissue structural alterations in *A. serricata* collected from Saanich Inlet, Holberg Inlet, Quatsino Sound and Granby Bay, British Columbia. (Number of clams < = 101). Only those correlations which are significantly greater than zero are shown. Those correlations with a Bonferroni-adjusted probability < 0.05 are indicated in bold with asterisks. Other values are for correlations with non-adjusted probabilities < 0.05.

	DTI	DCH	FTI	PDD	NVR	MAI	ID	CTEN	OEDEMA	MUCOHYP	FLAG	SEL
DTI	1.00											
DCH	-	1.00										
FTI	0.44*	-0.51*	1.00									
PDD	-	0.54*	-0.49*	1.00								
NVR	-	-	-0.29*	-	1.00							
MAI	-	-	-	-	-	1.00						
ID	-	-	-	-	-	-	1.00					
CTEN	-	-	-	0.25	-	-	-	1.00				
OEDEMA	-	0.28	-	0.37*	-	-	-	0.59*	1.00			
MUCOHYP	-0.24	-	-	-	-	-	-	-	0.42*	1.00		
FLAG	-0.25	-	-	-	-	-	-	-	-	0.24	1.00	
SEL	-	-0.39*	-	-0.27	-	-	-	-	-	-	0.28	1.00

The muscle atrophy index and incidence of intestinal cells were not correlated with any other variables.

Those variables thought to be sensitive to metal uptake (Chapter 2, page 59, 60), including nephrolith volume ratio and incidence of tertiary lysosomes in the stomach, were not correlated. However, N.V.R was correlated with the percentage of fragmented tubules. S.E.L. was inversely correlated with digestive cell height; this might have been a secondary effect of the strong seasonal response by both variables.

A multivariate analysis of tissue structure in A. serricata.

A principal component analysis (PCA) was applied to the six variables scored as other than presence or absence to (a) assess the interrelationship between tissue variation, and (b) assess the interrelationship between clams sampled from different sites and during different seasons.

The associated eigenvalues and amount of variability accounted for in the unrotated and rotated solution are provided in Table 8.

The interrelationship between tissue variables is illustrated by plots of the loading of individual variables on principal components (Figure 16). Digestive cell height (DCH) and proximal digestive duct density (PDD) were very closely related. The digestive tubule index (DTI) and fragmentation tubule index (FTI) were slightly less closely related. The muscle atrophy index (MAI) and nephracyte volume ratio (NVR) were not closely related to each other or to the other variables. Based on the prior univariate analyses, it would appear that MAI is unrelated to site, size, season, or sex and the variability is considered to be primarily 'noise'. The volume density of nephroliths in the kidney is not closely related to other quantitative measure of putative lesions and probably does not represent a response to stress per e. However, based on the one-way ANOVA, between-site differences suggest that NVR is partially controlled by environmental influences.

Table 8: Summary of principal components analysis of quantitative descriptors of tissue structure in clams, *A. serricata*, inhabiting natural sediment, and deposited copper-mine tailings or slag.

	Factor 1	Factor 2	Factor 3
A) Percent of total variance explained:			
Unrotated	36.3	19.3	17.9
Varimax-rotation	29.6	24.1	19.7
B) Eigenvalues:			
Factor 1		2.176	
Factor 2		1.156	
Factor 3		1.073	
Factor 4		0.769	
Factor 5		0.448	
Factor 6		0.378	

The multivariate relationship between clams collected from different sites in different seasons, based on tissue structure, is illustrated in Figures 17 and 18. Clams sampled when they were reproductively active (closed symbols) or reproductively quiescent (open symbols) plotted out at opposite ends of the first principal component. However, the polarity of reference site clams (SI) collected in fall or spring along the first principal component is opposite to that of clams from Lower Holberg Inlet.

Clams from lower Holberg Inlet, particularly those from stations H1 and H2, tended to plot out separately from clams sampled from upper Holberg Inlet, Quatsino Sound, or Saanich Inlet. *A. serricata* collected near Anyox, Granby Bay can be seen aggregated within the entire group of clams.

Figure 16 (A, B): The multivariate relationship between measures of tissue structural variation in *A. serricata*: Pairwise plots of loadings of six variables on the first three principal axes. The volume density of the proximal digestive ducts (PDD) and digestive cell height (DCH) are closely related.. The digestive tubule index (DTI) and fragmented tubule index (FTI) are also related. The muscle atrophy index (MAI) was interpreted to represent primarily error variability, while the nephrolith volume ratio (NVR) was possibly related to metal exposure of the clam rather than non-specific stress.
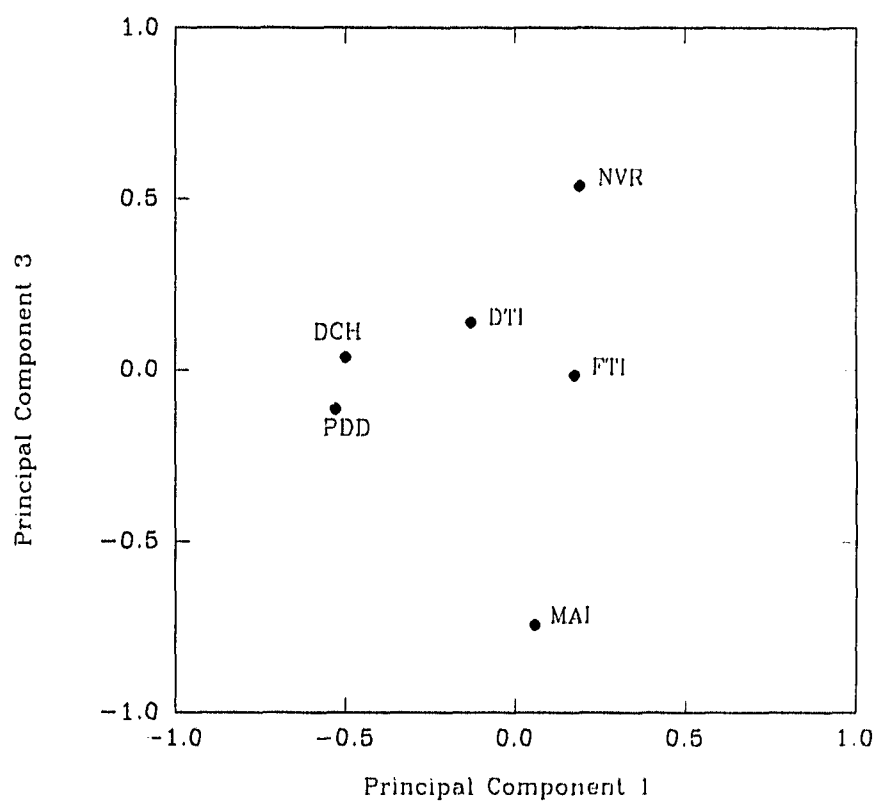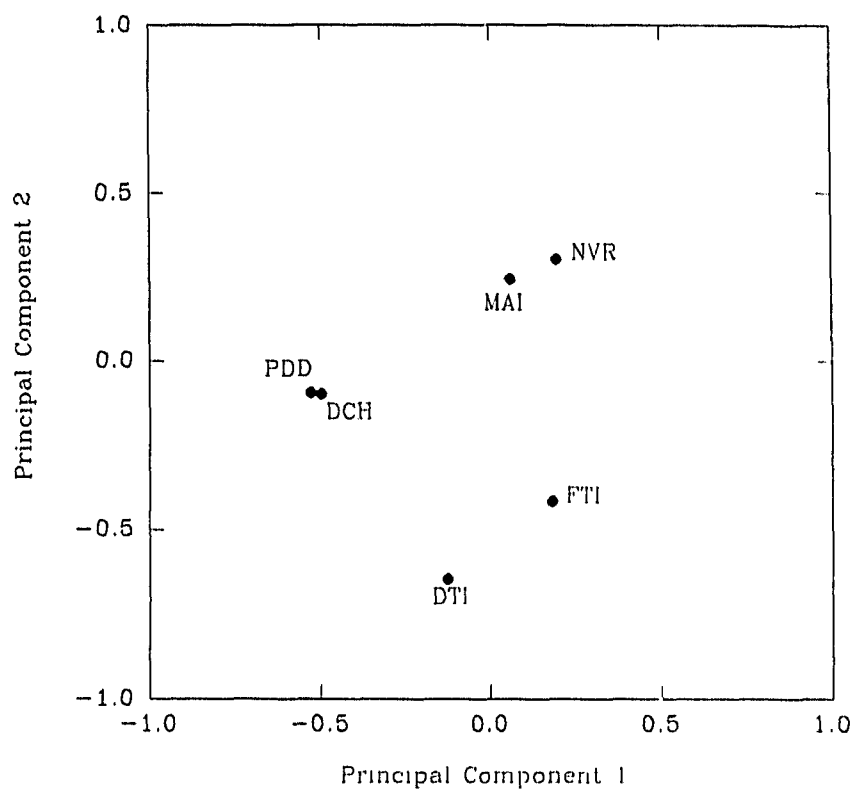
Individual clams were positioned along the first principal component both according to season and relative exposure to disturbance (Figure 17, 18). The spatial arrangement of *A. serricata* from Holberg Inlet along the second principal component partially parallels the spatial arrangement of sampling stations relative to the mine-tailings discharge. A pattern of distribution of clams along the third principal axis could not be discerned, even though this component accounted for 19.7 % of the total variance (Table 8).

The correlations between principal component loadings and distance of the clam from I.C.M.s tailings discharge pipe, clam size (MWVM), sex (male or female) and stage of reproductive cycle (SRC), are shown in Table 9. The first principal component is correlated with clam size, and marginally correlated with the stage of reproductive cycle. The second principal component is correlated with distance of the station from the mine-tailings discharge and clam size.

As indicated above, clam size, distance from the discharge pipe and some of the individual tissue variables co-vary. However, the correlation between distance from the discharge pipe and the first principal component was not significantly greater than zero. Therefore, one component of overall tissue variability appears to be influenced by the size, and age, of clams, independent of spatial effects.

Table 9: The relationship between multivariate measures of tissue structural variability and distance from the tailings discharge pipe, clam size, reproductive activity, and sex: Pearson correlations (N <= 101). Only those correlations which are statistically significant are shown.

Variable	Principal Component		
	1	2	3
Distance from discharge.[2]		-.52	
MWVM	-0.41	0.32	0.23[1]
SRC	-0.25[1]		
Sex			

1: Significant at $\alpha = 0.05$ if type 1 error is not adjusted for multiple comparisons.

2: Shortest distance along the trough of Holberg Inlet and Quatsino Sound stations. Both Saanich Inlet and Granby Bay clams were omitted in calculation of the correlation.

Figure 17: Multivariate relationship between *A. serricata* sampled from different sites and in different seasons based on tissue structure. Clams from the reference station are delineated by a dashed line for those collected while reproductively active (I) and reproductively quiescent (II). Clams sampled during the fall are indicated by solid symbols, and those sampled during spring are indicated by open symbols.

Clams collected from nearer to the mine-tailings discharge (stations H1 and H2) during fall are aggregated in the upper left quadrat of the plot. Clams collected from nearer to the mine-tailings discharge during the spring are grouped together in the upper right quadrat. *A. serricata* from Granby Bay are grouped together in an intermediate position between reference station and lower Holberg Inlet clams.

LEGEND: SI: * H1: ▲
QS: ♦ H2: ▼
GB: ● H3: ■
H4: ◆
H5: +
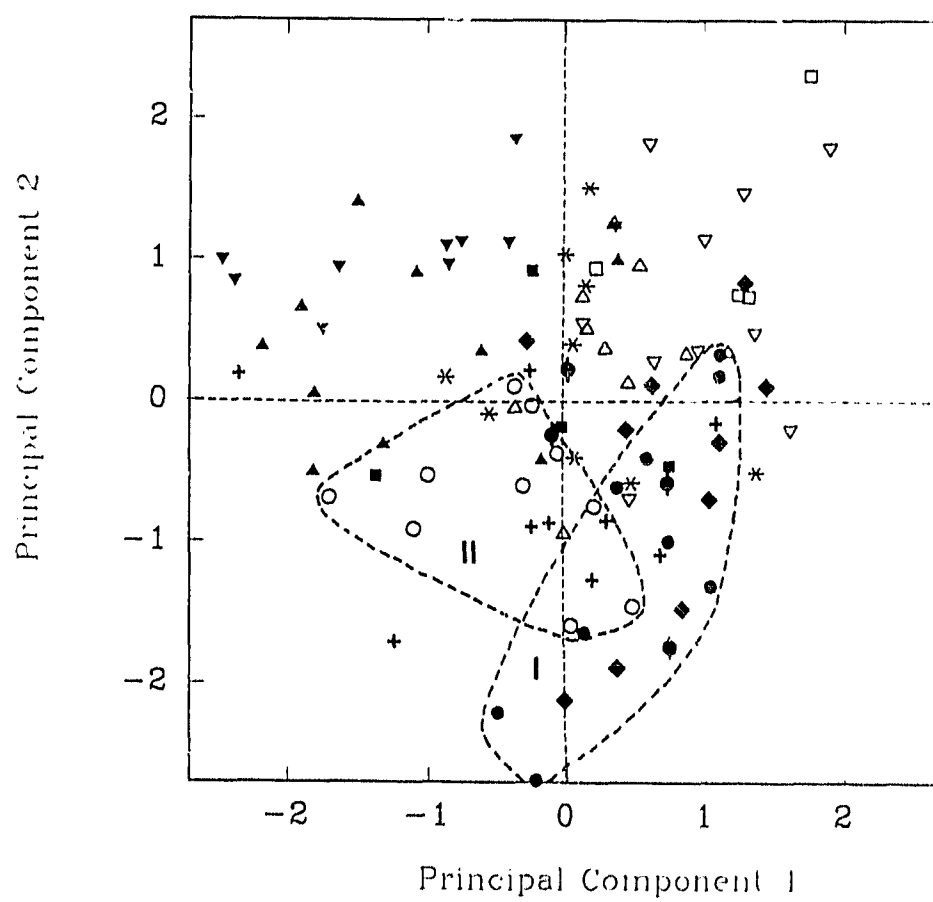
Figure 18: P.C.A. plot of the relationship, based on tissue structure, between *A. serricata* collected from different sites and in different seasons. The site and season means of principal component scores have been plotted rather than individual clam scores as in Figure 17 to illustrate overall between-site, between-season trends.
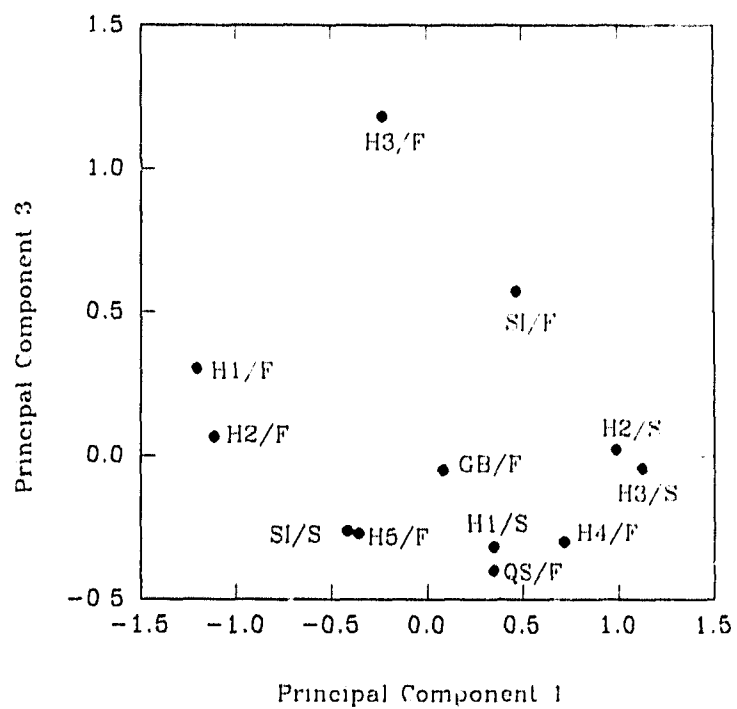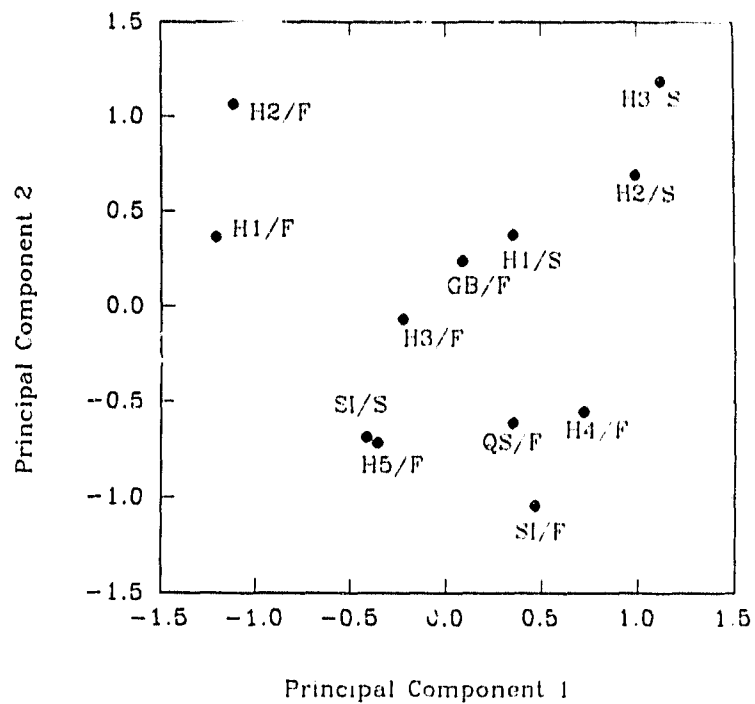
Discussion

Axinopsida serricata living within deposited mine-tailings exhibit variation in tissue structure which is distinct from that of clams living within less disturbed sites. The pattern of between-site differences as a whole reflects differences in exposure of clams to mine-tailings. Based on both univariate and multivariate analyses of tissue structure, two or three groups of clams can be distinguished: (1) clams from lower Holberg Inlet (stations H1, H2 and possibly H3) living in rapidly accumulating tailings deposits; (2) clams from Mill Bay, Saanich Inlet and clams from outer Quatsino Sound and upper Holberg Inlet (SI, QS, H4, H5); and (3) clams from Granby Bay.

Granby Bay was originally chosen as a potential worst-case of exposure to metals within marine sediments. *A. serricata* collected from Granby Bay contained degenerative tissue conditions which were more extreme than in clams from Saanich Inlet; however, Granby Bay clams were in better overall condition than those collected from lower Holberg Inlet. Degenerative changes in some tissues were most pronounced in lower Holberg Inlet (e.g. changes in the digestive diverticula and hyperplasia of mucocytes); for other degenerative changes (e.g. ctenidial fragmentation and lateral cell oedema), Granby Bay clams were most severely affected. Specific histopathological effects at these two sites are probably mediated by differences in the nature of the stressors.

Lower Holberg Inlet station H1 had a sediment particle size distribution and organic content which reflects substantial mine-tailings deposition. Stations in Holberg Inlet which were further from the discharge pipe (H2-H3) had sediment characteristics which were more similar to the reference site (SI). Since profound changes in the sediment characteristics were noted only in station H1, it might be predicted that deleterious changes in the tissue structure of *A. serricata* would be limited to this station only.

The incidence data (ctenidial disruption, oedema of lateral cells, hyperactivity of mucocytes, and stomach epithelium tertiary lysosomes) provide evidence that clams from station H1 were significantly different in their tissue structure from clams collected further up Holberg Inlet. However, non-dichotomous variables (digestive tubule index, digestive cell height, fragmented tubule index, and

nephrolith volume ratio) provide evidence that deleterious tissue effects extend beyond station H1 to stations H2 and H3, which are further removed from the discharge pipe. In addition, the simultaneous examination of tissue structure (Figure 18) shows that *A. serricata* from station H2 are, in fact, more dissimilar to the reference station clams than *A. serricata* from station H1. A better understanding of the etiology of tissue structural changes might provide an understanding of the observed spatial pattern of tissue variability.

The tissue structure of *A. serricata* is strongly influenced by season. The effects of exposure to mine-tailings and seasonally-dependent reproductive activity on tissue structure were additive for some tissue conditions (digestive tubule index, nephrolith volume ratio, ctenidial disruption, oedema of ctenidial lateral cells). Although degenerative changes were more severe in reproductively active clams, a coherent spatial pattern could be discerned in either season. However, for digestive cell height and proximal digestive duct density, the effect of tailings-exposure and reproductive activity were greater than their additive effects. Those clams living closest to the mine-tailings discharge exhibited very high between-season variation compared to their counterparts from relatively unaffected areas.

Clams collected during the fall were expected to be in worse condition than those from the same sites collected in the spring because of the additional stress imposed by expenditure of energy for gametogenesis and massive release of gametes during spawning. These expectations were born out by the results.

The sex of *A. serricata* or infection by a flagellate did not directly influence tissue structural variability in this study. Correlations between size (MWVM) and some histopathological effects were significantly greater than zero, but this was a secondary consequence a larger average size at stations H1 and H2 closest to the discharge pipe. If clam size is causally related to tissue deterioration, then correlations between the two should be significant both across and within sites. The absence of any significant correlation in two subsets of the data suggests that clam size does not influence tissue variation in a linear fashion.

Increase in body size in molluscs is associated with increased age, although it may also reflect other physiological conditions such as nutrition and stress. Age could influence tissue structure by prolonging the time of exposure to a chronic

stressor. Older, potentially senescent clams may be more susceptible to stress-related changes. Kirkwood (1987) proposed that the evolutionary optimiza.ion of life-history traits involves a trade-off between allocation of energy for reproduction and allocation of energy for somatic repair and maintenance. Progression of senescence should be inversely related to allocation of energy for maintenance repair of cells and tissues. Maynard Smith (1962) defined senescence as the sum of effects which, with increasing age, increase susceptibility to other factors which may cause death. Since *A. serricata* is a small, relatively short-lived iteroparous clam which has been described as r-selected (Reid and Brand, 1986), it might be expected that energy allocation for maintenance repair would be limited. A reasonable prediction for this clam would be that metabolic/detoxification waste products and structural damage would accumulate with age as is typical with r-selected species. However, no clear effect of size on tissue structure was observed.

The flagellates described in this study did not appear to cause additional tissue deterioration. However, the incidence of infection increased closer to I.C.M.s mine-tailings discharge. Moller (1990) found that the incidence of infectious disease in flounders, *Platichthys flesus*, from the Elbe River, in Germany, was related to undernourishment due to a depauperate zoobenthic food source. At Holberg Inlet station H1, dilution of sediment organic matter by rapidly accumulating mine-tailings may limit food availability for *A. serricata*. However, there was no obvious localized effect on oogenesis, which might also be expected if the clams were undernourished. It is possible that tailings-induced stress in *A. serricata* affects the ability of the clams to clear the ctenidial envelope and mantle cavity, possibly through impairment of ciliary beating or some other defense mechanism.

Changes in tissue structure which co-vary may reflect a similar underlying etiology. The digestive tubule index, digestive cell height, fragmentation tubule index, disruption of ctenidial filaments, and hyperactivity of ctenidial mucocytes all appear to represent tissue-level changes which are stress responses, i.e. are specific responses to a non-specific disturbance.

Changes in digestive tubule morphology and copious mucus production within the mollusca have been recognized as stress responses by Fantin *et al.* (1982), Lowe *et al.* (1981), Bayne (1980) and Jeffries (1972). An increase in the volume and

basiphilic staining intensity of ctenidial mucocytes of the bivalve *Venus verrucosa* was observed by Axiak *et al.* (1988) on exposure to petroleum hydrocarbons.

Oedema of ctenidial lateral cells may represent a contaminant-specific tissue response to elevated copper or zinc concentrations in sediment interstitial water. Sunila (1986a) found in a laboratory study of the long-term effects of copper or cadmium on the gill of the mussel, *Mytilus edulis*, that short-term, relatively low doses of copper caused severe oedema of ctenidial lateral cells while cadmium caused dilation of the branchial vessel and no oedema. In a separate study, Hietanen *et al.* (1988) found that acute exposure to zinc also resulted in oedema of lateral cells, dilation of the branchial vessel, and inflammation by haemocytes. Engel and Fowler (1979) noted that exposure to cadmium in the oyster *Crassostrea virginica* did not result in oedema of ctenidial cells as indicated by swelling and measures on the hydration state whereas exposure to copper did.

The volume of extracellular nephroliths in the kidney and the abundance of tertiary lysosomes in stomach epithelial cells may be responses to metal uptake and processing. NVR was uncorrelated with the other stress responses with the exception of digestive tubule fragmentation. Seiler and Morse (1988) found that the kidney cells of soft-shell clams, *Mya arenaria,* collected from polluted sediments had qualitatively higher numbers of intracellular granules. Rheinberger *et al.*. (1979) also observed a relationship between the size and abundance of kidney granules and degree of pollution.

Sunila (1987) observed a consistent atrophy of muscle bundles in *Mytilus edulis* collected from a severely polluted site and attributed this to starvation. Between-station, between-season differences in muscle atrophy in *A. serricata* were not statistically significant; MAI did not parallel a spatial pattern of tailings influence or seasonal pattern of reproductive stress. Therefore, variation in the volume density of muscle bundles in the foot retractor muscle of *A. serricata* in Holberg Inlet or Granby Bay is not interpretted to be pathological. The incidence of hyalinocyte-like cells in the posterior intestine (IC) was not elevated in lower Holberg Inlet, and the interpretation of these cells as haemocytes undergoing diapedesis merits a re-examination.

4. Changes in population attributes associated with changes in tissue structure: I. A baseline study of the ecology of A. serricata in Mill Bay, Saanich Inlet.

Methods

Investigation of life-history traits.

A. serricata were collected from Mill Bay, Saanich Inlet, (see page 15 in Chapter 1) at intervals of approximately one month from October, 1987, to October, 1989. Three replicates were obtained using a 0.1 m^2 Van Veen grab deployed from the M.S.S.V. John Strickland and screened through a 0.5 mm mesh. The material retained by the screen was immediately fixed in 10% Ca_2CO_3-buffered formalin.

All *A. serricata* were separated from the screened samples at a later date and the numbers counted as an abundance measure. For each replicate, the shell lengths of all *A. serricata* were measured to the nearest 0.05 mm. using an ocular micrometer in a dissecting scope. To assess life-history characteristics, a size frequency distribution of shell length was constructed from three pooled replicates, employing a shell length interval of 0.2 mm.

Various population attributes can be deduced from a size frequency distribution provided that individual cohorts, or age classes, can be distinguished. In this study, graphical methods as outlined by Cerrato (1980) were used to distinguish age classes. When a cumulative frequency distribution is plotted as a percentage of the total on probability paper, a variable which approximates a normal distribution will plot as a straight line. For those populations that have more than one distinguishable age class (i.e., non-continuous recruitment and a maximum life span which encompasses several generations), each age class approximates a normal distribution and the entire population will consist of a series of overlapping normal distributions. Each mode (age class) can be distinguished as the portion of the entire cumulative frequency distribution which falls between inflection points. Once individual modes have been identified, other population attributes such as recruitment, growth, survivorship and longevity can often be deduced. The possibility of and circumstances leading to inappropriate conclusions based on this method are discussed by Grant (1989).

The shell of *A. serricata* is small and extremely fragile and the external hinge is very weak. Therefore, within the pool of dead clams, hinged shells and intact unhinged shells are taken to represent recent mortalities. The death assemblage was examined in samples from December, 1987, June, 1988, and October, 1988. A size frequency distribution of the death assemblage was constructed based on shell length.

In November, 1987, ten additional *A. serricata* were collected for histological examination of mature oocytes. Preliminary observations of the embedded and sectioned specimens showed that male and female *A. serricata* were reproductively ripe in October and November (see page 115). The clams were fixed in Lillie's phosphate-buffered 10% formaldehyde, embedded in paraffin, sectioned at 4 to 6 μm, and stained with Harris' haematoxylin and eosin.

Oocyte size and appearance were compared to those of three other broadcast-spawning bivalves: the Lucinid *Parvilucina tenuisculpta*, collected from near Crofton, B.C.; the Tellinid *Macoma carlottensis* collected from Howe Sound, B.C. (Bright and Ellis, 1989); and the Mytilid *Mytilus trossulus* (=*edulis*)[2] collected from Howe Sound. All of the clams were fixed in 10% buffered-formalin and embedded in paraffin. For *A. serricata*, a mean and maximum diameter for mature oocytes was obtained by measuring 20 oocytes in each of three females, using the diameters across sections of oocytes which also contained a large area of germinal vesicle. Since oocytes are not perfectly spherical, the diameter was estimated as the average of two distances across the oocyte which were perpendicular to each other.

Animal-sediment relations.

The behavioural interaction of *A. serricata* with the surrounding substrate might be both an important influence on population attributes and a mediator of disturbance-induced histological effects.

---

2. McDonald and Koehn (1988) provided electrophoretic evidence from eight enzyme loci that mussels from the Pacific coast of North America were distinct from *Mytilus edulis* in the Atlantic. North of California, *edulis*-like mussels are similar electrophoretically to mussels from the Baltic Sea and parts of eastern Canada. McDonald and Koehn proposed the name *M. trossulus* Gould, 1850, based on priority.

The burrowing behaviour of *A. serricata* was observed by placing clams (which had been collected at 90 m depth from Mill Bay, Saanich Inlet 48 h earlier) in a viewing chamber. The chamber consisted of two sheets of plate-glass separated by 7 mm thick spacers between which sediment from Mill Bay, Saanich Inlet was sandwiched. The entire chamber was placed in a 10° C. running seawater system and allowed to equilibrate for 48 h. before introduction of the clams.

Results

Thorough sorting and identification of other macrofauna present within the samples was not attempted; however, an informal list of species co-dominant with *Axinopsida* in Mill Bay includes *Acila castrensis* and *Nucula tenuis* (Bivalvia), *Chaetoderma argenteum* (Aplacophora), *Ophelina accuminata, Cossura longicirrata, Nephtys* sp. and *Pectinaria* sp,(Polychaeta), *Brisaster latifrons* (Echinoidea), and unidentified foraminiferans.

Animal-sediment relations.

Allen (1958) described the burrowing behaviour of *Thyasira flexuosa*. After burrowing into the sediment, *T. flexuosa* excavates an inhalant channel from the subsurface clam burrow to the sediment surface using its long vermiform foot. The walls of the inhalant channel are consolidated by cementing together sediment particles with mucus produced by the foot. Other authors have assumed a similar burrowing behaviour in other Lucinaceans.

However, the burrowing behaviour of *A. serricata* departs markedly from this description. A typical burrow for *A. serricata* is shown in Figure 19a. A central chamber, here filled with faecal pellets (f), was established at approximately 2 cm depth. *A. serricata* uses its foot to excavate a large number of channels outward from the central chamber. Some of the channels are over 3 cm. in length, which in comparison to a maximum shell length of less than 0.6 cm. for *A. serricata* (personal observation) is quite long. The extensibility of the foot is undoubtedly related to an extensive haemocoel. Clams do not obviously excavate channels toward the surface. If anything, there may be a tendency to direct excavations downward into relatively more anoxic sediment (Figure 19 a-c). Also, the excavated channels do not appear to be consolidated by a cementing of the sediment on the channel walls. However,

Figure 19: Laboratory observations of burrowing behaviour by the clam, *A. serricata*, and associated changes in the sediment redox potential discontinuity.

(A) Photograph showing an irregularly shaped burrow containing faecal pellets (f).

(B) Radiating network of foot excavations from the central burrow (see also (A) above). There appears to be a net downward bias in direction of excavation.

(C) Bioturbation has, over a three week period, resulted in a sediment vertical profile within the burrowing chamber of approximately 3 cm. of light-coloured, well oxygenated sediment over top of darker, oxygen-depleted sediment. Arrow points to redox potential discontinuity. Double arrow points to deep excavations (approximatley 5 cm depth), which extend into the oxygen-depleted zone.
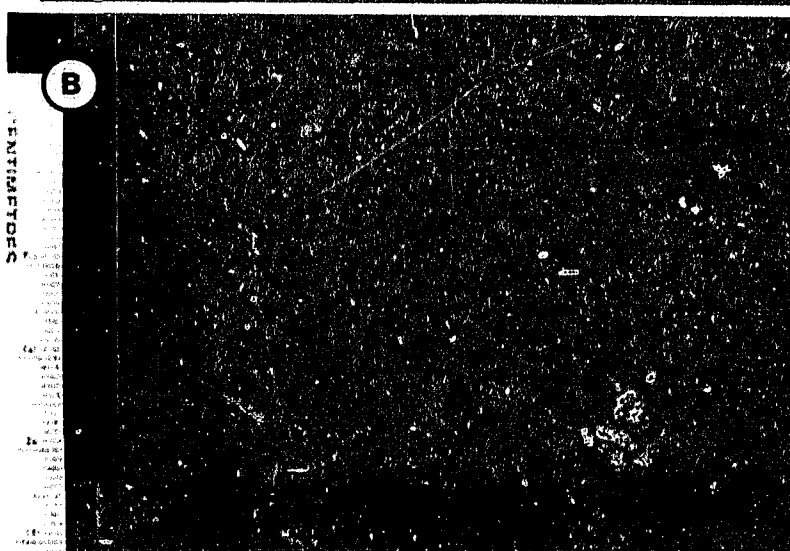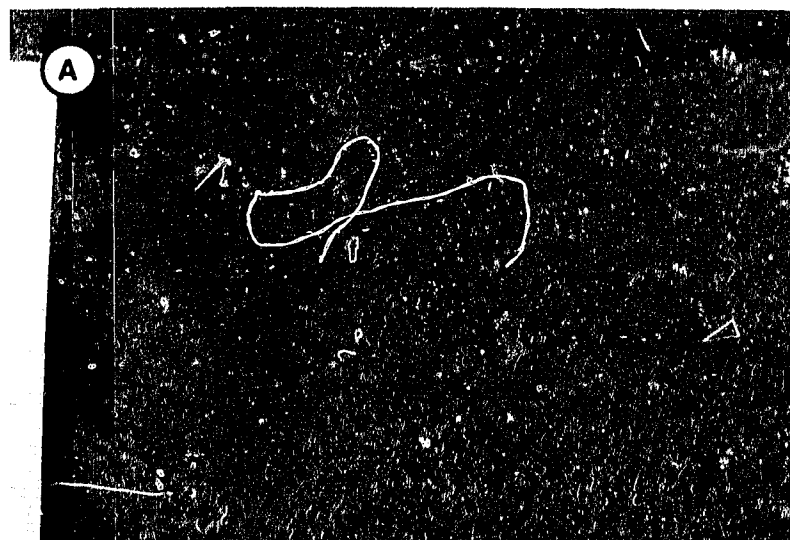
the extensive presence of mucus glands near the tip of the foot suggests that some consolidation may have occurred, albeit at a level too fine to discern macroscopically.

The net result of burrowing activity by several *A. serricata* was formation of an anastomosing network of fine channels throughout the upper 3 cm. of sediment. A lower density of excavations was observed below 3 cm. depth. These excavations had a primarily vertical orientation. The subsequent oxygenation of near-surface sediments induced by bioturbation can be seen in Figure 19c. Approximately 3 cm. of lightly-coloured, oxygenated sediments were seen to overlie darker, more anoxic sediments.

Temporal patterns of abundance.

The temporal trend from 1987 to 1989 in the mean density of *Axinopsida serricata* in Saanich Inlet is illustrated in Figure 20. The mean density varied from approximately 450 to 1500 clams/m^2. A long-term trend in clam density was not observed over the two year study period. At each sampling, *A. serricata* density varied widely between replicates indicating a patchy distribution. An annual minimum mean density was observed in July of each year. This may reflect the timing of movement into the population, with substantial recruitment to the substrate commencing in July.

Growth and maximum longevity.

Figure 21 shows a typical frequency distribution of the shell length of *A. serricata*, based on a single sampling. The youngest cohort was easily distinguished, but subsequent modes were less discernible. The cumulative frequency distribution, when plotted on probability paper, had four inflection points suggesting a total of five discernible size classes. The extracted modes, when replotted (dashed lines) had an approximately normal distribution as indicated by the linearity. The modal mean was taken as the point at which each modal line intersected the 50% point. An estimate of the standard deviation of shell length for each mode was derived from modal size range spanned by the mode between the 15.87% and 84.13% probability points (see Cerrato, 1980).

Figure 20: Temporal variation of the mean density and standard deviation (vertical bars) of *A. serricata* in Mill Bay, Saanich Inlet from November, 1987, to August, 1989. Density estimates were based on three replicates at each sampling date.

Clam abundance / 3 replicates
(0.3 sq. m.)
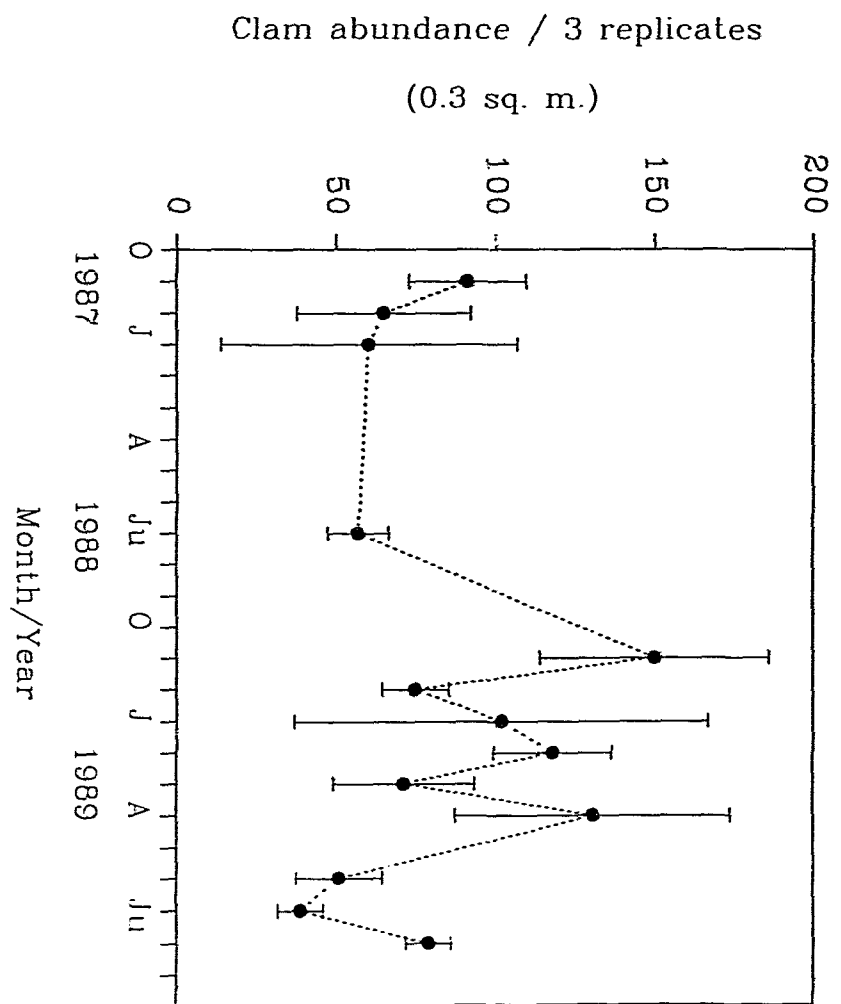
Figure 21: A typical frequency distribution of shell length of *A. serricata* from Mill Bay, Saanich Inlet (October, 1988). Individual cohorts can be separated based on inflection points of a cumulative percent frequency curve. The mean shell lengths for five size classes are indicated by arrows.

A. serricata Mill Bay. Oct. 1988

Cumulative Frequency (%)

Number

Shell Length (mm)

At least five size classes could generally be distinguished for most sampling dates (Figure 21). In approximately 20 percent of the clams collected from Mill Bay in October, 1988, growth rings could be distinguished on the shells. The largest collected clams collected had up to four rings, each ring likely corresponding to a season of very slow growth. A size frequency distribution of shell length at each of the rings as well as at the outer shell periphery (Figure 22) was also multimodal. Although the distinction between modes was less clear than for total shell length, modes for growth rings occurred at approximately 3, 4, and 5 mm. corresponding to the modal means for the 2+, 3+, and 4+ year classes of the histogram illustrated in Figure 21. There was no clear delineation between modes from approximately 2 to 3 mm. ring length. However, there was also considerably greater uncertainty associated with the identification of growth rings nearer the umbo: in *Axinopsida*, erosion of the shell near the umbo usually occurs.

A typical growth curve derived from the size-frequency data of *A. serricata* sampled in October, 1988, is shown in Figure 23. Histological observations (Bright, unpublished) indicated that spawning of gametes commenced in November; at this time of year, a minority of the follicles in a small proportion of the individuals show partial spawning. The shape of the growth curve for the shell of *A. serricata* is typical of the bivalvia (Cerrato, 1980). The maximum life-span of *A. serricata* in Mill Bay is estimated to be 5+ years.

The growth curve was constructed based on an assumption that individual modes of the size frequency distributions and rings on the shell correspond to annual cohorts and annual growth increments respectively. Evidence for this is provided by plotting the mean shell length and associated standard deviation for each cohort over time (Figure 24). The increase in shell length over a twelve month period is similar in magnitude to the estimates of the difference in shell length from one modal mean to the next in size-frequency distributions. The modal mean for each cohort in, for example, July of 1989 was not substantially different from the modal mean of the adjacent older cohort sampled one year earlier (July, 1988) as indicated by the overlap in standard deviation.

Figure 22: The frequency distribution of the shell length at discernible bands (or growth rings) on the shells of *A. serricata* from Mill Bay (collected during October, 1988). Frequencies of smaller (< 3 mm.) bands are under-represented due to the tendency of *A. serricata* shells to erode in the umbonal region.
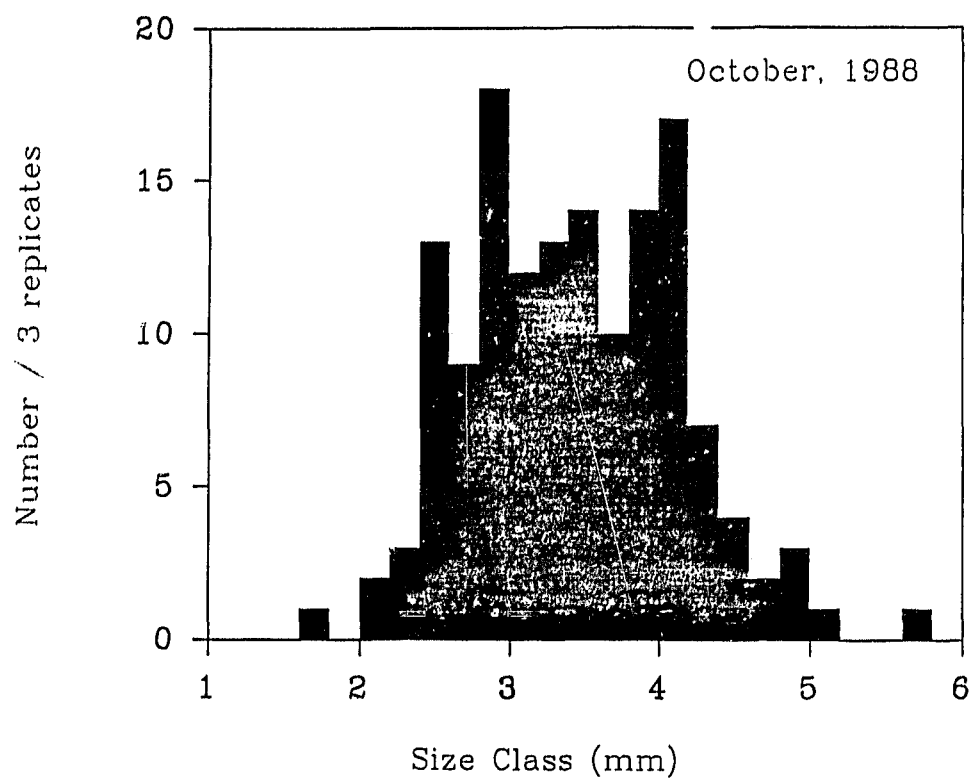
Figure 23: A typical growth curve for Mill Bay *A. serricata* reconstructed from the frequency distribution of shell length in samples collected in June, 1988. The time of fertilization of newly released gametes was taken as November based on histological observations. The vertical bars represent the standard deviation of each mode estimated by graphical methods (Cerrato, 1980).

Mill Bay, Saanich Inlet
June, 1988
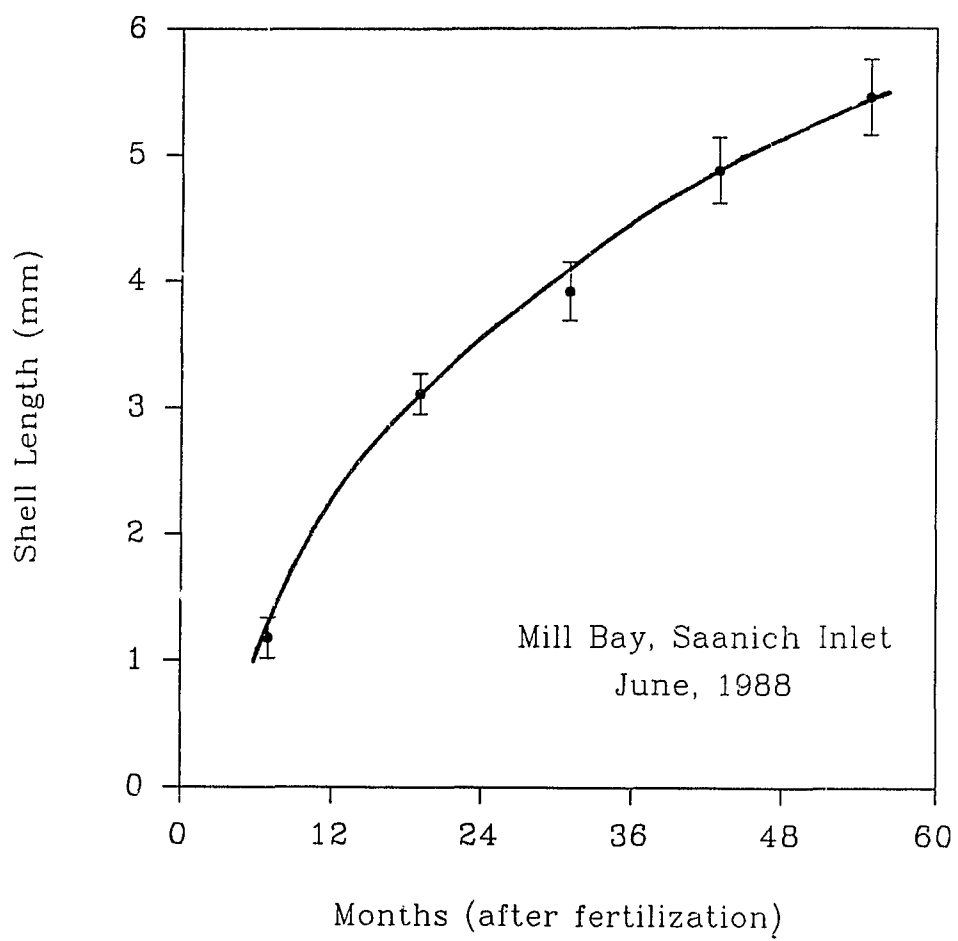
Figure 24: Seasonal growth of cohorts of *A. serricata* collected from Mill Bay, Saanich Inlet. Individual cohorts were distinguished by decomposing shell length frequency distributions at each date using graphical methods (Cerrato, 1980). Different cohorts are identified using different symbols. The standard deviation about the mean shell length is indicated by vertical bars. The curves have been hand-fitted. The interpolations from January to August, 1988, are indicated by a dotted line to indicate a greater uncertainty in hand-fitting the curves.
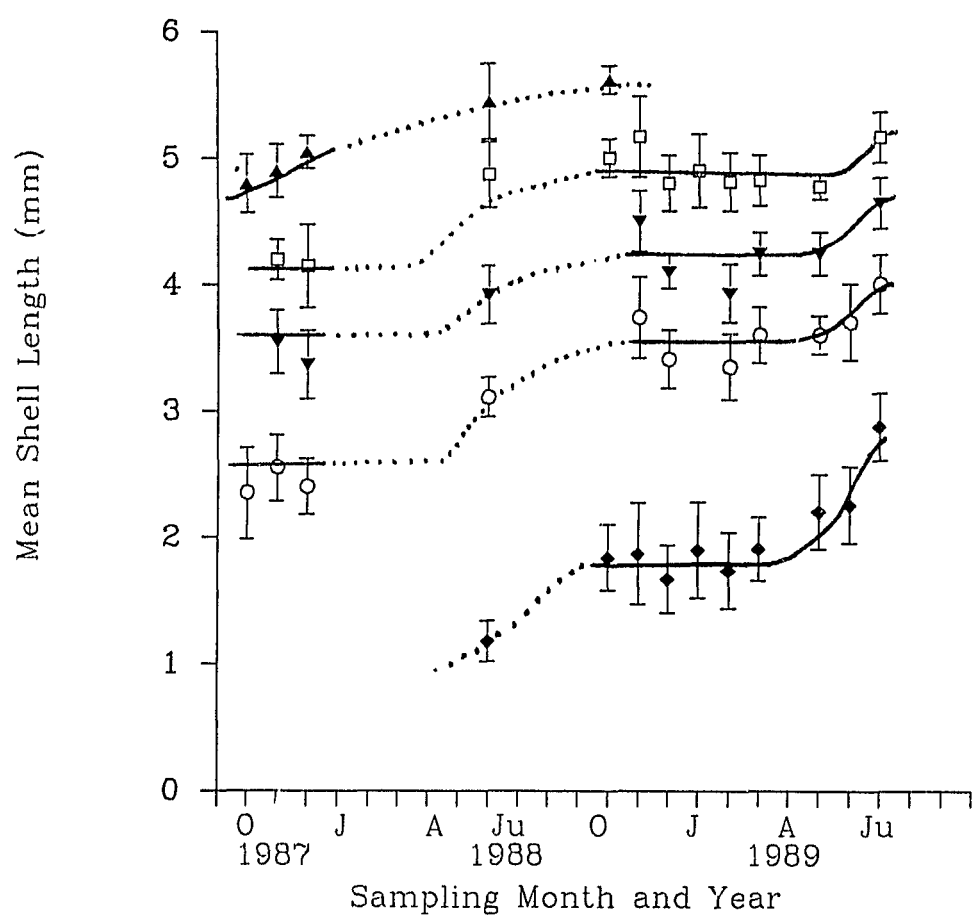
No growth was observed to occur in any of the cohorts from October to March. Rapid, episodic growth appeared to occur between March and July. Since the population was not sampled in August or September, the actual time of year in which shell growth ceases could not be clearly defined.

Reproduction.

The life-history traits of a population are related to the ability to disperse. In a broadcast spawner, dispersal is related to the number of gametes released, energy investment in individual gametes, success of fertilization, and prolongation of a pre-settlement stage by dependence on energy reserves, feeding ability, mobility, et cetera. Figure 25 provides a visual comparison of mature but unspawned oocytes in four bivalves, including *A. serricata*, the Mytilid *Mytilus trossulus*, the Tellinid *Macoma carlottensis* which has also been described as an opportunist, and *Parvilucina tenuisculpta*, a Lucinid which contains endosymbiotic tissue in gill filaments (Reid and Brand, 1986). In all species, the oocytes were deemed to be mature because partial spawning was evident in at least some of the reproductive follicles of each individual. Furthermore, the oocytes had a more rounded transverse profile than oocytes still attached by stalks to the follicles walls.

The average diameter of mature oocytes in *A. serricata* was 77 μm (n = 60, sd = 15.4) with a maximum observed diameter of 101 μm. Since, spawned and unspawned oocytes are not typically spherical, many authors report the diameter of oocytes as the largest of three major axes (Hermans, 1979). The maximum dimension encountered in transverse sections of *A. serricata* oocytes was 119 μm. Also, the diameter of oocytes in formalin-fixed, paraffin-embedded tissue is probably slightly smaller due to shrinkage than in living, shed oocytes.

Oocyte size in *A. serricata* is considerably larger than that of many other bivalves having planktotrophic larvae (reviewed by Gustafson, 1985). Ockelmann (1965) proposed that egg size in bivalves is related to the type of larval development (as planktotrophic, lecithotrophic or direct). Ockelmann proposed that a bivalve with a ripe egg diameter of 40 to 85 μm will have planktotrophic development. An egg diameter of 90 to 140 μm suggests lecithotrophic develoment (i.e., non-feeding larva). An egg diameter of greater than 150 μm is usually found in bivalves with direct-developing young.

Based on Ockelmann's criteria, the egg size in *A. serricata* suggests a lecithotrophic mode of larval development. However, Hermans (1979) points out that in broadcast spawning species, there is always a lecithotrophic larval stage which may or may not be followed by a planktotrophic stage. A planktonic stage further prolonged by feeding cannot be ruled out for *A. serricata* without further study.

The actual number of gametes produced was not measured, but the gonad in reproductively mature *A. serricata* comprises in both males and females an estimated 40 to 80+ percent of the volume of the visceral mass exclusive of stomach and foot musculature (see Chapter 5). Therefore overall fecundity is probably considerably higher in this clam that in the other bivalves examined relative to clam size and investment in other tissues.

Figure 25: A comparison of size and appearance of mature but unspawned oocytes of the bivalves (A) *Axinopsida serricata* (Thyasiridae), (B) *Parvilucina tenuisculpta* (Lucinidae), (C) *Macoma carlottensis* (Tellinidae), and (D) *Mytilus trossulus* (Mytilidae). Oocytes of *A. serricata* have a comparatively large, yolk-rich cortex which may enhance dispersal ability of fertilized larvae. Based on Ockelmann's (1965) criteria, *A. serricata* may have lecithotrophic develoment. Scale bars = 50 μm.
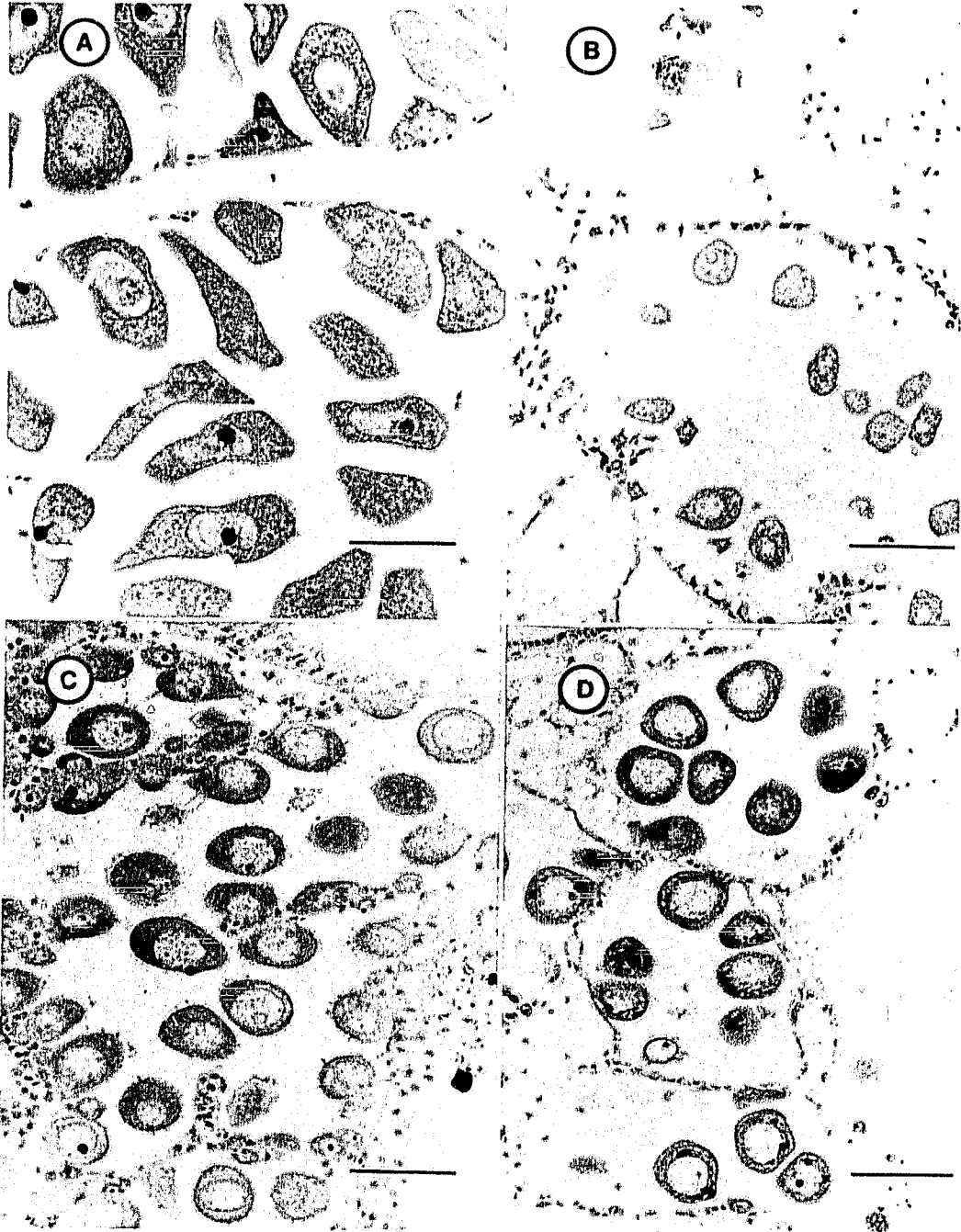
Recruitment.

The earliest months in which a newly settled (0+) year class could be found were June (1988) or July (1989). However, shell length was already approximately 0.8 to 1 mm. which is very large compared with that of other newly-settled bivalves (Loosanoff *et al.*, 1966). For example, Loosanoff *et al.* observed a maximum shell length prior to metamorphosis and settlement of 300 μm for *Mytilus edulis*, 230 μm for *Mya arenaria* and *Mercenaria mercenaria*, and 270 μm for *Arca transversa*.

Lopez-Jamar (1987) stated that the shell length at settlement for *Thyasira flexuosa* was approximately 180 μm, but the clams were not quantitatively sampled when screened through a 0.5 mm screen until they attained a length of 0.8 mm. Nonetheless, large quantities of particles considerably smaller than 0.5 mm were often retained by the screen in this study. Therefore, if *A. serricata* were present in the sediment at a shell length of less than 0.6 to 0.8 mm, then at least some of the clams should have appeared in screened samples.

The size of the prodissoconch (that portion of the shell nearest the umbos which is produced prior to settlement) can often be used to deduce the size of bivalves at settlement. In *A. serricata* from Mill Bay, a prodissoconch could not be unequivocally identified. In approximately 10% of clams examined, a small area with a less glossy periostracum and less distinct concentric banding was identified. The length of this region, i.e. the maximum distance along the longitudinal plane, varied from approximately 300 to 450 μm.

In some specimens, a different band occurred at a shell length of 800 to 1100 μm. The edge of this region was set off by a well defined concentric ring, and the shell on either side of the band was similar in appearance. This band was possibly a growth ring formed during the first winter after settlement .

Most infauna and permanently attached epifauna are considered to be sessile once settlement has occurred. In a stable population which does not experience immigration or emigration, the shape of the survivorship curve can usually be assessed by either following a cohort over time, or by examining the relative abundance of all cohorts during a single sampling interval.

The estimated abundances of two cohorts (clams newly settled in spring or summer of 1987 and 1988) were followed over a two or one year period respectively (Figures 26 and 27). In a static population, these curves should reflect survivorship. However, either a temporal increase or no trend was observed in the relative or absolute abundance of the cohorts. This suggests a prolonged movement into the population. Immigration to the population following the initial appearance of a cohort might be partially accounted for by delayed larval settlement. Yet, the observed immigration to a cohort after it is 1 year old indicates that *A. serricata* migrates after larval settlement has occurred.

Recruitment to the population in and following the summer of 1987 was very similar in magnitude to that of 1988 (compare Figures 26 and 27). Therefore, annual recruitment was considered to be relatively uniform over the study period.

Mortality.

The possibility that *A. serricata* migrates in the post-larval stage precluded an attempt to measure survivorship using cohort analysis.

Since no shell growth occurs for six months of the year or more, it is possible that the death assemblage reflected the age-dependent mortality within the living population. A typical length frequency distribution of the dead shells is shown in Figure 28 from collections made in June, 1988. The length frequency distribution of the living population is shown for comparison. The frequency in each size interval of unhinged shells was divided by two and added to that of hinged shells in order to construct the overall frequency distribution. A size-frequency distribution based only on hinged valves was very similar in shape (not shown).

The largest frequency of shell length occurred at 4+ mm. corresponding to clams which were 3+ years of age in the living population. A smaller peak occurred at a size corresponding to a 2+ year-class. It is tempting to suggest, based on the dead shell assemblage, that mortality is relatively low in the youngest cohorts and increases dramatically following the third year of growth. However, smaller shells were undoubtedly more fragile than larger shells and the absence of smaller shells was at least partially an artifact of differential destruction within the sediments and during sampling.

Figure 26: Abundance (as percent abundance of the total clam abundance or cohort abundance/ m^2) of a single cohort of *A. serricata* followed over two years. Larval settlement date estimated to occur in the summer of 1987. Vertical bars represent standard deviations based on three replicates at each sampling date. The lines predicted by a least-squares regression are shown.

For both relative cohort abundance (A) and absolute cohort abundance (B), a statistically significant increase was observed as a function of time since settlement, as follows:

(A): Relative abundance (%) = 15.60 + 1.07(Time in months).
    Prob. = 0.008, N = 39.

(B): Cohort density (no./0.1 m^2) = 9.97 + 1.02(Time in months).
    Prob. = 0.013, N = 39.
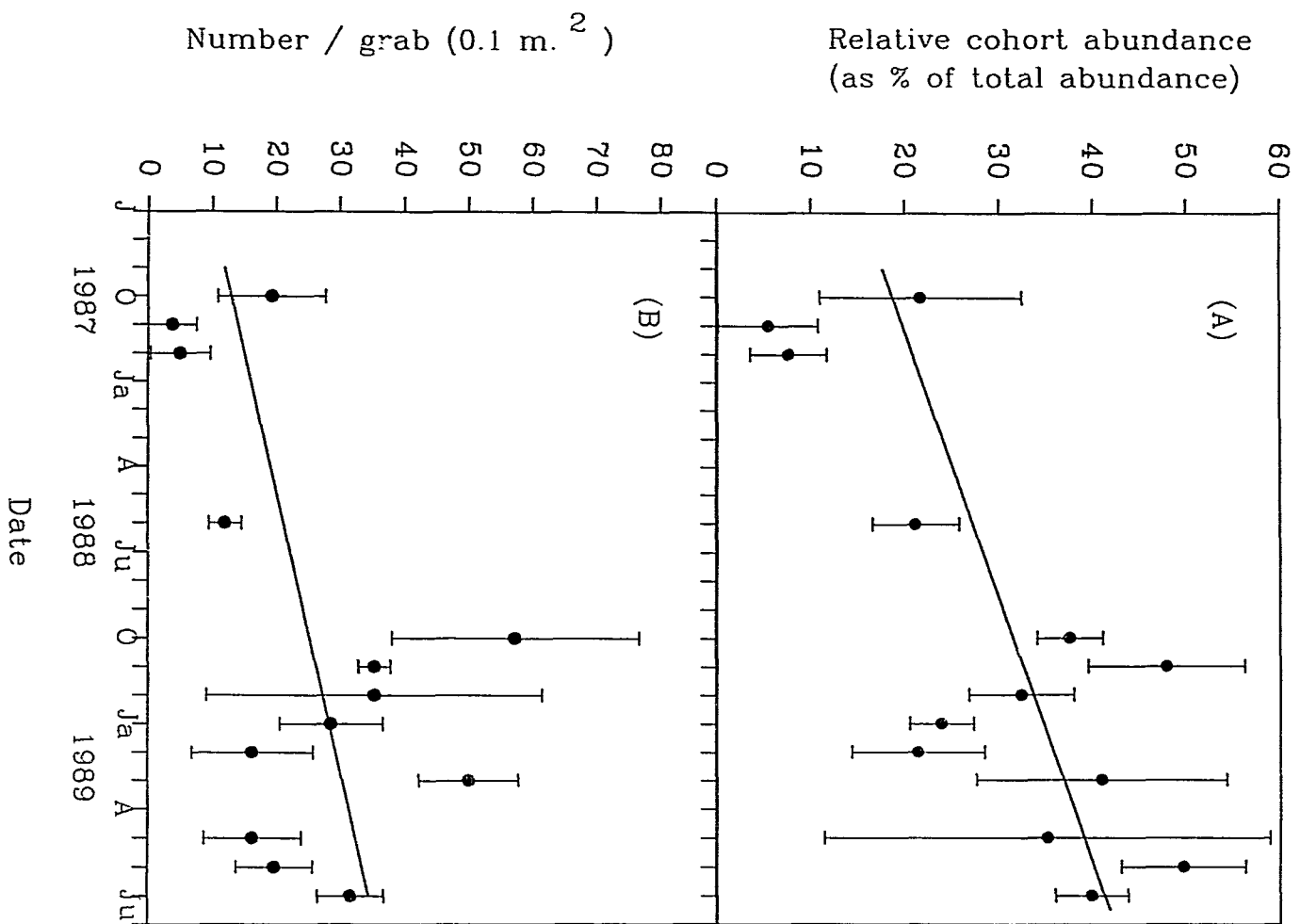
Figure 27: Abundance (as abundance relative to total clam abundance or cohort abundance/ m^2) of a single cohort of *A. serricata* followed over two years. Larval settlement was estimated to occur in the summer of 1988. Vertical bars represent standard deviations based on three replicates at each sampling date.

For relative cohort abundance (A) or absolute cohort abundance (B), there was no statistically significant linear trend related to time elapsed since clam settlement (N = 30, Prob. = 0.25 and 0.50 respectively).

As for Figure 26, note that the graph does not resemble a survivorship curve, as would be expected in a closed, stable population, and cannot be interpreted as such.

Number / grab (0.1 m.^{2})

Relative cohort abundance
(as % of total abundance)

(A)

(B)

Date

1987

1988

1989

Ja

Ap

Ju

O

Ja

Ap

Ju

124
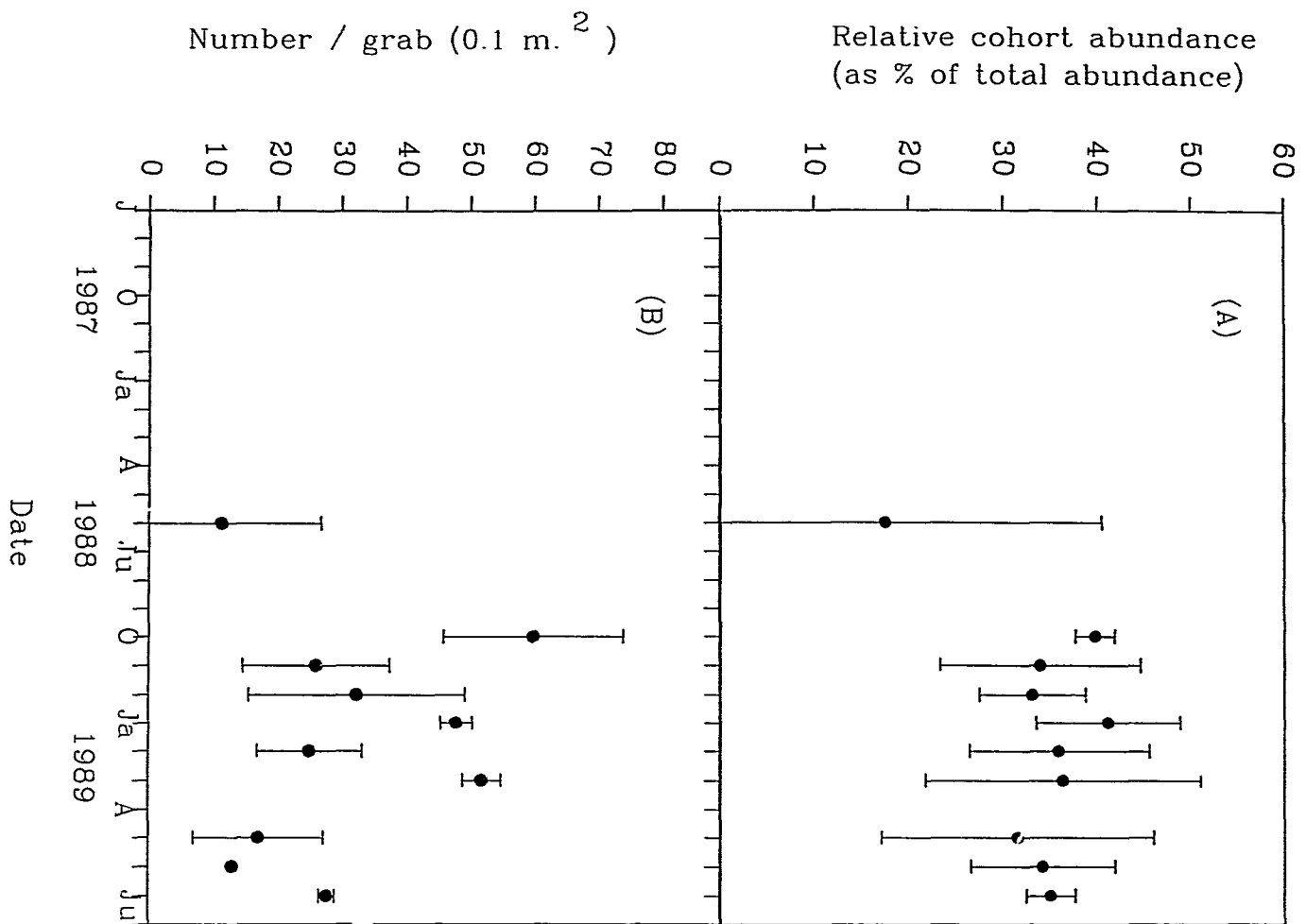
Figure 28: Frequency distribution of shell length of a death assemblage (A) and living population (B) of *A. serricata* from Mill Bay, Saanich Inlet (June, 1988). The blackened bars in (A) indicate mortalities attributed to gastropod predation based on the presence of circular bore holes in the clam shell. The relative absence of smaller empty shells was probably related to increased destruction of smaller, more fragile shells.

Figure caption showing two histograms: (A) Death assemblage and (B) Living clams, with y-axis labeled "Number / 3 replicates" and x-axis labeled "Shell length interval (mm)".
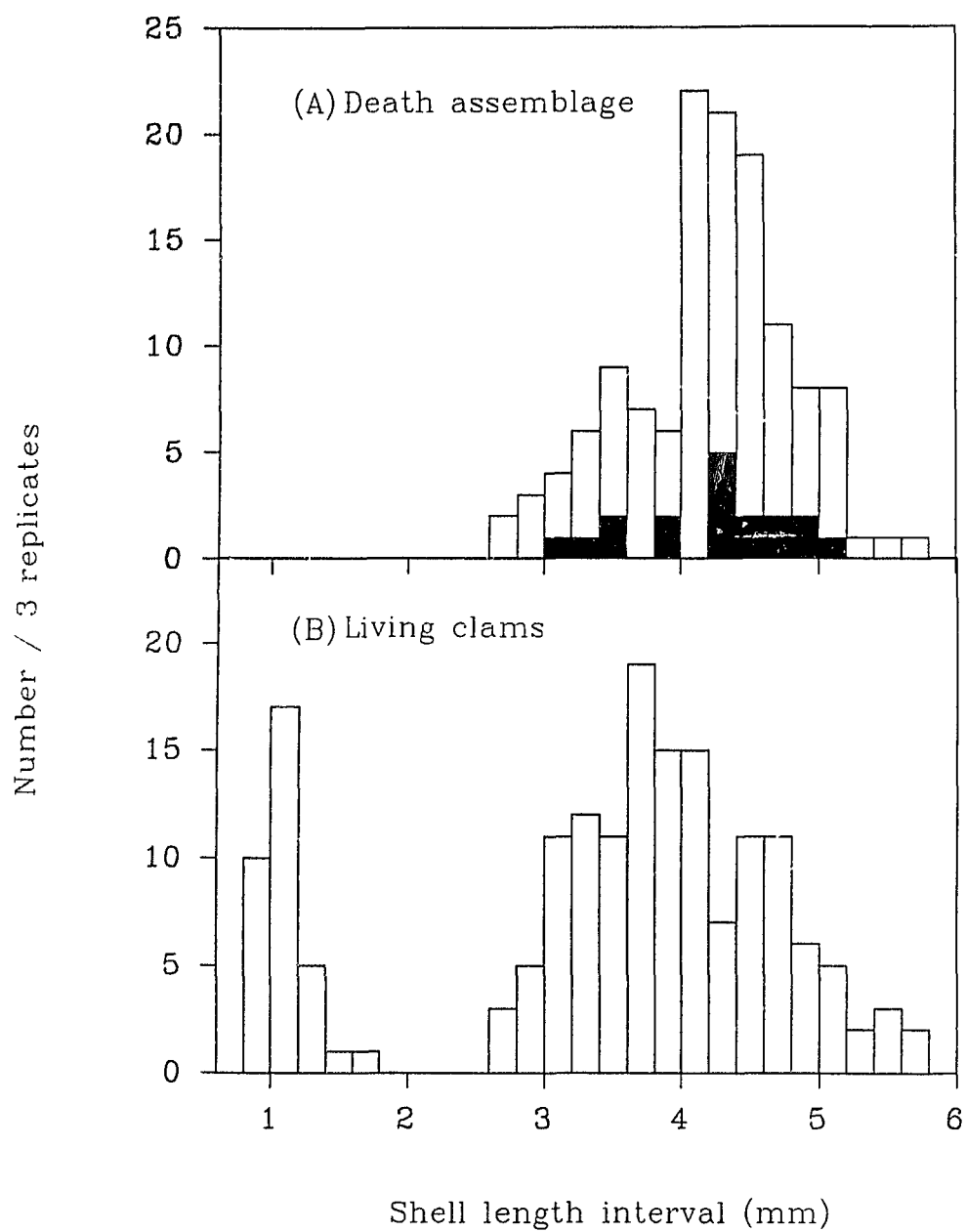
Some shells (hinged and unhinged) contained bore holes caused by predatory gastropods Figure 28). Based on the relative incidence of bore holes, an estimated 12% of mortality within the older (2+) year classes was caused by snail predation.

Discussion

The examination of a population of *A. serricata* from Mill Bay, Saanich Inlet provides the first detailed study of life-history characteristics for a small thyasirid. Life-history attributes of *A. serricata* include a small body size with shell length not exceeding 6 mm., a maximum longevity of five years or greater, and high reproductive output both in terms of partitioning of energy into gonad and investment in individual ova (for females). The size of unspawned oocytes provides some evidence that *A. serricata* may have a lecithotrophic larval stage.

Growth is episodic, occurring primarily in the warmer summer months while spawning occurs annually after growth has stopped (November-December). Annual growth increments are visible on the shells of some individuals.

That portion of *Axinopsida's* life-cycle between spawning and appearance of young at the sampling location remains unknown. The smallest detectable cohort had a shell length of approximately 0.8 to 1.0 mm., which is large for newly settled bivalve larvae. Furthermore, most lecithotrophic of planktotrophic veliger larvae are planktonic for a period of a few hours to a few days. If *A. serricata* commences spawning in November, then a prolonged planktonic existence to the following summer is unlikely.

In addition, a temporal increase rather than decrease in the relative or absolute abundance of a newly settled cohort followed over two years strongly suggests that post-settlement immigration to the population occurs for this species. Population structure might be influenced by immigration of *A. serricata* from shallower to deeper sediments.

Habitats that have undergone disturbance are often inhabited by organisms that are able to discover the area and rapidly colonize following the disturbance by virtue of rapid production and dispersal of progeny (Pianka, 1970; Grassle and

Sanders, 1973; Parsons, 1982). These opportunists, or r-selected species, have evolved strategies which for the population as a whole favor high intrinsic rates of population growth at the expense of an ability to sequester resources for longer periods of time and compete with other species.

Axinopsida serricata has been called an r-selected species (Reid and Brand, 1986) based on its wide-spread distribution in disturbed habitats. Elucidation of the life-history allows an examination of the hypothesis that the distributional ecology of *A. serricata* in British Columbia is related to life-history traits which have collectively been termed r-selected.

Life-history correlates of opportunism usually include a short maximum life-span, rapid development, small body size, high fecundity with little parental investment and a logarithmic decline in survivorship (Pianka, 1970).

Powell and Cummins (1985) and more recently Heller (1990) provided a compilation of data on the estimated maximum longevity of marine molluscs. Although some members of the Bivalvia are notoriously long-lived (e.g. geoducks, *Panope generosa*, with a maximum longevity of greater than one hundred years), a maximum life span of 3-4 years occurs with the highest frequency. Many bivalves have estimated maximum life spans of 1 year or less.

This can be compared with the maximum longevity of *A. serricata* which is here estimated to exceed five years. Since *A. serricata* in British Columbia is very often the last bivalve to disappear in the face of ongoing disturbance (Ellis and Hoover, in press; personal observation), then it might be expected to have a very short longevity.

The possibility that *A. serricata* has a lecithotrophic larval development would also appear to be at odds with it being an r-selected species. *Thyasira gouldi*, which has a much larger maximum body size, was observed by Blacknell and Ansell (1974) to undergo direct development within demersal egg capsules unattached to the parent. Therefore, planktotrophic development may be rare within thyasirids.

In Rupert and Holberg Inlets, the response of abundance of *A. serricata* to mine tailings discharge closely resembles a general model of marine macrobenthic

community responses to organic enrichment (see Chapter 5 below) as proposed by Pearson and Rosenberg (1978). Gray (1989) referred to this pattern as a "retrogression to dominance by opportunistic species".

The salient features of taxa defined *a posteriori* as opportunists based on changes in abundance relative to other community related events are as follows. In unstressed, undisturbed environments an opportunist occurs widely in very low abundances (i.e. has a cosmopolitan distribution). With increased environmental disturbance, both the number of species present as well as total community biomass will increase slightly preceding a marked decrease with increased disturbance. For the opportunist, maximum abundance occurs at a point along a gradient of increasing disturbance after overall species richness and total biomass have declined significantly. For marine benthic macroinvertebrates, this 'peak of opportunists' is often characterized by a three-fold increase in abundance over that of undisturbed areas, with single-species densities approaching thousands of individuals/ m^2. Greater magnitudes of disturbance result in a catastrophic decrease in abundance of opportunists leading ultimately to a community devoid of macroinvertebrates.

Retrogression to dominance by opportunists is a pattern presently recognized by a number of scientists who are interested in 'environmental stress' (Gray, 1989; Rapport, 1989). Little attempt has been made to reconcile categorizing a species as an opportunist based on community data with that based on life-history data and on physiology. The life history attributes of *A. serricata* suggest that the label of opportunist is not justifiable. Observations on the life histories of most opportunists identified through community responses are lacking. One exception is the polychaete *Capitella capitata* (Grassle and Grassle, 1978; Tsutumi, 1987). Investigations of physiological/autecological correlates of opportunism as defined by community response in a large number of marine benthic species presently recognized as opportunistic would be extremely useful.

The categorization of organisms along an r-selected/ K-selected continuum may be oversimplified. However, the concept is pervasive in association with a wide range of other biological theories. Rhoads and Boyer (1982) compared patterns of bioturbation in marine infaunal macroinvertebrates between pioneering species and equilibrium species. Most early colonizing species, principally polychaetes, protect

themselves from unpredictable changes in concentrations of dissolved oxygen, sulfides, and organic decomposition products by building tubes which partially isolate the organism from the immediate sedimentary environment.

For *A. serricata*, the tendency appears to be in the opposite direction; burrowing behaviour and functional morphology seem to reflect an attempt by the clam to form a stronger as ociation with the surrounding environment. The mantle cavity is poorly adapted to isolate the soft tissues from the surrounding pore water, and radiating excavations likely enhance exposure to surrounding sediment and pore water.

Vermeij (1978) suggested a third category of life history traits which he labelled "stress-tolerant". Some environments are physiologically stressful; however, the underlying disturbance is one which occurs frequently and may be more predictable, at least for biota with an evolutionary history associated with that disturbance. For example, animals living in the intertidal zone can be considered stress tolerant, above and beyond the relative categorization of life-history traits as r- or K-selected. *Axinopsida* is not an intertidal species, but the concept of stress tolerance is more appropriate than that of opportunism, given the life history.

5. Changes in population attributes associated with changes in tissue structure: II. Between-site variation in fecundity, growth, and abundance related to the spatial distribution of tissue structure alterations.

The digestive diverticula, ctenidium, and kidney of *Axinopsida serricata* exhibited between-station variation in structure (see Chapter 3). The incidence and severity of deleterious changes in these tissues increased near I.C.M.'s mine-tailings discharge. Based on the incidence and severity of tissue structure alterations, *A. serricata* from lower Holberg Inlet station H1 and H2 closer to the discharge pipe were in poorer condition than clams from the reference site (SI) and from other stations in upper Holberg Inlet (H4, H5) and Quatsino Sound (QS). This chapter explores whether a similar pattern of impact occurs at the population level.

Methods

The study sites.

Growth and abundance of *A. serricata* were measured at the sites in which histological effects were investigated. Stations SI (Saanich Inlet), GB (Granby Bay), QS (Quatsino Sound) and stations H1 to H5 (Holberg Inlet) were sampled in November, 1987 as summarized in Table 1 (page 23 in Chapter 1) and shown in Figure 29 (below). Three replicate samples were obtained at each station using a 0.1 m.^{2} Van Veen grab in Saanich Inlet and a 0.1 m.^{2} Smith-McIntyre grab at all other stations.

The macrofauna and large debris were recovered by screening the samples through a 0.5 mm mesh screen, and were immediately fixed in 10% Ca_2CO_3-buffered formalin.

I.C.M.'s Environmental Control Division monitors benthic community structure at 25 stations in Rupert Inlet, Holberg Inlet, and Quatsino Sound in September of each year. Spatio-temporal trends in the abundance of *A. serricata* were reconstructed from the resulting data set, which extends back to 1971. I.C.M. monitoring personnel employ a 0.05 m.^{2} Ponar grab to obtain 3 replicate benthic samples, which are then screened through a 0.6 mm. screen.

Figure 29 shows the locations of the I.C.M. monitoring stations used in this

study. The depth and distance from the discharge pipe are shown in Table 10. The subset of stations chosen for the examination of abundance patterns included those along the trough (dotted line in Figure 29); shallow stations were largely ignored, although station 24 in upper Holberg Inlet was more shallow than the others. The rationale for this was that (1) tailings deposition occurs with greater magnitude along the trough, and (2) shallow stations tend to group separately from trough stations based on benthic community data (I.C.M., 1989; Taylor, 1986).

I.C.M.'s benthic stations 5, 3, and 24 are the same as stations H1, H3, and H4 respectively (Figure 29) sampled for this study. Shell length frequency distributions were obtained from collections of *A. serricata* archived by I.C.M. from stations 5, 3, and 24 for the years 1988, 1989, and 1990.

Figure 29: Benthic stations from Island Copper Mine's monitoring program which were used here to investigate spatial and temporal patterns of growth and abundance in *A. serricata*. The stations along the trough of Rupert and Holberg Inlets are connected by a dotted line. I.C.M. stations 5, 3, and 24 correspond to stations H1, H3, and H4 respectively as employed in this study.
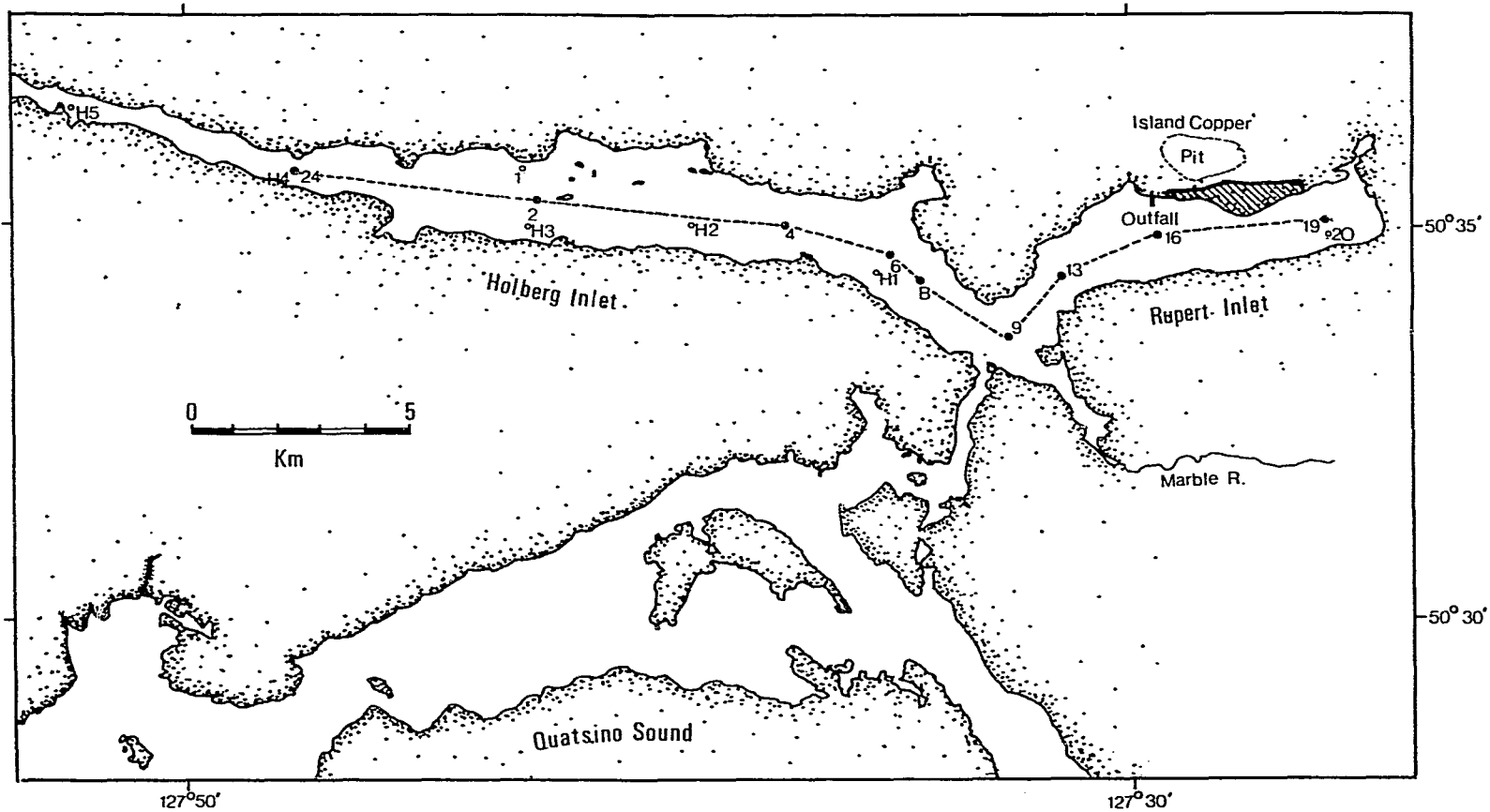
Table 10: List of sampling stations used to assess patterns of variation in population attributes (fecundity, growth, abundance) of *A. serricata*. "Variables" lists the variables examined at each station (F: fecundity; G: growth; S: average size; P: population structure; A: abundance).

Station	Location	Depth (m)	Distance from Discharge (km)	Variables
ICM 19	Rupert	71	2.9	A^1
ICM 16	"	78	0.7	A^1
ICM 13	"	120	3.0	A^1
ICM 9	"	136	4.6	A^1
ICM B	Holberg	151	7.0	A^1
ICM 6	"	137	8.4	A^1
ICM 4	"	118	10.4	G^2,A^1
ICM 2	"	98	16.5	A^1
H1(ICM 5)	Holberg	129	7.7	$F^3,G,S^4,A^{5,6},P^7$
H2	"	108	12.1	F,G,S,A^5
H3(ICM 3)	"	87	16.0	$F,G^8,S,A^{5,6},P$
H4(ICM 24)	"	76	21.8	$F,S,A^{1,5,6},P$
H5	"	38	26.8	F,G,S,A^5
QS	Quatsino	195	22.1	F,S,A^5
ICM 20	Rupert	40	4.2	G
ICM 1	Holberg	21	16.9	G
SI	Saanich	90	na	F,G,S,A^5
GB	Granby Bay	82	na	F,G,S

na: not applicable.
1: Includes data from commencement of tailings discharge (1971) to 1989.
2: Growth curves reconstructed from cohort analysis of September or November, 1987, collections.
3: All fecundity measurements were made on *A. serricata* collected in November, 1987.
4: Size as average shell length in November, 1987, collections.
5: Abundance (no./ m.^{2}) in November, 1987, collections.
6: Abundance (no./ m.^{2}) in I.C.M. collections from 1977 to 1990).
7: Includes at all stations examined the shell length frequency distributions from November, 1987 and from I.C.M. collections in September, 1988 to 1990.
8: A growth curve was also reconstructed using I.C.M.'s 1989 data.

Measures of fecundity.

Krebs (1978) defined fecundity as the "potential capability of an organism to produce reproductive units such as eggs, sperms, or asexual structures." In this study, the relative volume of gonad and the percent volume of oocytes within follicles of reproductively ripe *A. serricata* were taken as indicators of fecundity. Details of the collection and preparation of specimens for histological examination are provided on pages 22-24 in Chapter 2.

In *A. serricata*, the gonad of males or females only occurs in arborescent tufts of the visceral mass. In reproductively ripe clams, gonad typically extends from the base of the tufts near the stomach wall to near the tips of the arborescent tufts. An increase in gonad size with seasonally-dependent reproductive maturity coincides with a decrease in the volume occupied by the digestive diverticula. In clams at the same stage of reproductive development, between-site differences in stress could possibly influence the volume of gonad produced.

A gonad index (GI) was devised as the percent volume of visceral tufts in each clam occupied by gonad as opposed to digestive diverticula. The percent volume of gonad was estimated by measuring the percent area of gonad in the arborescent tufts in three randomly chosen transverse sections per clam. In all clams, sections were chosen from those that passed through the visceral mass near the geometric center of the clam and that contained a portion of the stomach.

The percent area of gonad and digestive diverticula were measured by tracing the outline of each onto paper using a projecting microscope. The outlines were then cut out and weighed to the nearest 0.1 g. on a balance. The percent weight of gonad within the arborescent tufts was taken to be directly proportional to the percent area of gonad in the section and to percent volume of gonad in the whole clam.

Fecundity might also vary with the quality of gametes within the follicles in addition to the volume of gonad produced. Therefore, a second measure called gamete-to-follicle ratio (GFR) was devised. GFR is a stereological estimate of the percent volume of follicles occupied by gametes. GFR was measured only in females.

GFR is the percentage of points under a 10 x 10 grid of the eyepiece graticule which fall on oocytes as opposed to other structures or space within the follicle. Measurements were made at 200x magnification on each of two randomly chosen areas in three randomly chosen sections (total number of replicates was 6).

To test the dependance on body size of fecundity as measured by GI and GFR, simple linear regressions were used employing the maximum width of the visceral mass (MWVM) as an indicator of size. MWVM was measured in transverse sections on prepared slides as described in Chapter 3 (page 63). The regressions were performed on *A. serricata* from all stations sampled in November, 1987.

Growth.

Growth curves for *A. serricata* from Saanich Inlet, Granby Bay and Rupert/Holberg Inlets were reconstructed from shell length frequency distributions. Details of the method employed are provided on pages 98 and 99 in Chapter 4.

Abundance.

The mean densities of *A. serricata* from stations with Saanich Inlet, Granby Bay, Quatsino Sound, or Holberg Inlet were calculated from the clam abundance in three replicates. Spatial trends in the abundance of *A. serricata* were assessed using the samples collected in November, 1989.

Temporal and spatial trends in abundance were evaluated using the data from I.C.M. collections taken in September of each year.

Spatial covariation between population attributes and tissue structure.

Chapter 4 describes the spatial distribution of histological effects associated with mine-tailings discharge. This distribution is compared to the spatial distribution of population characteristics defined below. I also attempted to make a more formal comparison of tissue-level and population-level effects by examining the correlation of fecundity, growth, and abundance at each site with a measure of average tissue deterioration.

Two measures of histopathology were compared to population attributes, as follows. The first measure is based on principal components analysis of six tissue variables (page 84 to 93 in Chapter 3). Scores on the second of three principal components were significantly correlated with distance from the tailings discharge pipe (page 89). Therefore, site-averaged scores on principal component 2 were compared with variation in population attributes.

The second measure of tissue effects used was the estimated population incidence of ctenidial lesions. The percent incidence of *A. serricata* exhibiting either oedema of lateral cells, hyperactivity of mucocytes, or both was compared to between-site variation in population attributes.

Results

Between-population differences in fecundity associated with mine-tailings discharge.

Station-to-station differences in the estimated fecundity of *A. serricata* are shown in Figure 30. The means for gonad index (GI: Figure 30a) were higher in Granby Bay and in Holberg Inlet stations H1 and H2 than in clams from the reference site (SI). Before subjecting the GI data to a one-way ANOVA, the homogeneity of group variances was examined using Bartlett's test; no departure from homogeneity of variance was detected (DF = 7, P = 0.15).

A one-way ANOVA showed significant between-station differences in GI (DF = 7, 59, p < 0.001). Group similarities within the stations were examined using a Tukey-Kramer post-hoc test. As shown by the lettering at the top of Figure 30a, Granby Bay and lower Holberg Inlet station (H1 and H2) clams had a similar relative volume of gonad. Granby Bay clams and station H2 clams had a significantly different GI from the reference station, although station H1 clams did not.

If GI varies between sexes, part of the between-site variability may be due to between-site differences in the ratio of males to females. Therefore, an independent sample t-test was used to compare all males combined (n = 30) with all females combined (n = 35). There was no difference between sexes in the percent volume of arborescent tufts occupied by gonad (DF = 63, P = 0.65).

Gamete-to-follicle ratio (GFR) was measured only in the female *A. serricata* collected. Figure 30(b) illustrates between-station differences in GFR. There was no detectable departure from homogeneity of group variance (Bartlett's test: DF = 7, P = 0.17). Significant between-station variation in GFR occurred, as indicated by a one-way ANOVA (DF = 7, 27; P = 0.001) accompanied by a Tukey-Kramer test. The GFR in female *A. serricata* from lower Holberg Inlet (H1 and H2) was significantly higher than in females from Quatsino Sound, Saanich Inlet, or Granby Bay.

Figure 30: Spatial patterns of fecundity in reproductively ripe *A. serricata* from Saanich Inlet (SI), Granby Bay (GB), Quatsino Sound (QS) or Holberg Inlet (H1-H5), British Columbia.

(A) Between-station differences in the relative volume of gonad (as opposed to digestive diverticula) in the arborescent tufts (i.e., GI) of *A. serricata* collected in November of 1987. The lettering (A, B, C) at the top of the figure shows group similarities based on a Tukey-Kramer post-hoc test. Vertical bars are standard deviations about the mean.

(B) Between-station differences in the percent volume of follicles occupied by oocytes (GFR) in *A. serricata* collected in November, 1987. The lettering (A, B) shows group similarities based on a Tukey-Kramer post-hoc test. Vertical bars are standard deviations about the mean.
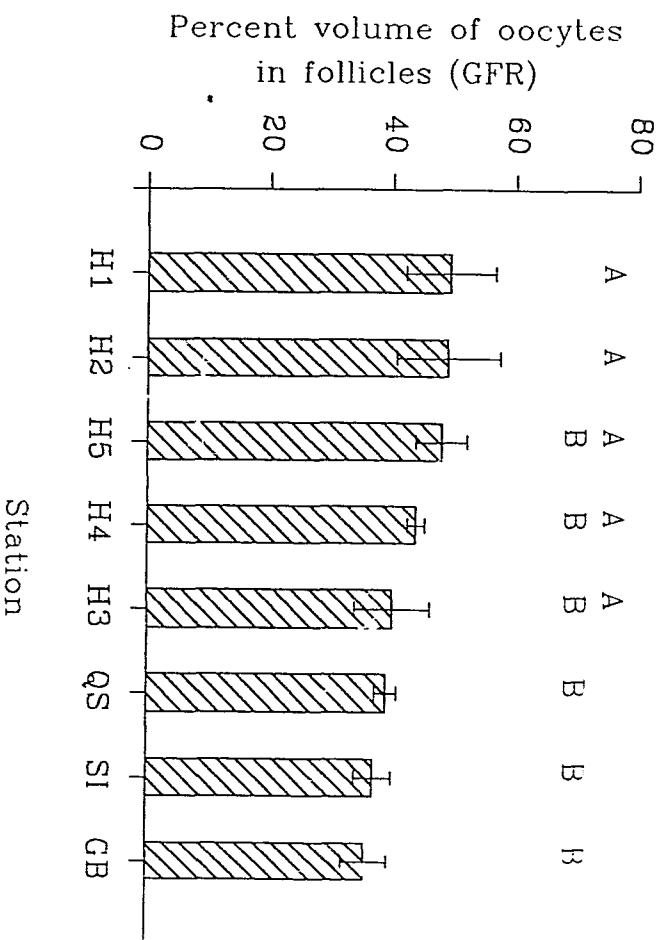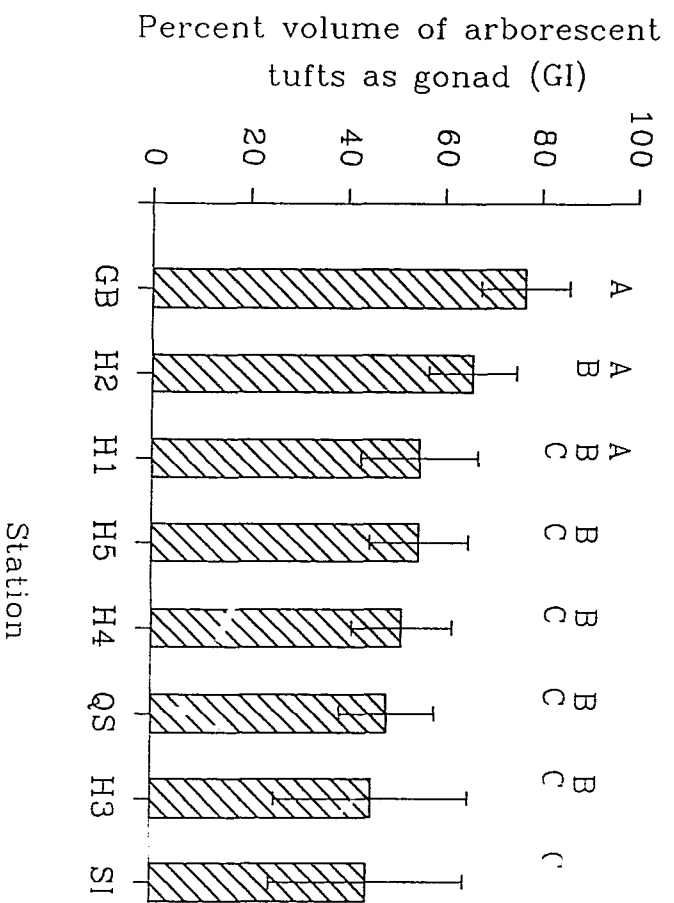
Neither GI nor GFR varied as a function of body size when clams from all November, 1987, collections were combined (for the regressions on MWVM, P = 0.09 and 0.45 respectively; n = 65 and 34 respectively).

The percent volume of gonad within arborescent tufts and percent volume of oocytes in follicles were multiplied to provide a combined index of fecundity (Figure 31). The results for Bartlett's test for homogeneity of group variance are as follows: DF = 7, P = 0.302. A one-way ANOVA of relative fecundity (= GI x GFR) showed significant between-sample differences in relative fecundity (DF = 7, 26; P = 0.017). However, a Tukey-Kramer test showed that the between-sample differences in relative fecundity do not reflect the spatial pattern of sampling stations relative to the mine-tailings discharge pipe (Figure 31). The relative fecundity of reference station clams (SI) was not significantly different from clams from any other station.

Figure 31: Between-station differences in the relative fecundity (Gl x GFR) of reproductively ripe *A. serricata*. As shown by the lettering at the top of the figure (A, B), the pattern of station similarities does not parallel the spatial distribution of sample locations relative to I.C.M.'s mine-tailings discharge. Vertical bars are standard deviations.
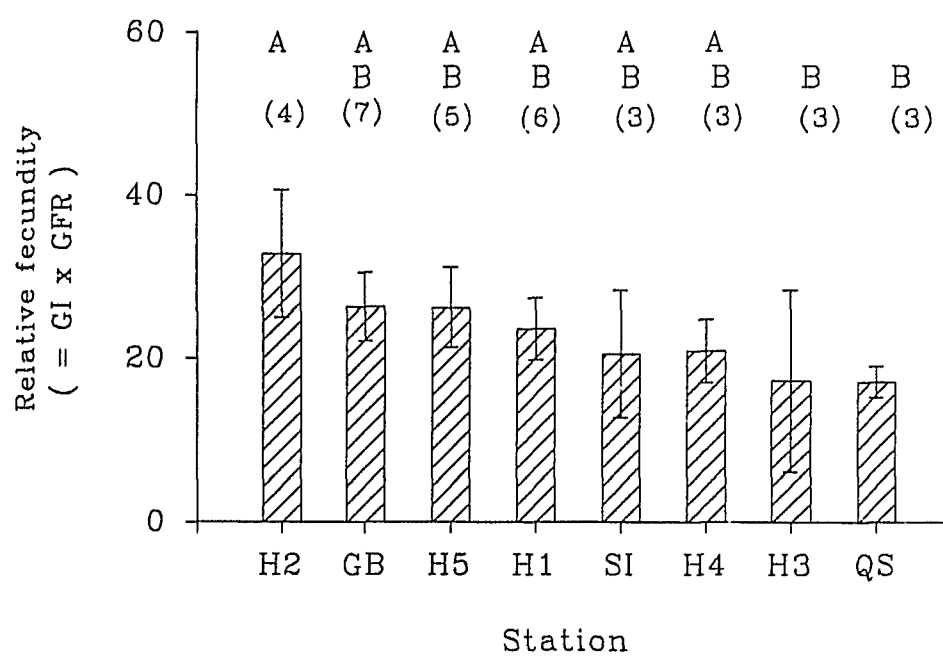
Spatial variation in recruitment and growth.

Figure 32 shows the frequency distributions of shell length of *A. serricata* for stations H1 to H5 and QS sampled during November, 1987. Several year classes of *A. serricata* were either missing or poorly represented at most of the Holberg Inlet stations (H1 to H4). In contrast, the population from Mill Bay, Saanich Inlet contained five well represented, easily distinguished cohorts at most sampling dates (see Figure 21, page 106 in Chapter 4). Therefore recruitment and/or mortality are interpreted to be more highly variable in Holberg Inlet, exclusive of station H5, than in the Mill Bay population.

The average size of *A. serricata* at each station might provide an indication of differences in growth. The mean shell length of *A. serricata* at station H1 was similar to that of *A. serricata* from Mill Bay (SI) and Granby Bay (GB) (Figure 33). In addition, mean shell length increased toward the discharge pipe (from station H4 to H1) rather than decreasing. However, the large average shell length in *A. serricata* from station H1 derives from the absence from the sub-population of more recent, smaller recruits (Figure 32). Therefore, mean shell length in Holberg Inlet *A. serricata* is not a reflection of spatial differences in growth.

Figure 32: Frequency distributions of shell length in November, 1987, samples of *A. serricata* from Holberg Inlet (H1 to H5) and Quatsino Sound (QS). A recently recruited cohort was absent from Station H1. A 0+ year class was present at all other stations. The poor representation of some year classes at stations H4 and H5 in particular suggest more variable annual recruitment and/or mortality than in Mill Bay, Saanich Inlet (see Figure 21 in Chapter 4).

Number of <u>A. serricata</u> in three replicates (0.3 m.2)
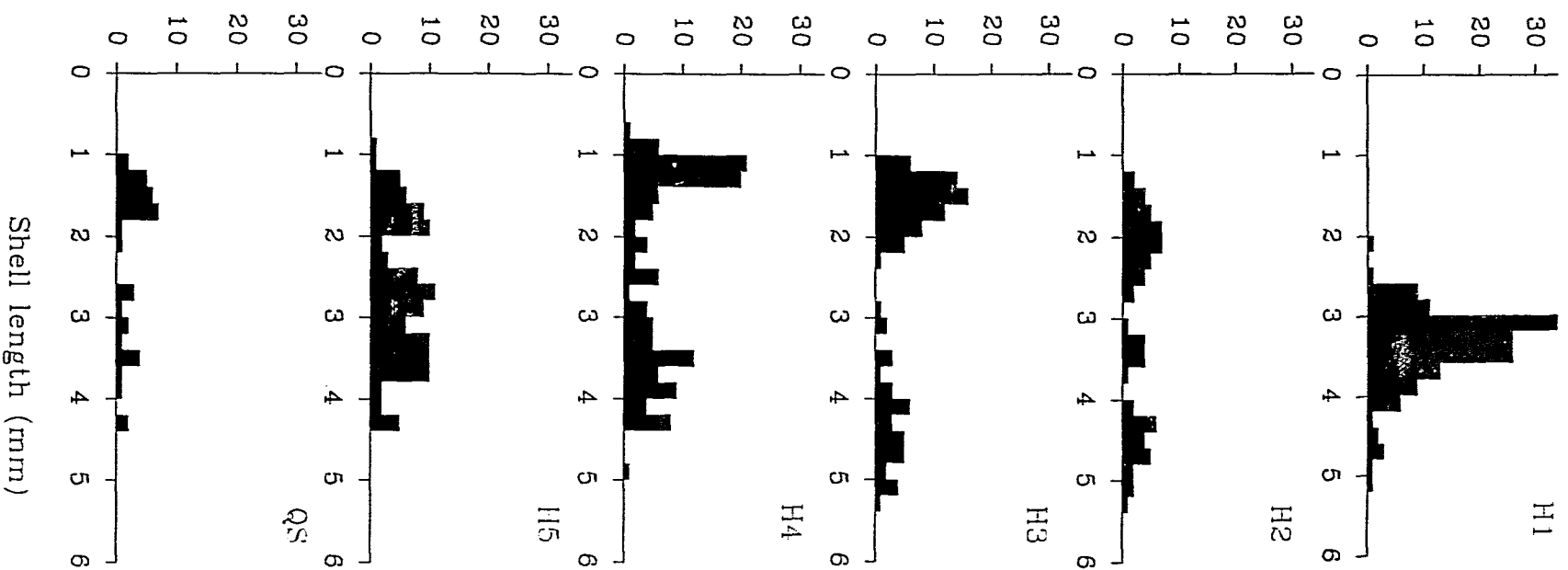
Shell length (mm)

Figure 33: Between-station differences in the average shell length of *A. serricata* in November, 1987 from Saanich Inlet (SI), Granby Bay (GB), Quatsino Sound (QS), and Holberg Inlet (H1 to H5). Vertical bars are standard deviations.

Mean shell length of <u>A</u>. <u>serricata</u>

Distance from the mine-tailings discharge
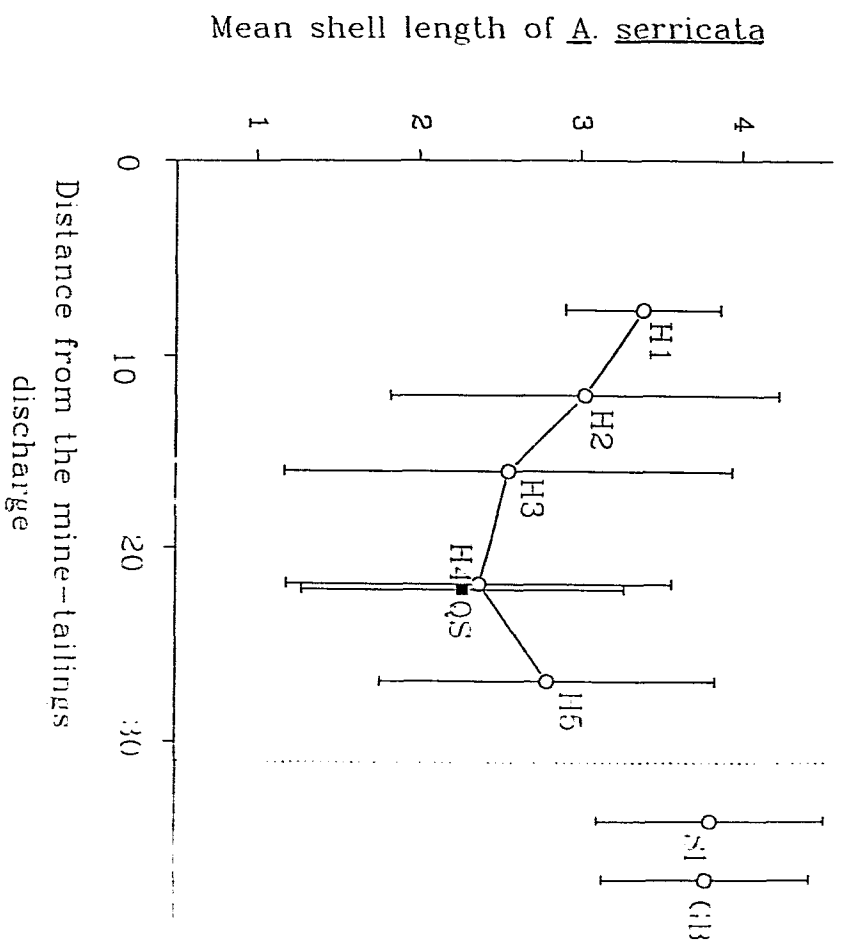
Figure 34 illustrates a generalized growth curve for *A. serricata* in Mill Bay, Granby Bay, and Rupert/Holberg Inlets. The growth curve for Mill Bay clams was derived by plotting the modal means and standard deviations of length frequency distributions from each of 13 sampling dates (shown in Figure 24 on page 113) against the clam age. The curve was hand-fitted.

At most of the Holberg Inlet stations, some cohorts were not easily discerned. Therefore, growth curves were difficult to reconstruct accurately. Growth curves were reconstructed from November, 1987, collections from stations H2, H3 and H5 (Figure 34). A growth curve for H3 reconstructed from I.C.M.'s September, 1990, benthic collections (not shown) was virtually identical to that shown here for the 1987 collections. Growth curves could not be reconstructed from the 1987 length frequency distributions at stations H1, H4 or QS, due to a low abundance which prohibited accurate identification of cohorts. For *A. serricata* from Granby Bay collected in November, 1987, cohorts were easily distinguished within the shell length frequency distribution, although the modal means for the first and fifth year class were not resolvable due to low abundance.

Based on I.C.M.'s benthic collections taken in September, 1988, growth curves for *A. serricata* (Figure 34) were reconstructed for I.C.M.'s stations 1, 4, and 20 (see Figure 29). Stations 1 and 20 are shallow stations, removed from the trough; however, high abundances of *A. serricata* at these stations permitted the confident reconstruction of growth curves. Station 4 occurs along the trough (Figure 29) at a depth of 118 m.

Each growth curve was constructed from a maximum of 3 to 5 data points (modal means). Sample sizes were, therefore, too small to statistically compare coefficients of curvilinear regressions between stations. However, a visual comparison of between-station differences in the growth of *A. serricata*, based on growth curves illustrated in Figure 34, provides the following information. (1) Shell length at a given age is higher in Mill Bay clams than in Holberg Inlet or Granby Bay clams. Age is taken here to mean the estimated time elapsed since spawning and fertilization occurred. (2) Shell length at a given age is lowest in *A. serricata* living at Rupert and Holberg Inlet stations ICM 1, ICM 4, ICM 20. Growth curves of *A. serricata* from these stations were not noticeably different based on an overlap

Figure 34: Growth curves of *A. serricata* in Mill Bay, Granby Bay and Rupert and Holberg Inlets as estimated from shell length frequency distributions. Vertical bars represent standard deviations around each modal mean. The growth curve for Mill Bay *A. serricata* was derived from frequency distributions from collections at thirteen sampling dates from October, 1987, to July, 1989. All others are based on three replicates collected in November, 1987 (H2, H3, H5, GB) or September, 1988 (ICM 1, ICM 4, ICM 20).

Clam size at a given age is lower at shallow Rupert and Holberg Inlet stations and not obviously related to mine-tailings exposure. The station depths are shown in Table 10 above.
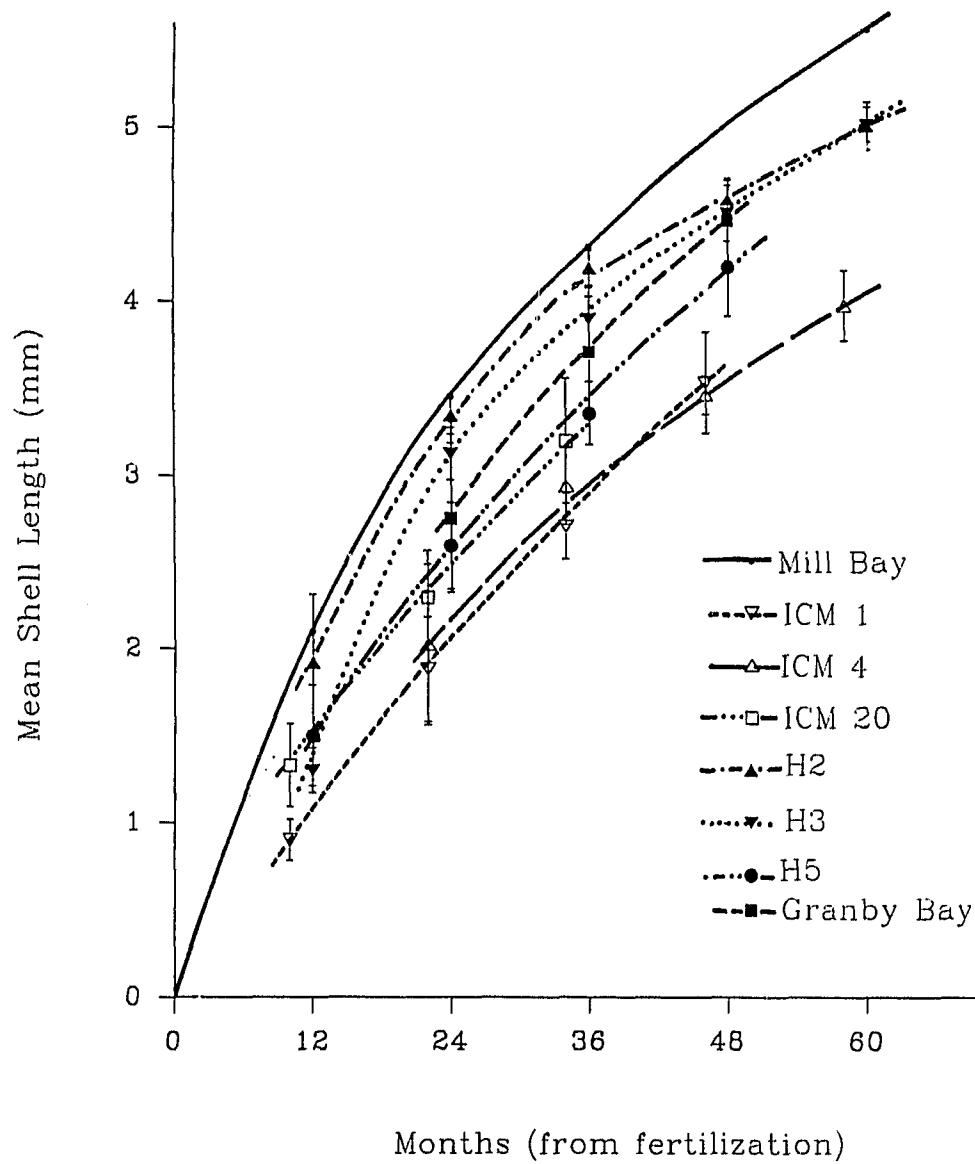
in the standard deviation at each mode. No difference in growth of *A. serricata* occurred within these stations in response to postulated differences in exposure to mine-tailings. (3) Shell length at a given age is higher at the Holberg Inlet trough stations H2, H3 and H5 than in shallow stations ICM 1 and ICM 20: growth in *A. serricata* may be influenced by depth-dependent environmental differences. This may also explain the slightly lower shell length at a given age observed at station H5, which is shallower than at H2 or H3. However, growth at ICM 4, a deeper trough station, was similar to ICM 1. (4) The growth curve of *A. serricata* in Granby Bay was similar to that of clams collected from Holberg Inlet station H3 and H5. (5) The decline with age of the instantaneous growth rate of *A. serricata* was similar in magnitude at all stations sampled except for station H2. Station H2 was the closest station to the discharge pipe where abundance of *A. serricata* facilitated reconstruction of a growth curve; the slope of the curve decreases more rapidly than at other stations. However, an apparent depression of growth in older clams from station H2 may have been an artifact arising from the erroneous identification of the fourth and fifth size classes. On the other hand, similar size classes were also present at station H4 (Figure 32).

If logistic growth curves are replotted as a Walford plot, i.e., size of a cohort at a given age interval as a function of size at the preceding age interval, then the resulting plot will usually be linear (see Cerrato, 1980). To further compare growth curves between the reference and tailings influenced stations, Walford plots were reconstructed for stations SI, H2, and H3 (Figure 35). The Saanich Inlet data used includes estimated age classes at thirteen different sampling dates from October, 1987, to July, 1989 (see Chapter 4). The station H3 data were derived from the November, 1987, collection. For station H2, data from the November, 1987 collection and I.C.M.'s September, 1989, collection were used.

The lines in Figure 35 for stations SI, H2, and H3 are calculated from simple least-squares regressions, as shown in Table 11. A visual comparison of the lines shows that growth rates along the trough of Holberg Inlet (H2, H3) and in Mill Bay, Saanich Inlet (SI) are the same. There is no appreciable difference in the hypothetical age at which growth ceases (i.e., where the growth curve becomes asymptotic) as indicated by the point at which the lines cross a line with a slope of 1 (dotted line in Figure 35). Furthermore, there is no difference in the immediate

post-settlement instantaneous growth rate as indicated by the y-intercepts. Therefore, growth rates did not vary between reference site clams and those from mine-tailings influenced stations.

Table 11: Simple regressions of the growth of *A. serricata* from Saanich Inlet and Holberg Inlet based on plots of size at age X + 1 year as a function of size at age X (i.e., Walford plots).

Stn	N	Prob.	r^2(adj.)	Model
SI	30	<0.001	0.91	Y = 2.09 + 0.66 X
H2	4	0.006	0.98	Y = 2.19 + 0.60 X
H3	6	<0.001	0.96	Y = 2.22 + 0.61 X

Figure 35: Walford plots for *A. serricata* from Mill Bay, Saanich Inlet (SI), and lower Holberg Inlet (H2, H3) based on shell length frequency distributions. Table 11 provides details of the regression lines shown.
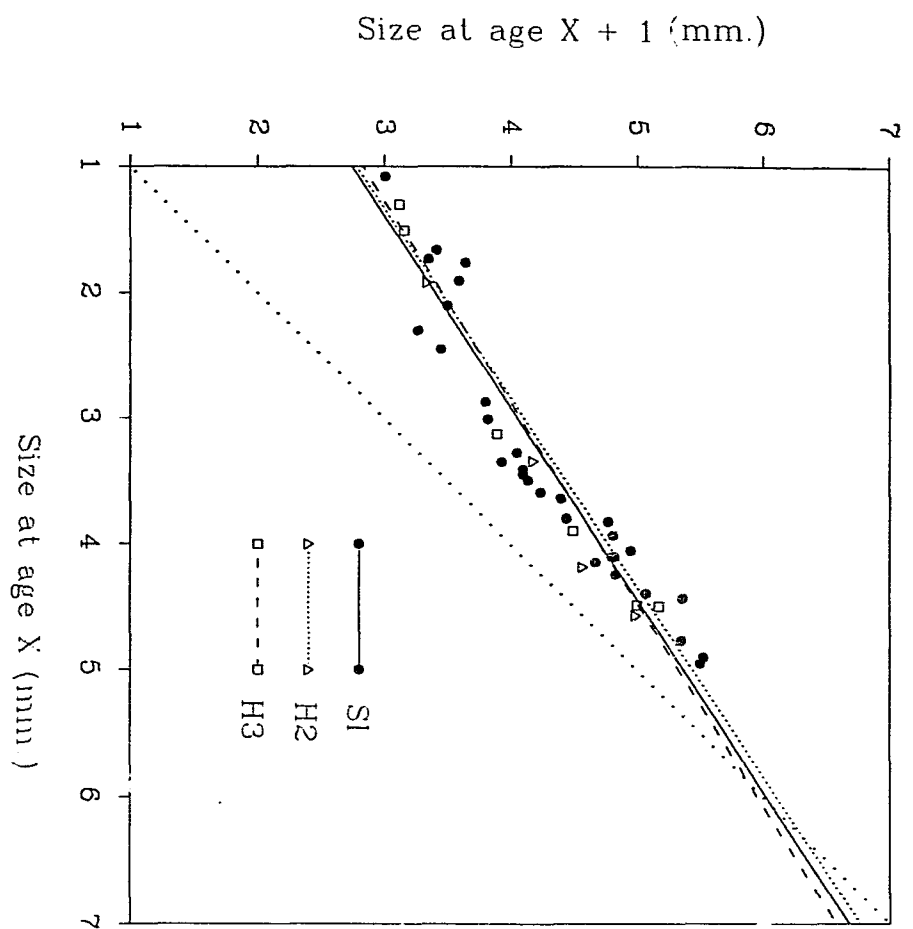
Temporal patterns of population structure.

A. serricata were sampled in November, 1987, for the examination of tissue structural alterations. Possible population effects were also examined by following the fate of the sub-populations at Holberg Inlet stations H1 (ICM 5), H3 (ICM 3), and H4 (ICM 24) from November, 1987, to September, 1990.

The sub-population of *A. serricata* from station H1 is in a state of decline, as illustrated in Figure 36. In 1987, the sub-population consisted of two or three older age classes. No recruitment has occurred at station H1 since before 1987. The total abundance of clams has steadily decreased and will decline to almost zero unless recruitment occurs in 1991.

In contrast, the youngest cohort was present in sub-populations from stations H3 and H4 examined from 1987 to 1990 (Figure 37 and 38 respectively). The low overall abundance of *A. serricata* at station H3 in September, 1989, may have resulted from sampling error (inadequate grab penetration, erroneous boat positioning, a patchy clam distribution, or a combination of these). However, macrofauna other than *A. serricata* were well represented in two of the three grab samples and the collection depth was similar to that of 1988 (I.C.M., 1990).

At station H3, the second and third age class were poorly represented in length frequency distributions from 1987, 1988, and 1990 (Figure 38). In spite of this, clams in the fourth and fifth year class were abundant. This might be related to a patchy distribution of annual settlement leading to spatial heterogeneity of population structure or to the recruitment of already settled, older clams.

Figure 36: Temporal change in the population structure of *A. serricata* from
station H1 in Holberg Inlet, British Columbia. Frequency distributions of
shell length were reconstructed from grab samples in November, 1987, and
September, 1988 to 1990.

Number of _A. serricata_ in three replicates

Shell length (mm)

1987

1988

1989

1990
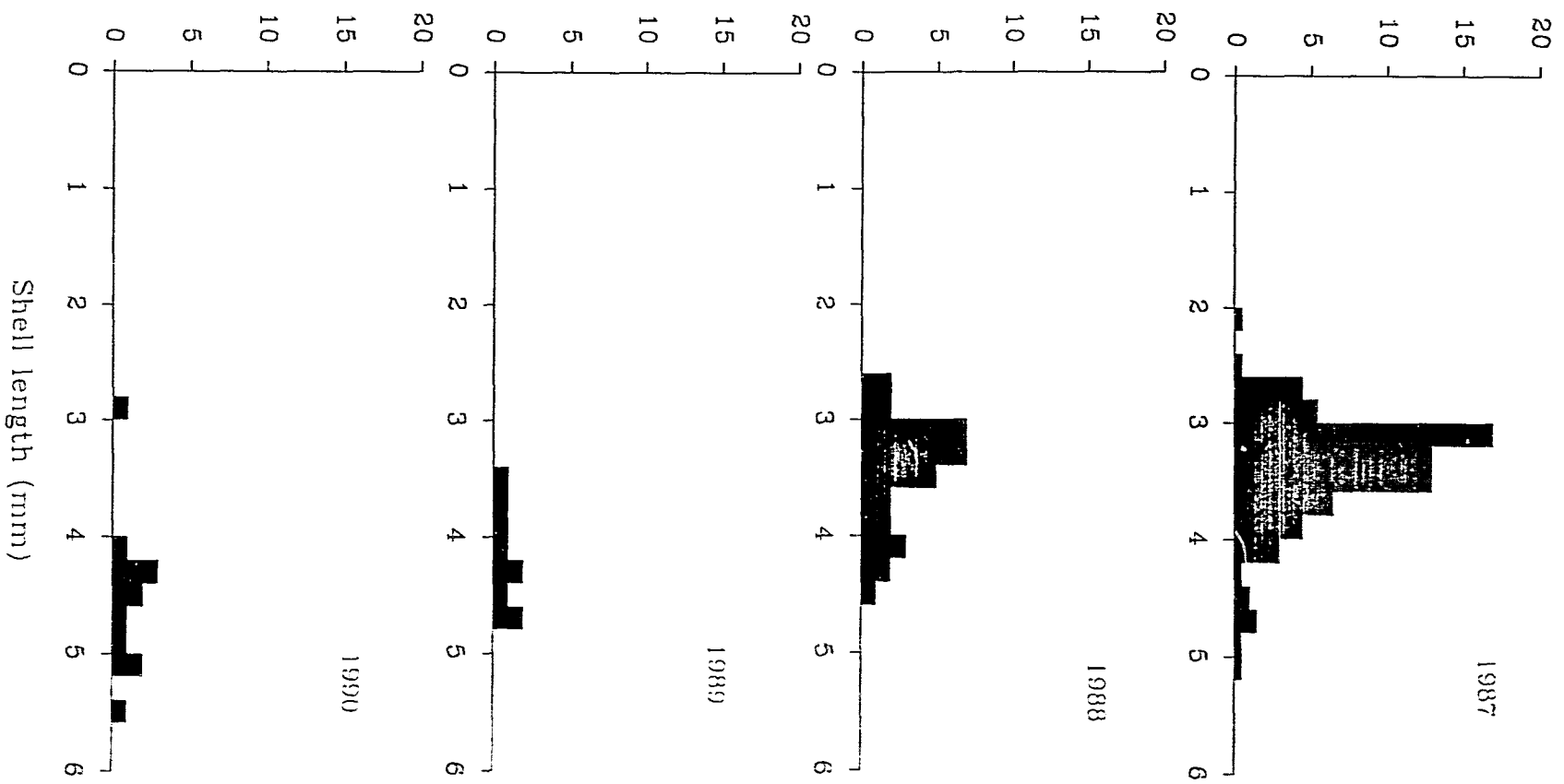
Figure 37: Temporal change in the population structure of *A. serricata* from station H3 in Holberg Inlet, British Columbia. Frequency distributions of shell length were reconstructed from grab samples in November, 1987, and September, 1988 to 1990.

Number of <u>A</u>. <u>serricata</u> in three replicates

Shell length (mm)

1987

1988

1989

1990
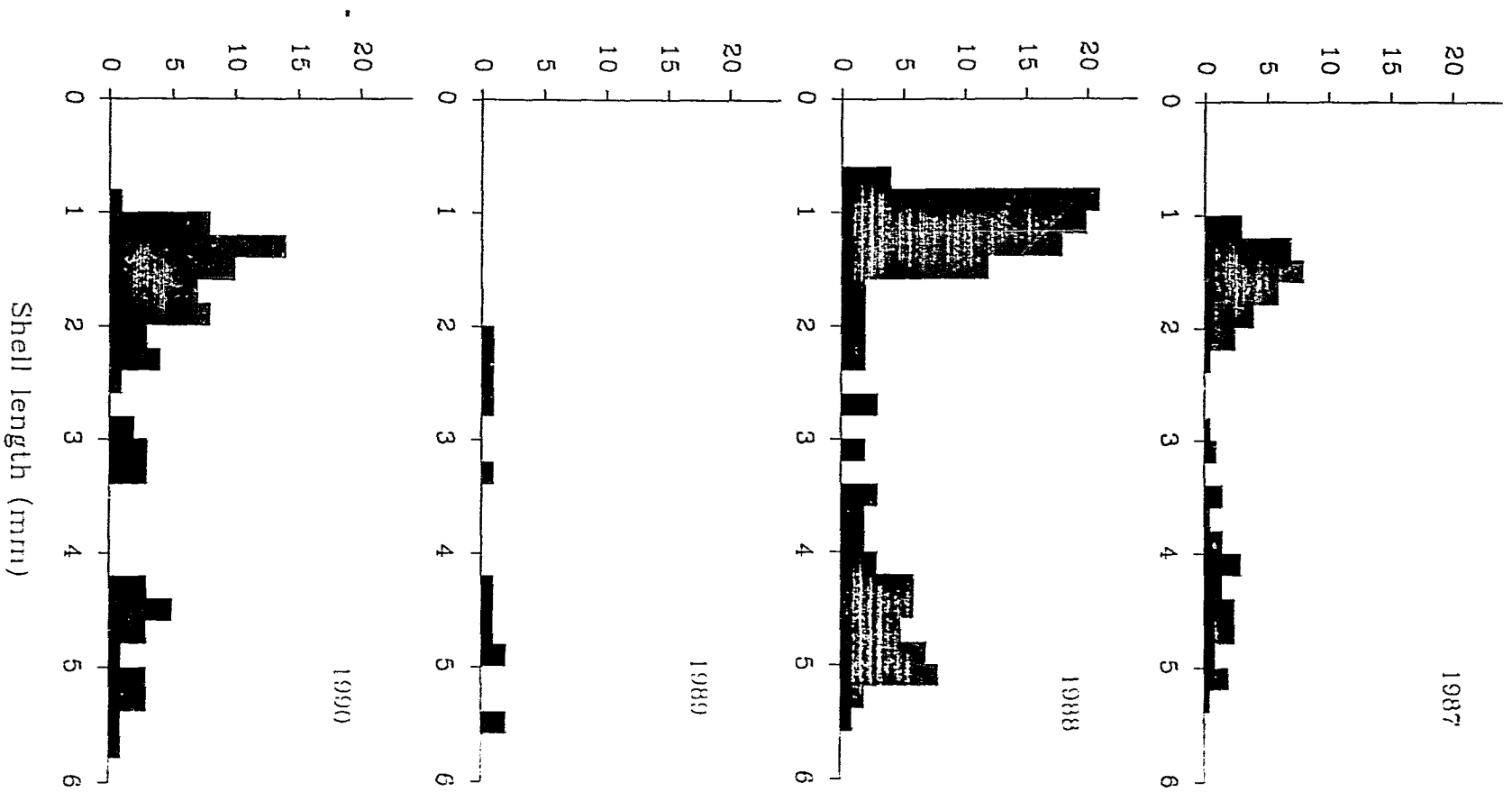
Figure 38: T·mporal change in the population structure of *A. serricata* from station H4 in Holberg Inlet, British Columbia. Frequency distributions of shell length were reconstructed from grab samples in November, 1987, and September, 1988 to 1990.

Number of _A. serricata_ in three replicates

Shell length (mm)

1987

1988

1989

1990
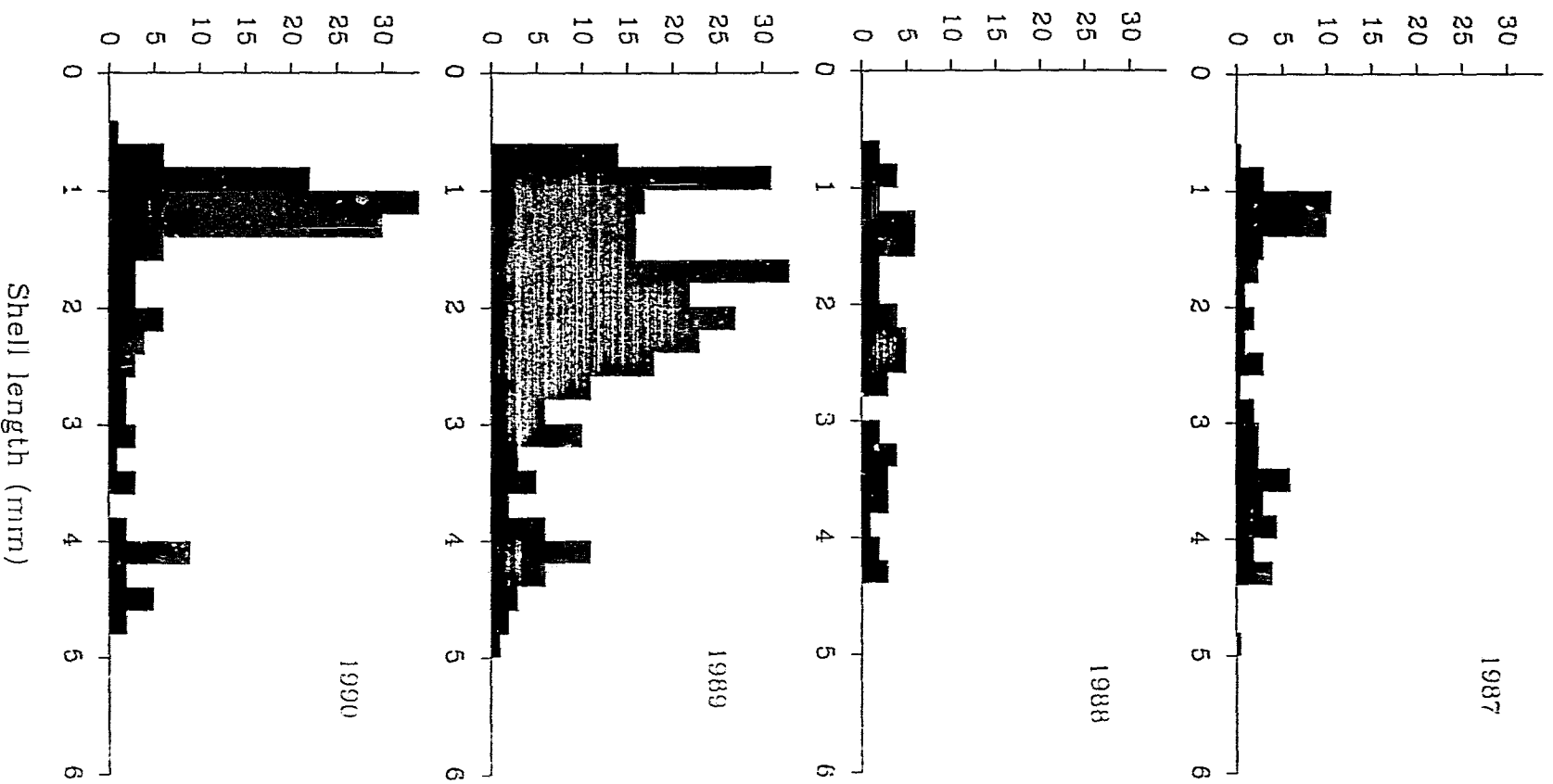
Spatio-temporal patterns of abundance.

The declining condition of the sub-population of *A. serricata* from station H1 is also apparent in the decline in total abundance since 1986, as shown in Figure 39. There is no apparent temporal trend in the abundance of the clams at stations H3 or H4, at least since 1983.

Figure 40 provides a simultaneous examination of spatial and temporal trend of *A. serricata* abundance along the trough of Rupert and Holberg Inlets. A near complete depression of clam abundance occurred at station 16 near the tailings outfall from 1971 to the present. Note that the initial abundance of *A. serricata* in 1971 at the outset of mine start-up was uniformly low in all of Rupert and Holberg Inlet. The smooth saddle-shaped area from 1972 to 1977 is an artifact of the lack of data collected during this period and three dimensional interpolation. An initial increase in abundance of *A. serricata* in Rupert and Holberg Inlets commenced in 1977 or slightly before.

There is an trend in peak abundance relative to mine tailings discharge, both spatially and temporally. For any given year, a peak abundance of *A. serricata* was observed in mid- to lower Holberg Inlet, with decreased abundances farther away from the discharge point. This pattern fits a community response defined by Pearson and Rosenberg (1978) as a 'peak of opportunists.' From 1977 to 1989, the peak of abundance migrated away from the point-source tailings discharge. Note the overall enhancement of abundance near the head of Holberg Inlet (ICM 24). Increased abundance of *A. serricata* occurs at most of the shallower I.C.M. monitoring stations (I.C.M., 1989).

Figure 39: Temporal trends of abundance (no./ m.^{2}) of *A. serricata* at Holberg Inlet, British Columbia, stations H1, H3, and H4. Vertical bars indicate standard deviation.
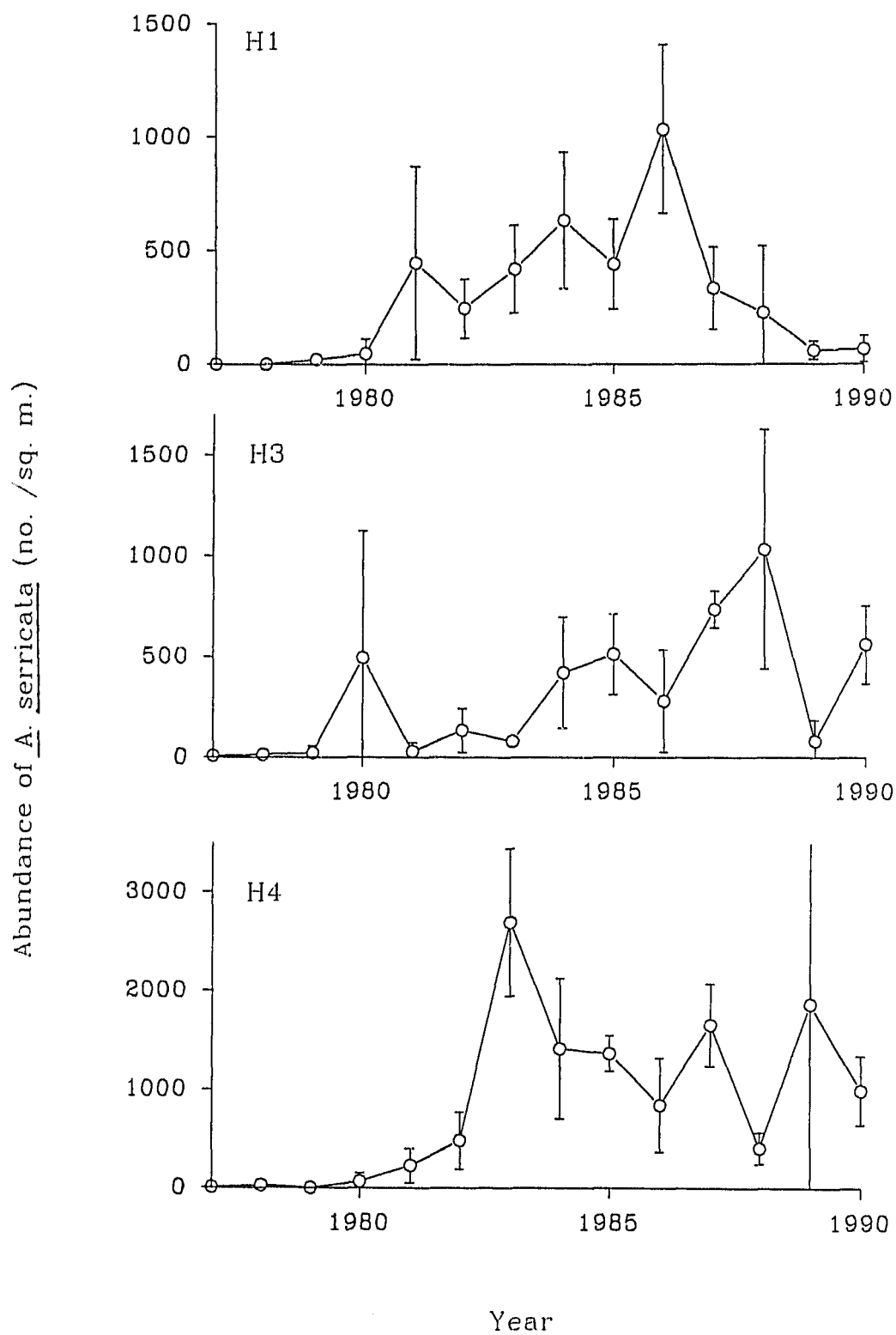
Figure 40: Spatio-temporal trends of abundance (no./m.^{2}) of *A. serricata* along the trough of Rupert and Holberg Inlets. The locations of the stations used are provided in Figure 29. The discharge of mine-tailings commenced in 1971, and data were plotted from collections made in September of each year, excluding 1973 to 1976 because taxa from benthic collections were only identified to class or phylum.

Note the peak of abundance commencing around 1980. This peak has migrated away from the tailings discharge since 1980. An overall increase in abundance of *A. serricata* in Rupert and Holberg Inlet has occurred since the commencement of mine-tailings discharge.

Number/sq.m.
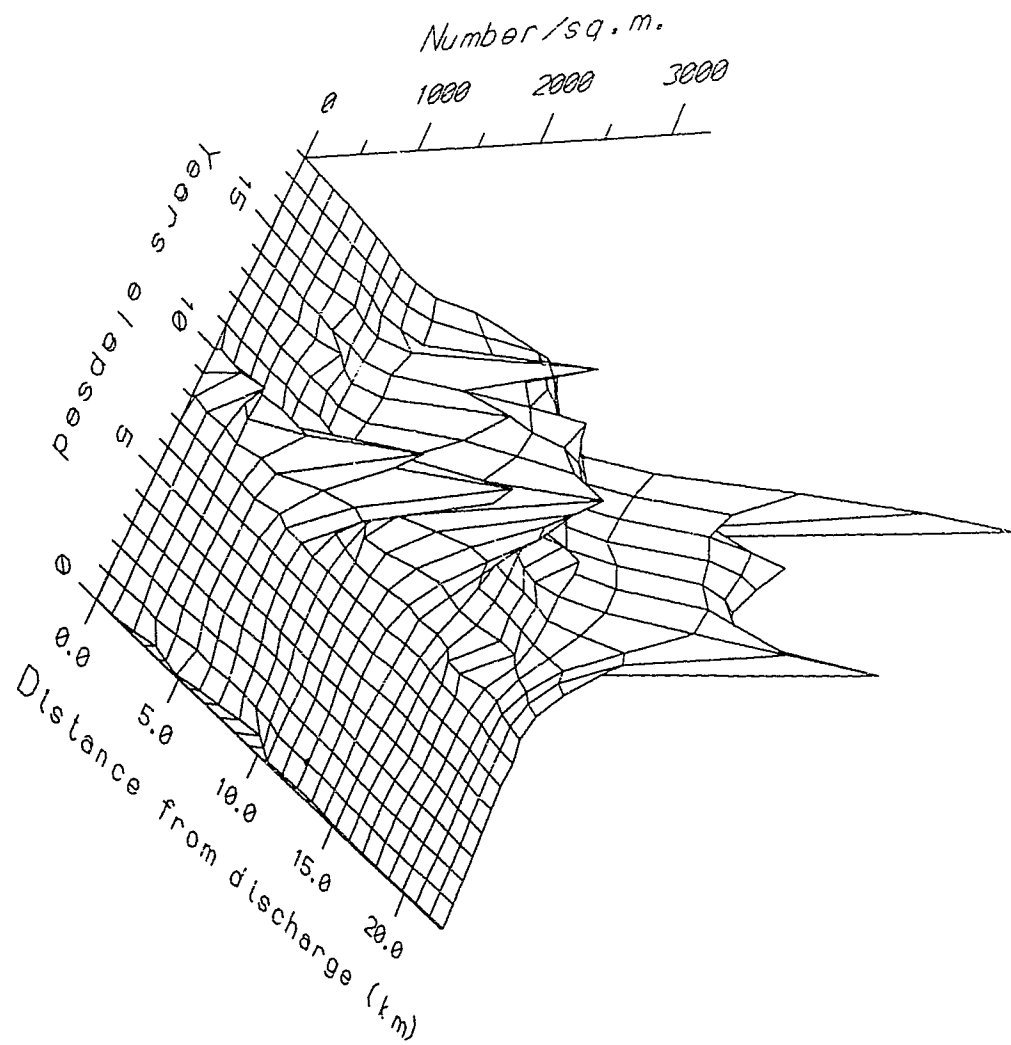
Deleterious changes in tissues and associated population-level effects.

Figure 41 shows the relationship between tissue structural variation in *A. serricata*, variation in population characteristics, and distance from I.C.M.'s mine-tailings discharge. A linear regression shows that the tissue variation captured by the second principal component of a P.C.A. of tissue structure (Chapter 3) varies as a function of distance from the discharge pipe (n = 6, prob. = 0.34, $r^2_{(adj.)}$ = 0.643). The incidence at each station of oedema of ctenidial lateral cells and/or hyperactivity of ctenidial mucocytes is not linearly correlated with distance from the discharge pipe (n = 6, prob. = 0.15), but there was a higher incidence of these ctenidial conditions at stations H1 and H2.

In contrast, the average abundance of *A. serricata* or relative fecundity were not related to the distance of the station from the discharge pipe (Figure 41). As shown above, the growth rates of clams at Holberg Inlet stations H2 and H3 were the same as growth rates of clams living in Mill Bay, Saanich Inlet. Therefore, spatial variation in fecundity, growth, and abundance do not parallel spatial variation in tissue structure. Furthermore, fecundity or abundance was not correlated with tissue variation as summarized by a principal components analysis or by the incidence of ctenidial lesions.

Figure 41. Spatial variation in the abundance and fecundity of *A. serricata* relative to a marine discharge of mine-tailings versus spatial variation in tissue structure. Vertical bars in the upper two plots represent standard deviations.

P.C.2 scores are the average scores at a station on the second principal component of a P.C.A. of six tissue variables. These scores vary linearly as a function of distance of the station from the tailings discharge pipe, as follows: P.C.2 = 1.38 - 0.83 x distance.
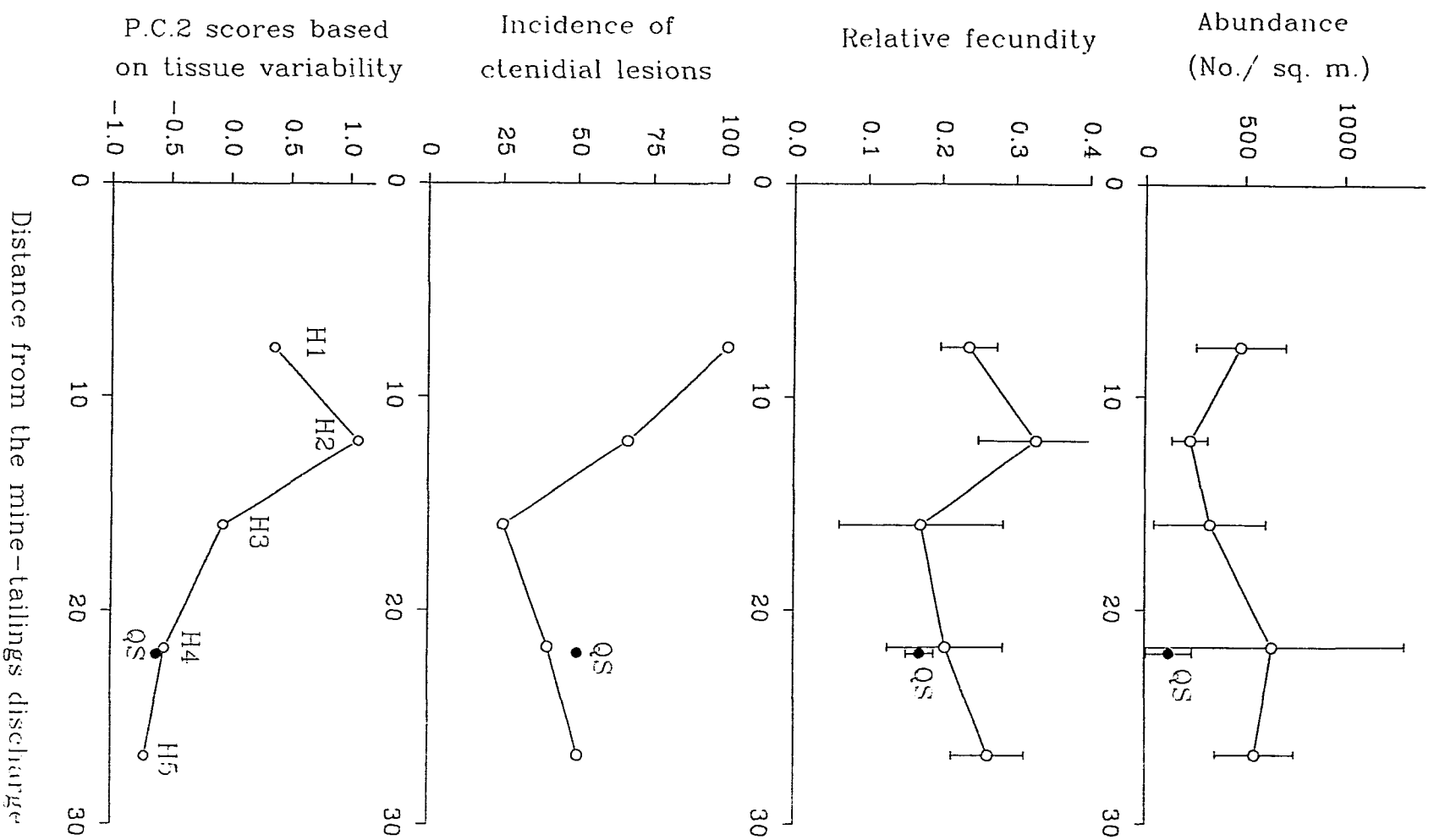
Discussion

Spatial variation in fecundity, growth, and abundance of *A. serricata* influenced by mine-tailings does not parallel spatial variation in tissue structure. Yet, variation of the digestive diverticula and ctenidium in lower Holberg Inlet *A. serricata* were interpreted to be maladaptive, or pathological (Chapter 2 and 3). If a condition is pathological, it should impose stress on an organism.

Selye (1957) defined stress as a characteristic pattern of hormonal and physiological responses precipitated by diverse environmental influences. Other researchers have provided alternate operational definitions of stress (see Brett, 1958; Sibly and Calow, 1989; Koehn and Bayne, 1989). Regardless of definition, it is generally accepted that prolonged stress or exposure to a stressor results in decreased fitness of an organism either by causing mortality or through alteration of energy expenditure for growth, maintenance repair, and reproduction.

The fecundity of *A. serricata* in Holberg Inlet and Quatsino Sound did not vary as a function of distance from I.C.M.s mine-tailings discharge. If anything, the gonad index (GI) and percent volume of oocytes in follicles (GFR) were higher close to the discharge pipe (stations H1 and H2) than farther afield.

Gonad index was estimated as the volume of gonad produced by individual *A. serricata* as a percent the volume of gonad and digestive diverticula combined. Therefore, a higher GI may reflect decreased volume of digestive diverticula rather than increased production of gonad.

In contrast, an elevated GFR at station H1 and H2 suggest that oocytes occupied a greater volume of space in the follicles, possibly because of increased investment in oocytes by the clams. Bayne (1985) suggested that there is evidence for the suppression of fecundity in molluscs subjected to environmental stressors. For example, Bayne *et al.* (1979) demonstrated that the number of eggs released by *Mytilus edulis* decreases with reduced scope for growth.

However, the other examples provided by Bayne (1985) suggest that, if anything, stress increases fecundity in marine molluscs. Intertidal bivalves (e.g. *Choromytilus meridionalis* studied by Griffiths, 1981; *Geukensia demissa* studied by Jordan and Valiela, 1982) and intertidal limpets (Sutherland, 1982) either maintain

or increase reproductive effort with increased height on the shore and increased aerial exposure.

Emmett *et al.* (1987) inferred that summertime mortalities of > 90% in cultured populations of *Mytilus trossulus* from British Columbia were related to reproductive stress; intense spawning coincides with the timing of high mortality.

In *A. serricata* and in other bivalves there is little evidence for compensatory variation in fecundity as a mechanism of reducing mortality. In fact, it is possible that stress increases reproductive output. This pattern might confer an evolutionary advantage by producing progeny which disperse from a stressful location.

The growth rates of *A. serricata* from lower Holberg Inlet stations H2 and H3 were similar to those of *A. serricata* from Mill Bay, Saanich Inlet. There were no between-station differences in growth rates which reflected the spatial pattern of tissue variation. Slower growth was observed at some stations (ICM 1, ICM 4, ICM 20) in Rupert and Holberg Inlets. The slower growth may have been a reflection of a coarser sediment, which is often correlated with depth of the seabed. However, the reason for slower growth of *A. serricata* at station ICM 4, at a depth of 118 m. is unknown.

There are apparently no other studies on field populations of marine invertebrates in which histopathological effects were adequately compared to growth. The incidence and severity of putative tissue lesions in *Macoma carlottensis* living in deposited tailings beds in Howe Sound, British Columbia, were not related to decreased abundance or to decreased growth as indicated by mean shell length (Bright and Ellis, 1989). However, growth at each station was not measured directly.

Within Rupert and Holberg Inlets, Jones and Ellis (1976) reported that size of the polychaete *Ophelina accuminata* (= *Ammotrypane aulogaster*) was inversely correlated with thickness of the deposited tailings blanket. The abundance of worms (no./m.^{2}) peaked at a point along a transect closer to the discharge than the peak in size, suggesting that the consequence of mine tailings discharge for body size (and growth) and population density were similar, but the geographical scales of impact were not equal. Since 1977, the abundance of *Ophelina* has declined to almost zero

in Rupert and Holberg Inlets and it is clear that other factors have also influenced population dynamics.

Fabrikant (1984) studied another lucinacean, *Parvilucina tenuisculpta*, near a sewage outfall in southern California. *P. tenuisculpta* (which contains sulfur-oxidizing endosymbiotic bacteria: Reid and Brand, 1986) responded to increased loading of organic matter and associated contaminants. The clams had increased growth rates due to increased availability of organic matter and sulfides. On the other hand, increased availability of toxic decomposition products of organic matter (*sensu* Bader, 1954) towards the sewage discharge were increasingly limiting to population density. Therefore a weak negative correlation was observed between mean shell length and population density. Fabrikant attempted to explain this apparent paradox by suggesting that resistance within the population was variable. Weaker members succumbed at lower levels of stress leaving more resistant individual with largely uninhibited growth rates. Similar results were obtained in this study for *A. serricata*. The largest average shell length occurred at station H1 closest to the discharge pipe, whereas peak abundance occurred farther away. However, increased average size in the face of decreased abundance cannot be reconciled by reference to increased availability of food substances since, in this case, the discharged material was inorganic tailings material.

In New York Bight, Steimle *et al* (1990) found little difference in growth rates or productivity to biomass (P:B) ratios across stations varying primarily in concentrations of sediment organic carbon and contaminants (trace metals, PAHs and PCBs) for 9 species of polychaetes including *Amastigos caperatus, Aricidea catherinae, Glycera dibranchiata, Nephthys picta, Lumbrineris acicularur ., Schistomeringo caeca, Spiophanes bombyx, Tharyx annulosus* and *Pherusa affinis*. This study suggested that certain population characteristics of some species are unresponsive to differences in contaminant exposure.

In contrast, Zajac and Whitlatch (1989) found that in Long Island Sound, dredge material disposal resulted in slowing or reversal of a previous trend toward population increase of *Nephthys incisa* up to 200 m. away from the dump site. Reduction of population growth rate (and productivity) was due to an initial failure

in recruitment immediately following dumping, and from decreased worm size and survivorship in the established population.

The temporal trend since 1987 indicates that a sub-population of *A. serricata* living in close proximity to I.C.M.'s mine-tailings discharge (station H1) is declining. This might be taken as evidence that tissue lesions exert deleterious effects on populations of *A. serricata*. However, the principle mechanism of population decline at station H1 was a failure of recruitment (Figure 36). It was not possible to determine if survivorship in the established population was also affected.

No relationship between tissue-level and population-level effects was found for *A. serricata* influenced by the marine discharge of mine-tailings. However, the manifestation of histopathological effects in 1987 occurred at a greater distance from the discharge pipe (to station H2 or H3) than the peak of abundance (ICM 4; Figure 29 and 40). For environmental monitoring, tissue structural variability of *A. serricata* appears to be more sensitive to environmental disturbance than its population parameters. The general discussion (below) pursues possible explanations for the apparent decoupling of tissue- and population-level responses to environmental stressors.

6. General Discussion and Conclusions.

Evolutionary and functional significance of tissue structure and body morphology in Axinopsida serricata.

The life-history characteristics of *A. serricata* are a mix of those characterizing an r-strategist (small body size, elevated abundance in disturbed habitats) and K-strategist (iteroparity, moderate maximum longevity, production of large, yolk-rich oocytes)(Chapter 4). Therefore, *A. serricata* is interpreted as stress-tolerant rather than as an opportunist. Differences between organisms in their tolerance to disturbance or stress are associated with their evolutionary history. The following discussion of Lucinacean evolution provides a basis for a broader interpretation of tissue and population response to stressors in *A. serricata*.

Reid and Brand (1986) proposed that the morphology and tissue structure of Lucinids is related to the co-evolution between sulfide-oxidizing chemoautrophic bacteria contained in the ctenidia and their clam host. They further suggested that the Thyasiridae and Ungulinidae each represent a monophyletic line in which a strategy of symbiosis characteristic of the hypothetical ancestor has largely been abandoned.

However, several features of *Axinopsida* lend credence to an alternate hypothesis. The lack of siphons in an infaunal species and relative lack of fusion of the mantle edges suggests that *Axinopsida* and other Lucinaceans have an impaired ability to isolate the mantle cavity from the surrounding sediment. The relative lack of specialization of cilia on laterofrontal cells of ctenidial filaments suggests a limited food sorting ability.

If *Axinopsida* is a bulk deposit feeder, there should be relatively large quantities of poorly sorted sediment in the digestive tract. Within histological sections, only a limited quantity of accumulated material comprised of sediment, diatom frustules and other debris was detectable in the posterior intestine (which confirms some ability to derive nutrition by conventional means). The intestine lacks the coiled elongation of many of the deposit-feeding Tellinidae such as *Macoma carlottensis* (Bright, 1987).

A sub-surface, bulk deposit feeder might be expected to place less investment in elaboration of sensory structures and nervous tissue; however, the size of the ganglia in *Axinopsida* relative to the small body size and the extensive development of sensory structures including statocysts and ciliary bundles on the middle fold of the mantle edge suggests a reasonably elaborate ability to respond to environmental cues.

Finally the enigmatic shape within Thyasirids of the visceral wall as extens' ... into arborescent tufts requires some explanation. Bernard (1972) suggested that an increased diameter of digestive tubules and ducts as seen in the Thyasirids was often associated with a macrophagous feeding habit in other bivalves. Based on an examination of gut content in histological sections, this theory seems very unlikely. Furthermore, the convoluted architecture created in the mantle cavity by the arborescent tufts is at odds with the need to efficiently sort and transport food and debris in a conventional filter- or deposit-feeder.

The most plausible explanation for these features is that *Axinopsida* and Thyasirids in general have evolved a body form which optimizes the uptake of dissolved organic matter (D.O.M.) from sediment interstitial waters, which is produced as a byproduct of microbial activity. Extension of the digestive diverticula into arborescent tufts would increase dramatically the surface area available for nutrient uptake relative to body volume or mass. This would also help to explain differences between Thyasirid species in the degree of branching of arborescent tufts (see Bernard, 1972). Larger Thyasirids have a more finely divided surface of the visceral mass which might be expected since body mass and nutrient requirements would increase proportionally with the cube of shell length, and a finer division of arborescent tufts would further increase surface to volume ratio. The least extensive elaboration of arborescent tufts occurs in very small Thyasirids, for example *Adontorhina* spp. (Scott, 1986).

A hypothesis of adaptation to uptake of D.O.M. is also appealing in examining the general evolution of Lucinacea. Reid and Brand (1986) suggested that a hypothetical ancestor of the Lucinacea was one which possessed a loose, ectosymbiotic relationship with sulfide-oxidizing chemoautotrophs. They further hypothesized that emergence of the Lucinacea, involving paedomorphic alterations

of ctenidia, siphons, gut, and mantle cavity, was driven by sulfide-oxidizing symbiosis.

An alternate hypothesis is that emergence of the Lucinacea, involving paedomorphosis, occurred in response to uptake of D.O.M. Pequignat (1973), Manahan et al. (1982) and Jorgensen (1983) documented the uptake of D.O.M. by bivalves. Manahan (1982) and Jaekle and Manahan (1989) documented D.O.M. uptake by bivalve veligers and pediveligers, which may contribute to nutrition along with pedal feeding (King, 1987) before development of a functional, filtering gill. The Lucinacean ancestor might resemble very small modern day Thyasirids or Ungulinids. Major sources of D.O.M. within marine sediments include decomposition of deposited organic matter by heterotrophic bacteria and synthesis by sulfide-oxidizing chemoautotrophic bacteria. The relative importance of these sources would depend on the chemical composition of the sediment interstitial water, i.e. on concentration of sulfide, and on redox potential, which are in turn influenced by depth and organic input. Bacterial decomposition of organic matter is mediated by the progressive utilization of different electron acceptors of decreasing free energy availability (O_2, nitrate, Mn^{4+}, Fe^{3+}, sulfate; Pedersen, 1983).

An evolutionary scheme for progressive elaboration of endosymbiosis with chemoautotrophs in the Lucinacea, and to a lesser extent in some of the larger Thyasirids (Reid and Brand, 1986) from a D.O.M.-utilizing ancestor is as follows. Emergence of a Lucinacean ancestor may have occurred as a permanent extension of the surface or near-surface pediveliger stage of a eulamellibranch ancestor, i.e through paedomorphosis. Divergence was accompanied by elaboration of the foot into a complex, vermiform organ capable of being inserted downward into the sediment. This may have initially resulted in increased efficiency of pedal-feeding, and provided channels for passage of interstitial water from a larger volume of sediment to body surfaces capable of D.O.M. uptake. Formation of extensive radial channels outward from the visceral mass by the foot would have the obvious advantage of increasing the volume of sediment pore water used for nutrition. If the sphere of influence of the clam is calculated using either a cube of a radius approximated by one half the width of the actual burrow or by the extensibility of the foot (under 5 mm. compared with over 30 mm. respectively), the advantage to forming radial networks of foot excavations becomes obvious.

Any increase in body size would require both the elaboration of body surface, possibly as arborescent tufts and a more typical eulamellibranch ctenidium, and a larger concentric radius of foot excavations, both vertically and horizontally. There may have been a related, independent evolutionary trend toward deeper burrowing into the sediment related to decreased vulnerability to epifaunal predators. In either case, increased exposure to increasingly sub-oxic to anoxic interstitial water would be a consequence of downward foot excavations.

Adaptations of the foot were likely accompanied by elaboration of the mantle edge as seen in all modern Lucinaceans in order to minimize inundation of the mantle cavity with sediment. Elaboration includes a general increase in thickness, extensive ciliation of the inner lip, and development of mantle glands (Allen, 1958).

Free sulfide is a powerful inhibitor of the cytochrome-C oxidase system (Hand and Somero, 1983; Powell and Somero, 1986) and an ability to tolerate through detoxification high environmental sulfide concentrations has been documented for several marine invertebrates. For an infaunal species living in fine sediments which are chemically reduced at a shallow depth (within the first few millimeters to centimeters of the sediment surface in many near-shore environments), the evolution of a sulfide-detoxification mechanism is a prerequisite to expanded exploitation of the habitat. Exploitation of increasingly anoxic, deeper burrows may have lead to utilization of the foot in excavating upwards in order to maintain contact with oxygen-rich surface water. Construction of anterior inhalant chambers with the foot probably occurred primitively as an anastomosing network of unconsolidated excavations as shown by *Axinopsida*, and in an advanced state as shown by *Thyasira flexuosa* and *Lucina pennsylvanica* (Allen, 1958) in which the integrity of a single or slightly-branched channel is maintained through cementation of sediment particles by mucus secretions from the foot.

Evolution of endosymbiosis within the Lucinidae probably occurred through an increasingly intimate association between sulfide-oxidizing autotrophic bacteria in the surrounding sediment and ultimately within the mantle cavity itself, and decreased reliance on decomposition of organic matter by heterotrophic bacteria. Elaboration of the subfilamentar tissue of the gill may represent a more recent adaptation associated with internalization of the symbiont.

The Ungulinidae may have diverged as particulate matter deposit-feeders from a D.O.M.-utilizing ancestor before radiation of the Thyasirids in response to D.O.M. utilization and the Lucinids in response to sulfide-oxidizing symbiosis. The complexity and extensibility of the foot in both the Ungulinidae and the Lucinidae are reduced in comparison to the Thyasiridae, which relates well to the proposed impetus for divergence.

In comparing this hypothetical scenario with that proposed by Reid and Brand (1986), major differences arise in the order in which features were thought to evolve. As proposed here, symbiosis was never adopted by ancestral Ungulinids. The presence of bacteria within gill filaments of some modern Thyasirids may represent a relatively recent development. A D.O.M.-utilizing ancestor appears to also explain the presence in the superfamily of an anterior inhalant opening, reduction of the stomach, and elaboration of the mantle margin and sense organs. Allen (1958) noted that the exhalant siphon present only in Lucinids is different in structure from that of the other eulamellibranchia. It is more likely that the siphon arose in a previously siphonless ancestor within the Lucinidae rather than being secondarily lost from a Lucinid-like ancestor in the Ungulinidae and Thyasiridae. Finally, the postulated uptake of D.O.M. in the Thyasiridae helps explain the arborescent tufts and adaptations of the foot.

Mechanisms for isolation between the clam and its surrounding environment are poorly developed in *A. serricata*. The burrowing behaviour of *A. serricata* possibly alters chemical diagenesis of sediment by creating networks of channels into the sediment at depths of up to 5 cm. or greater. This in turn would alter sediment porosity, redox potential and rates of pore-water diffusion and exchange with the overlying water column.

Aller (1982) discusses chemical alterations to sediments by macrobenthos. The walls of infaunal burrows, in the absence of a mucous lining, are permeable to solute diffusion associated with sedimentary reactions. For *A. serricata*, metals such as copper and zinc having greater solubility in oxidized form are expected to occur in elevated concentrations in the burrow. Therefore, *A. serricata's* evolutionary history may provide pre-adaptation for stress.

Decoupling of tissue variation and population-level effects in <u>Axinopsida serricata</u> living in deposited copper-mine tailings.

Axinopsida serricata collected along a hypothetical gradient of impact from copper mine-tailings discharge exhibited tissue structural variation. Digestive tubules, digestive ducts, mineralized granules in the kidney, tertiary lysosomes in stomach epithelial cells, and ctenidial cells exhibited variation, both seasonally and across stations.

Structural alterations which were considered to be pathological, i.e., which were tissue lesions, included increased swelling, fragmentation, and necrosis of digestive tubule digestive cells, oedema of ctenidial lateral cells, hyperactivity of ctenidial mucocytes, and disruption of filaments. However, there is no evidence that increased incidence and severity of these lesions influence population characteristics of *A. serricata*. This section explores the lack of spatial congruence between, or decoupling of, tissue and population variation.

Three lines of evidence indicate that extremes of tissue variation in *A. serricata* were pathological. (1) Spatial variation in the incidence and severity of tissue structural alterations were significantly correlated with distance of the sub-population from mine-tailings discharge. The tissue structure was quantified in a blind study, thus limiting subjectivity. (2) Tissues (digestive tubules and ctenidia) were generally in poorer condition just prior to the estimated time of spawning. This is predicted based on the additional stress imposed by seasonally-dependent reproductive output, and further suggests that tissue variation in *A. serricata* is related to stress. (3) Structural variations of the digestive tubules and ctenidia of *A. serricata* were qualitatively similar to histopathological effects described for other molluscs (reviewed in Chapter 1, and by Lowe (1988), Bright (1987), Bright and Ellis (1989)). Similar changes to digestive tubules and ctenidia have been extensively described in both field populations and in laboratory studies of molluscs exposed to toxins.

However, the spatial distribution of lesions in Holberg Inlet *A. serricata* was not reflected in spatial patterns of fecundity, growth, or abundance. The effect of a stressor at one level of biological organization should manifest itself at a higher subsequent level of organization if stress is prolonged or of sufficient magnitude.

Within an organism, stressors which affect fitness should also influence the population as a whole. There are three or more possible explanations for the apparent absence of population-level effects in this study, as discussed below.

Calow (1989) points out that stress can also enhance the long-term fitness of groups by acting as a selective pressure, and suggests a need to delineate between proximate and ultimate effects of stress. The chronic disturbance imposed by nineteen years of discharging copper-mine tailings to Rupert and Holberg Inlets may have selectively removed sensitive individuals of *A. serricata*, particularly in lower Holberg Inlet. However, tissue lesions were observed in the surviving clams at stations H1 and H2, and a majority of the *A. serricata* appeared to be affected (for example, the incidence of oedema of ctenidial lateral cells at station H1 was 70%). Therefore, altered tissue structure does not appear to substantially alter overall physiological functioning in *A. serricata*.

A delay between the manifestation of tissue structural change in individual *A. serricata* and manifestations of decreased fitness at the population level could explain an apparent absence of relationship between tissue-level and population-level effects. Delayed escalation of tissue-effects to the population level probably would not exceed the maximum longevity of the species (which for *A. serricata* is 5+ years). However, tailings have been deposited almost continuously in Holberg Inlet since the 1970s, a span of almost 20 years. On the other hand, the magnitude of stress induced by tailings deposition in lower Holberg Inlet stations H1 and H2 may have increased to critical levels for *A. serricata* only recently. This interpretation is indirectly supported by the temporal pattern of abundance of *A. serricata* in Rupert and Holberg Inlet (Figure 40 in Chapter 5). The peak of abundance has migrated away from the discharge pipe in recent years.

Kirkwood (1987) proposed that opportunistic species optimize the energy available for reproduction through strategies of neglect. Proportionately less energy is channeled into somatic maintenance and repair than in longer-lived, more K-selected species. A similar strategy by *A. serricata* might explain lack of variation in growth or reproduction associated with tissue lesions. Accumulation of somatic damage might not seriously decrease the lifetime fitness of a bivalve with a short maximum longevity. However, if somatic repair is neglected, then tissue damage

should accumulate with age. As noted in Chapter 3, the size of *A. serricata* was not causally related to the incidence or severity of tissue lesions.

The apparent decoupling of tissue-level and population-level effects of mine-tailings exposure in *A. serricata* may be related to selection of stress-tolerant individuals, a time lag in manifestation of decreased fitness at the population-level, homeostatic maintenance of growth and reproduction through strategies of neglect, or other mechanisms.

If an area is disturbed sufficiently to cause stress in resident organisms, there are two possible outcomes: either compensatory mechanisms are effective in limiting long-term consequences of stress, or a cascade of effects is manifested at a higher level of biological organization. Koehn and Bayne (1989) categorized responses of organisms to stress based on a hypothetical cascade of cause and effect as primary, secondary, or tertiary effects. In marine molluscs, Moore *et al.* (1987) have hypothesized a causal link between lysosomal membrane destabilization, protein catabolism, cell autolysis, and tissue level effects in digestive diverticula and gonad. There is, however, insufficient literature relating these effects to those at higher levels of biological organization.

Based on diverse studies of the responses of biota located along an increasing gradient of a stressor, it is apparent that there are at each level of biological organization internally consistent, interpretable patterns of impact. However, it is presently difficult to predict possible effects on one level of organization based on observations at another.

Conclusions.

The digestive diverticulum, ctenidium, kidney, and stomach epithelium in *Axinopsida serricata* exhibit variation in tissue structure. Tissue variation between sub-populations of *A. serricata* is influenced by the distance from a submarine discharge of copper-mine tailings and by the season of collection. Reproductively active clams were in worse condition, based on their tissue structure, than reproductively quiescent clams from the same location. Seasonally-dependent gamete production may place additional stress on individual clams.

The tissue variation measured in *A. serricata* was not related to infection of the ctenidial pouch by a flagellate or to the sex of the clam. Furthermore, tissue variation did not appear to be causally related to clam size as might be expected since larger, older clams have been exposed to the stressor(s) for a longer period of time.

Reid and Brand (1986) described *A. serricata* as an r-selected species. However, a maximum longevity of 5+ years and reproductive investment in large (maximum measured diameter exceeded 100 μm in formalin-fixed tissue), yolk-rich oocytes are at odds with the categorization of *A. serricata* as an opportunist.

Several of the tissue changes described for *A. serricata* have been described by other authors as pathological (see above); these include vacuolation, fragmentation and necrosis of digestive cells, oedema of ctenidial lateral cells, and hyperactivity of ctenidial mucocytes. However, sub-populations in lower Holberg Inlet with elevated incidence and severity of these putative lesions did not experience a demonstrable decrease in fecundity, growth, or abundance.

The apparent decoupling of tissue-level and population-level effects on *A. serricata* of mine-tailings discharge may have been related to (1) a time-lag in manifestation at the population-level, (2) selection of stress-tolerant individuals in tailings-influenced areas, (3) minimization of effects on growth and fitness through the neglect of somatic maintenance and repair, or (4) some other unknown mechanism. The anatomy and burrowing behaviour of *A. serricata* suggest that the mantle cavity may be routinely exposed to dissolved metals, sulfides, and organic decomposition products associated with sediment diagenesis. Therefore, the enhanced distribution of *A. serricata* in disturbed habitats could be partially attributable to pre-adaptation to stress.

7. LITERATURE CITED

Abbott, R.T., 1974. American Seashells. Van Nostrand Rheinhold Co., N.Y.

Allen, J.A., 1958. On the basic form and adaptations to habitat in the Lucinacea. Philisophical Transactions of the Royal Society (London), 241: 421-484.

Aller, R.C., 1982. The effects of macrobenthos on chemical properties of marine sediment and overlying water, in Animal-Sediment Relations. P.L. McCall and M.J.S. Tevesz, eds. pp. 53-102.

Auffret, M., 1988. Histopathological changes related to chemical contamination in *Mytilus edulis* from field and experimental conditions. Marine Ecology Progress Series 46: 101-107.

Axiak, V., J.J. George and M.N. Moore, 1988. Petroleum hydrocarbons in the marine bivalve *Venus verrucosa*: accumulation and cellular responses. Marine Biology 97: 225-230.

Bader, R. 1954. The role of organic matter in determining the distribution of pelecypods in marine sediments. Journal of Marine Research 13: 32-47.

Barnes, R.D., 1980. Invertebrate Zoology, 4th ed. Saunders College, Philadelphia.

Bayne, B.L. et al, 1978. The Effect of Stress and Pollution on Marine Animals.

Bayne, B.L., D.L. Holland, M.N. Moore, D.M. Lowe and J. Widdows, 1979. Further studies on the effects of stress in the adult on the eggs of *Mytilus edulis*. Journal of the Marine Biological Association of the U.K. 58(4): 825-843.

Bayne, B.L., 1980. Physiological measurements of stress. Rapport et Proces-Verbaux des Reunions. Conseil Internationale pour l'Exploration de la Mer 179: 56-61.

Bayne, B.L., 1985. Responses to environmental stress: tolerance, resistance and adaptation. In Marine Biology of Polar Regions and Effects of Stress on Marine Organisms. J.S. Gray and M.E. Christiansen, editors. John Wiley and Sons, N.Y. pp. 331-349.

Bernard, F.R., 1972. The genus *Thyasira* in western Canada (Bivalvia: Lucinacea). Malacologia 11(2): 365-389.

Blacknell, W.M. and A.D. Ansell, 1974. The direct development of the bivalve *Thyasira gouldi* (Philippi). Thalassia Jugosl. 10: 23-43.

Bodammer, J.E., 1979. Preliminary observations of teh cytopathological effects of copper sulphate on the chemoreceptors of *Callinectes sapidus*. In Marine Pollution: Functional Responses. F.J. Vernberg et al, eds. Pergamon Press, N.Y. pp. 223-237.

Boyden, C.R., 1977. Effect of size upon metal content of shellfish. Journal of the

Marine Biological Association of the U.K. 57: 675-714.

Boyden, C.R. and D.J.H. Phillips, 1981. Seasonal variation and inherent variability of trace elements in oysters and their implications for indicator studies. Marine Ecology - Progress Series 5: 29-40.

Brand, D., 1990. Prevalence of idiopathic lesions in English Sole, *Parophrys vetulus*, from Vancouver Harbour and selected inlets along the British Columbia coastline. Unpublished report to Environmental Protection Service, Environment Canada.

Brett, J.R., 1958. Implications and assessments of environmental stress. in The Investigations of Fish-Power Problems, H.R. MacMillan Lectures in Fisheries. P.A. Larkin, ed. University of British Columbia. pp. 1-111.

Bright, D.A., 1987. The histology of *Macoma carlottensis* Whiteaves, 1880 (Bivalvia: Tellinidae) and histopathology related to mine-tailings discharge. M.Sc. Thesis, University of Victoria. 134 pp.

Bright, D. A. and D.V. Ellis, 1989. Aspects of histology in *Macoma carlottensis* (Bivalvia: Tellinidae) and *in situ* histopathology related to mine-tailings discharge. Journal of the Marine Biological Association, U.K., 69: 447-464.

Brinkhurst, R.O., B.J. Burd and R.D. Kathman, 1987. Benthic studies in Alice Arm, B.C. during and following cessation of mine tailings disposal, 1982-1986. Canadian Technical Report of Hydrography and Ocean Sciences No. 89: 45 pp.

Brunnetti, R. I. Gola and M. Majone, 1986. Sister-chromatid exchange in developing embryos of *Mytilus galloprovincialis* Lmk. (Bivalvia). Mutation Research 174: 207-211.

Buchanan, J.B., 1984. Sediment analysis. In Methods for Study of Marine benthos. N.A. Holme and A.D. McIntyre, eds. Blackwell Scientific. pp. 41-65.

Burd, B.J. and R.O. Brinkhurst, 1990. Vancouver Harbour and Burrard Inlet Benthic Infaunal Sampling Program, October 1987. Canadian Technical Report of Hydrography and Ocean Sciences No. 122, 49 pp.

Byers, S.C., E.L. Mills and P.L Stewart, 1978. A comparison of methods of determining organic carbon in marine sediments, with suggestions for a standard method. Hydrobiologia.

Cajarville, M.P., J.A. Marigomez and E. Angulo, 1989. A stereological survey of lysosomal structure alterations in *Littorina littorea* exposed to 1-napthol. Comparative Biochemistry and Physiology 93C(2): 231-237.

Calabrese, A., J.I. McInnes, D.A. Nelson, R.A. Greig and P.P Yevich, 1984. Effects of long-term exposure to silver or copper on growth, bioaccumulation, and histopathology in the blue mussel *Mytilus edulis*. Marine Environmental Research 11: 253-274.

Calow, P., 1989. Proximate and ultimate responses to stress in biological systems. Biological Journal of the Linnean Society 37: 173-181.

Cerratto, R.M. 1980. Demographic analysis of bivalve populations, in Skeletal Growth of Aquatic Organisms. Biological Records of Environmental Change. D.C. Rhoads and R.A. Lutz, eds. Plenum Press, N.Y. pp. 417-465.

Conlan, K.E., 1977. The effects of wood deposition from a coastal log handling operation on the benthos of a shallow sand bed in Saanich Inlet, British Columbia. M.Sc. Thesis, University of Victoria. 202 pp.

Conlan, K.E. and D.V. Ellis, 1979. Effects of wood waste on sand-bed benthos. Marine Pollution Bulletin 10(9): 262-267.

Cossa, D., E., Bourget and J. Piuze, 1979. Sexual maturation as a source of variation in the relationship between cadmium concentration and body weight of *Mytilus edulis* L. Marine Pollution Bulletin 10: 174-176.

Dethlefsen, V., 1978. Occurrence and abundance of some skeletal deformities, diseases, and parasites of major fish species in dumping areas off the German coast. International Coucil for Exploration of the Sea (C.M. Papers and Reports), E:8, 17 pp.

Ellis, D.V., 1989. Environments at Risk. Springer-Verlag, N.Y.

Ellis, D.V., 1987. A decade of environmental impact assessment at marine and coastal mines. Marine Mining, 6: 385-417.

Ellis, D.V. and P.M. Hoover, 1990. Benthos on tailings beds from an abandoned coastal mine. Marine Pollution Bulletin 21: 477-480.

Ellis, D.V. and P.M. Hoover, in press. Benthos recolonizing mine tailings in British Columbia fjords. Marine Mining.

Emmett, B., K. Thompson and J.D. Popham, 1987. The reproductive and energy storage cycles of two populations of *Mytilus edulis* (Linne) from British Columbia. Journal of Shellfish Research 6(1): 29-36.

Engel, D.W. and B.A. Fowler, 1979. Copper and cadmium induced changes in the metabolism and structure of molluscan gill tissue, in Marine Pollution Functional Responses. F.J. Vernberg et al., eds. Pergamon Press, N.Y. pp. 239-256.

Engel, D.W., W.G. Sunda and B.A. Fowler, 1981. Factors affecting trace metal uptake and toxicity to estuarine organisms. I. Environmental parameters. In Biological Monitoring of Marine Pollutants. J.F. Verberg et al., eds. Academic Press, pp. 127-144.

Fabrikant, R., 1984. The effect of sewage effluent on the population density and size of the clam *Parvilucina tenuisculpta*. Marine Pollution Bulletin 15(7): 249-253.

Fantin, A.M. Bolognani, L. Benedetti, L. Bolognani and E. Ottaviani, 1982. The effect of lead pollution on the freshwater gastropod *Viviparus viviparus* L.: biochemical and histochemical features. Malacologia 22(1-2): 19-21.

Fischer, H. 1983. Shell weight as an independent variable in relation to cadmium content of molluscs. Marine Ecology - Progress Series 12: 59-75.

Fries, C.R. and M.R. Tripp, 1977. Cytological damage in *Mercenaria mercenaria* exposed to phenol, in Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems. D.A. Wolfe, ed. Pergamon Press, Oxford. pp. 174-181.

George, S.G, T.L. Coombs and B.J.S. Pirie, 1982. Characterization of metal-containing granules from the kidney of the common mussel, *Mytilus edulis*. Biochimica Biophysica Acta 716: 61-72.

George, S.G. and A. Viarengo, 1983. A model for heavy metal homeostasis and detoxification in mussels.

Goldberg, E.D., 1980. The International Mussel Watch. Report of a Workshop Sponsored by the Environmental Studies Board. Commission on Natural Resources. National Research Council. National Academy of Sciences. Washington, D.C. 248 pp.

Grant, R., 1989. The use of graphical methods to estimate demographic parameters. Journal of the Marine Biological Association, U.K., 69: 367-371.

Grassle, J.F. and J.P. Grassle, 1978. Life histories and genetic variation in marine invertebrates, in Marine Organisms: Genetics, Ecology and Evolution. NATO Conference Series, B. Battaglia and J.A. Beardmore, eds., Plenum Press, N.Y.

Grassle, J.F. and H.L. Sanders, 1973. Life histories and the role of disturbance. Deap-Sea Research 20: 643-659.

Green, R.H., 1979. Sampling Design and Statisitical Methods for Environmental Biologists. J. Wiley and Sons, Toronto, 257 pp.

Gray, J.S., 1989. Effects of environmental stress on species rich assemblages. Biological Journal of the Linnean Society 37: 19-32.

Gustafson, R.G., 1985. Development, morphogenesis, and putative symbiont transmission in the percalymma larva of *Solemya reidi* (Bivalvia, Cryptodonta, Solemyidae). Ph.D. Thesis, University of Victoria.

Griffiths, R.J., 1981. Production and energy flow in relation to age and shore level in the bivalve *Choromytilus meridionalis*. Estuarine and Coastal Shelf Science 13: 477-493.

Hand, S.C. and G.N. Somero, 1983. Energy metabolism pathways of hydrothermal vent animals: adaptations of a food-rich and sulfide-rich deep-sea environment. Biological Bulletin 165: 167-181.

Hay, A.E. 1981. Submarine channel formation and acoustic remote sensing of suspended sediment and turbidity currents in Rupert Inlet, B.C. Ph.D. thesis, University of British Columbia.

Heller, J. ,1990. Longevity in molluscs. Malacologia 31(2): 259-295.

Hermans, C.O., 1979. Polychaete egg sizes, life histories and phylogeny. In Reproductive Ecology of Marine Invertebrates, The Belle W. Baruch Library in Marine Science Number 9, S. E. Stancyk, editor. University of S. Carolina Press, S.C. pp. 1-10.

Hietanen, B., I. Sunila and R. Kristoffersson, 1988. Toxic effects of zinc on the common mussel Mytilus edulis L. (Bivalvia) in brackish water. I. Physiological and histopathological studies. Annales Zoologici Fennici 25: 341-347.

Hinton, D.E. and J.A. Couch, 1984. Pathobiological measures of marine pollution effects. In Concepts in Marine Pollution Measurements, H.H. White, editor. Maryland Seagrant Publication, pp. 7-32.

Hoff, J.T, J.A.J. Thompson and C.S. Wong, 1982. Heavy metal release from mine tailings into sea water- a laboratory study. Marine Pollution Bulletin 13(8): 283-286.

Homer, B.L. and K. R. Pierce, 1989. Morphometric cytochemistry of diminution of catalase-containing peroxisomes in copper-loaded liver. Histochemical Journal 21: 63-71.

Island Copper Mine, 1986. Island Copper Mine Annual Environmental Assessment Report for 1985. Vol. 2. Unpublished.

Island Copper Mine, 1987. Island Copper Mine Annual Environmental Assessment Report for 1986. Vol. 2. Unpublished.

Island Copper Mine, 1988. Island Copper Mine Annual Environmental Assessment Report for 1987. Vol. 2. Unpublished.

Island Copper Mine, 1989. Island Copper Mine Annual Environmental Assessment Report for 1988. Vol. 2. Unpublished.

Jackim, E., G. Morrison and R. Steele, 1977. Effects of environmental factors on radiocarbon uptake by four species of marine bivalves. Marine Biology 40: 303-308.

Jaekle, W.B. and D.T. Manahan, 1989. Feeding by a "non-feeding" larva: uptake of dissolved amino acids from seawater by lecithotrophic larvae of the gastropod Haliotis rufescens. Marine Biology 103: 87-94.

Jeffries, H.P., 1972. A stress syndrome in the hard clam Mercenaria mercenaria. Journal of Invertebrate Pathology 20(3): 242-251.

Jones, A.A. and D.V. Ellis, 1976. Sub-obliterative effects of mine-tailings on marine

infaunal benthos. II: Effect on the productivity of *Ammotrypane aulogaster*. Water, Air, and Soil Pollution 5: 299-307.

Jones, I.M. and F.L. Harrison, 1987. Variability in the frequency of sister-chromatid exchanges in the larvae of *Mytilus edulis*: implications for field monitoring, Journal of Experimental Marine Biology and Ecology 113: 283-288.

Jordan, T.E. and I Valiela, 1982. A nitrogen budget of the ribbed mussel, *Geukensia demissa*, and its significance in energy flow in a New England salt marsh. Limnology and Oceanography 27: 75-90.

Jorgensen, C.B., 1983. Patterns of uptake of dissolved amino acids in mussels (Mytilus *edulis*). Marine Biology 73: 177-182.

Julshamn, K. 1981. Studies on major and minor elements in molluscs in western Norway. III. Effects of size and age on the content of 10 elements in oyster (*Ostrea edulis*). taken from unpolluted waters. Fiskeridirectoratets Skrifter Serie Ernaering, 1: 199-214.

Kathman, R.D., R,O Brinkhurst, R.E Woods and D.C. Jeffries, 1983. Benthic studies in Alice Arm and Hastings Arm, B.C. in relation to mine tailings dispersal. Canadian Technical Report of Hydrography and Ocean Sciences No 22. 30 pp.

Kathman,R.D., R.O. Brinkhurst, R.E. Woods and S.F. Cross, 1984. Benthic studies in Alica Arm, B.C., following cessation of mine tailings disposal. Canadian Technical Report of Hydrography and Ocean Sciences No. 37. 57 pp.

King, J.J.,1987. Juvenile feeding ontogeny of the geoduck *Panope abrupta* (Bivalvia: Saxicavacea) and comparative ontogeny of feeding in bivalves. M.Sc. Thesis, University of Victoria. 261 pp.

Kirkwood, T.B.L., 1987. Maintenance and repair processes in relation to senescence: adaptive strategies of neglect, in Environmental Physiological Ecology. P. Calow, ed. Cambridge University Press, N.Y. pp. 53-66.

Koehn, R.K. and B.L. Bayne, 1989. Towards a physiological and genetical understanding of the energetics of the stress response. Biological Journal of the Linnean Society 37: 157-171.

Krebs, C.J., 1978. Ecology: The Experimental Analysis of Distribution and Abundance. Harper and Row, N.Y. p. 622.

Langton, R.W., 1975. Synchrony in the digestive diverticula of Mytilus *edulis*. Journal of the Marine Biological Association, U.K. 55: 221-229.

Levings, C.D., E.P. Anderson and G.W. O'Connell, 1985. Biological effects of dredged material disposal in Alberni Inlet, in The Role of the Oceans as a waste-disposal option. G.Kullenberg, ed. Reidel Publ. Co., Boston. pp. 131-155.

Lie U. and D.S. Kisker, 1970. Species composition and structure of benthic infauna communities off the coast of Washington. Journal of the Fisheries Research Board of Canada 27: 2273-2285.

Littlepage, J., 1978. Oceanographic and marine biological surveys, Alice and Hastings Arm, B.C. 1974-1977. Unpublished report for Climax Molybdenum Corp. B.C. Ltd.

Loosanoff, V.L., H.C. Davis and P.E, Chanley, 1966. Dimensions and shapes of larvae of some marine bivalve mollusks. Malacologia 4: 351-435.

Lopez-Jamar, E., G. Gonzalez and J. Mejuto, 1987. Ecology, growth and reproduction of *Thyasira flexuosa* (Bivalvia, Lucinacea) from Ria de la Coruna, north-west Spain. Ophelia 27(2): 111-126.

Lowe, D.M., 1988. Alterations in cellular structure of *Mytilus edulis* resulting from exposure to environmental contaminants under field and experimental conditions. Marine Ecology Progress Series 46: 91-100.

Lowe, D.M., M.N. Moore and K.R. Clarke, 1981. Effects of oil on the digestive cells in mussels: quantitative alterations in cellular and lysosomal structure. Aquatic Toxicology 1: 213-226.

Lowe, D.M. and R.K. Pipe, 1986. Hydrocarbon exposure in mussels: a quantitative study of the responses in the reproductive and nutrient storage cell systems. Aquatic Toxicology 8: 265-272.

Majone, F., R. Brunneti. I Gola and A.G. Levis, 1987. Persistence of micronuclei in the marine mussel *Mytilus galloprovincialis* after treatmetn with mytomycin C. Mutation Research 191: 157-161.

Malins. D.C., B.B. McCain, J.T. Landahl, M.S. Myers, M.M Krahn, D.W. Brown, S.-L. Chan and W.T. Roubal. 1988. Neoplastic and other diseases in fish in relation to toxic chemicals: an overview. Aquatic Toxicology 11: 43-67.

Malins, D.C., B.B. McCain, D.W. Brown, A.K. Sparks, H.O. Hodgins and S.-L. Chan. 1982. Chemical contaminats and abnormalities in fish and invertebrates from Puget Sound. NOAA Technical Memorandum OMPA-19. 168 pp.

Manahan, D.T., 1982. The role of dissolved organic matter in the nutrition of bivalve larvae. Ph.D. Thesis, University of Wales, U.K.

Manahan, D.T., S.H. Wright, G.C. Stephens and M.A. Rice, 1982. Transport of dissolved amoni acids by the mussel *Mytilus edulis*: demonstration of net uptake from natural seawater. Science 215: 1253-1255.

Marigomez, J.A., E.Angulo and E. Moya, 1986. Copper treatment of the digestive gland of the slug *Arion ater* L. 2. Morphometrics and histophysiology. Bulletin of Environmental Contamination and Toxicology 36: 608-615.

Marigomez, J.A., M.M. Vega, M.P. Cajarville and E. Angulo, 1989. Quantitative

responses of the digestive-lysosomal system of winkles to sublethal concentrations of camium. Cellular and Molecular Biology 35(5): 555-562.

Mason, A.Z. and J.A. Nott, 1980. A rapid, routine technique for the x-ray microanalysis of microincinerated cryosections: an SEM study of inorganic deposits in tissues of the marine gastropod *Littorina littorea* (L.). Journal of Histochemistry and Cytochemistry 28(12): 1301-1311.

Mason, A.Z., K. Simkiss and K.P. Ryan, 1984. The ultrastructural localization of metals in specimens of *Littorina littorea* collected from clean and polluted sites. Journal of the Marine Biological Association of the U.K. 64: 699-720.

Maung Myint, U. and P.A. Tyler, 1982. Effects of temperature, nutritive and metal stressors on the reproductive biology of *Mytilus edulis*. Marine Biology 67: 209-223.

McDonald, J.H. and R.K. Koehn, 1988. The mussels *Mytilus galloprovincialis* and *M. trossulus* on the Pacific coast of North America. Marine Biology 99: 111-118.

Moore, M.N. and K.R. Clarke, 1982. Use of microstereology and quantitative cytochemistry to determine the effects of crude-oil-derived hydrocarbons on lysosomal structure and function in a marine bivlave mollusc, *Mytilus edulis*. Histochemistry Journal 14: 713-718.

Moller, H., 1990. Association between diseases of flounder (*Platichthys flesus*) and environmental conditions in the Elbe estuary, FRG. Journal du Conseil Internationale pour l'Exploration de la Mer 46: 187-199.

Moore, M.N., 1980. Cytochemical determination of cellular responses to environmental stressors in marine organisms. Rapport et Proces-Verbaux des Reunions. Conseil Internationale pour l'Exploration de la Mer 179: 7-15.

Morton, B., 1970. A note on the cytological structure and function of the digestive diverticula of *Macoma balthica* correlated with rhythm of the tide. Malacological Review 3: 115-119.

Nicolaidou, A., M.A. Pancucci and A. Zenetos, 1989. The impact of dumping coarse metalliferous waste on the benthos in Evoikos Gulf, Greece. Marine Pollution Bulletin 20(1): 28-33.

Nissenbaum, A. and D.J. Swaine, 1976. Organic matter-metal interactions in recent sediments: the role of humic substances. Geochimica et Cosmochimica Acta 40: 809-816.

Ockelmann, K.W., 1965. Developmental types in marine bivalves and their distribution along the Atlantic coast of Europe. In Proceedings of the First European Malacology Congress (1962), pp. 25-35.

Owen, G., 1955. Observations of the stomach and digestive diverticula of the Lamellibranchia. I. The Anisomyaria and Eulamellibranchia. Quarterly Journal of Microscopical Science 96: 517-537.

Pal, S.G., 1972. the fine structure of the digestive tubules of *Mya arenaria* L. II. Digestive cells. Proceedings of the Malacological Society of London 40: 161-170.

Parsons, P.A., 1982. Adaptive strategies of colonizing animal species. Biological Review of the Cambridge Philosophical Society 57: 117-148.

Pearson T.H. and R. Rosenberg, 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanography and Marine Biology Annual Review 16: 229-311.

Pedersen, T.F., 1983. Interstitial water metabolite chemistry in a marine mine-tailings deposit, Rupert Inlet, B.C. Canadian Journal of Earth Science 21: 1-9.

Pedersen, T.F., 1985. Early diagenesis of copper and molybdenum in mine tailings and natural sediments in Rupert and Holberg Inlets, British Columbia. Canadian Journal of Earth Science, 22(10): 1474-1484.

Pedersen, T.F. and A.J. Losher, 1988. Diagenetic process in aquatic mine tailings deposits in British Columbia. In: Chemistry and Biology of Solid Waste. W. Salomons and U. Forstner, editors. Springer-Verlag, Berlin, pp. 238-258.

Pequignat, E., 1973. A kinetic and autoradiographic study of the direct accumulation of amino acids and glucose by the organs of the mussel *Mytilus edulis*. Marine Biology 19: 227-244.

Pesch, G.G. and C.E. Pesch, 1980. *Neanthes arenoceodentata* (Polychaeta, Annelida), a proposed cytogenetic model for marine genetic toxicology. Canadian Journal of Fisheries and Aquatoc Science 37: 1225.

Peters, E.C., 1988. Recent investigations on disseminated sarcomas of marine bivalve molluscs, in Disease Processes in Marine Bivalve Molluscs. pp. 74-92.

Pianka, E., 1970. On "r" and "K" selection. American Naturalist 104: 592-597.

Pickard, G.L., 1963. Oceanographic features of inlets in Vancouver Island, British Columbia. Journal of the Fisheries Research Board of Canada 20(5): 1109-1144.

Pirie B.J.S. and S.G. George, 1979. Ultrastructure of the heart and excretory system of *Mytilus edulis* (L.). Journal of the Marine Biological Association, U.K. 59: 819-829.

Powell, E.N. and H. Cummins, 1985. Are molluscan maximum life spans determined by long-term cycles in benthic communities ? Oecologia 67: 177-182.

Powell, M.A. and G.N. Somero, 1986. Adaptations to sulfide by hydrothermal vent animals: sites and mechanisms of detoxification and metabolism. Biological Bulletin 171: 274-290.

Presley, B.Y., Y. Kolodny, A. Nissenbaum and I.R. Kaplan, 1972. Early diagenesis in

a reducing fjord, Saanich Inlet, British Columbia. 2. Trace-element distribution in interstitial water and sediment. Geochimica Cosmchimica Acta 36: 1073.

Rapport, D.J., 1989. Symptoms of pathology in the Gulf of Bothnia (Baltic Sea): ecosystem response to stress from human activity. Biological Journal of the Linnean Society, 37: 33-49.

Reid, R.G.B. and D.G. Brand, 1986. Sulfide-oxidizing symbiosis in Lucinaceans: implications for bivalve evolution. The Veliger, 29(1): 3-24.

Reid, R.G.B. and D.G. Brand, 1989. Giant kidneys and metal-sequestering nephroliths in the bivalve *Pinna bicolor*, with comparative notes on *Atrina vexillum* (Pinnidae). Journal of Experimental Marine Biology and Ecology 126: 95-117.

Reynolds, P.D., 1990. Functional morphology of the heart/kidney complex, digestive system and mantle of *Dentalium rectius* (Mollusca, Scaphopoda). Ph.D. Dissertation, Universtiy of Victoria.

Rheinberger, R., G.L. Hoff and P.P Yevich, 1979. the kidney of the quahog (*Mercenaria mercenaria*) as a pollution indicator, in Animals as Monitors of Environmental Pollutants: Animal Models and Wildlife as Monitors. Academy of Sciences, Washington, D.C. pp. 119-129.

Rhoads, D.C. and L.F. Boyer, 1982. The effects of marine benthos on physical properties of sediments: a successional perspective, in Animal-Sediment Relations. P.L. McCall and M.J.S. Tevesz, eds. pp. 3-52.

Richards, F.A., 1965. Chemical observations in some anoxic, sulfide-bearing basins and fjords. In Proceedings of the Second International Water Pollution Research Conference, Tokyo 1964, pp. 215-243.

Robinson, W.E. and R.W. Langton, 1980. Digestion in a subtidal population of *Mercenaria mercenaria* (Bivalvia).Marine Biology 58: 173-179.

Robinson, W.E., 1983. Assessment of bivalve intracellular digestion based on direct measurements. Journal of Molluscan Studies, 49: 1-8.

Ruddell, C.L. and D.W. Rains, 1975. The relationship between zinc, copper and the basophils of two crassostreid oysters, *C. gigas* and *C. virginica*. Comparative Biochemistry and Physiology Series A 51: 585-591.

Sahota, T.S., F.G. Peet, A. Ibaraki and S.H. Farris, 1985. Chromatin distribution pattern and cell functioning. Canadian Journal of Zoology 64: 1908-1913.

Scarpato, R., L. Migliore, G. Alfinito-Cognetti and R, Barale, 1990. Induction of micronuclei in gill tissue of *Mytilus galloprovincialis* exposed to polluted marine waters. marine Pollution Bulletin 21(2): 74-80.

Scott, P.H., 1986. A new species of *Adontorhina* (Bivalvia: Thyasiridae) from the

Northeast Pacific, with notes on *Adontorhina cyclia* Berry 1947. The Veliger 29(2): 1-46.

Seiler, G.R. and M.P. Morse, 1988. Kidney and hemocytes of *Mya arenaria* (Bivalvia): normal and pollution-related ultrastructural morphologies.Journal of Invertebrate Pathology, 52: 201-214.

Selye, H.,1957. The Stess of Life. McGraw-Hill Co. Ltd., London.

Sibly, R.M. and P. Calow, 1989. A life-cycle theory of responses to stress. Biological Journal of the Linnean Society 37: 101-116.

Simkiss, K. and A.Z. Mason, 1983. Metal ions: Metabolic and toxic effects. in The Mollusca, Vol. 2: Environmental Biochemistry and Physiology. P.W. Hochachka, ed. Academic Press, N.Y. pp 101-164.

Sindermann, C.J. 1980. The use of pathological effects of pollutants in marine environmental monitoring programs. Rapport et Proces-Verbaux des Reunions. Conseil Internationale pour l'Exploration de la Mer 179: 129-134.

Southward, A.J. and E.C. Southward, 1970. Observations on the role of dissolved organic compounds on the nutrition of benthic invertebrates. Sarsia 45: 69-96.

Steimle, F.W. Jr., P. Kinner, S. Howe and W. Leathem, 1990. Polychaete population dynamics and production in the New York Bight associated with variable levels of sediment contamination. Ophelia 31(2): 105-123.

Sunila, I. and R. Lindstrom, 1985. The structure of the interfilamentar junction of the mussel (*Mytilus edulis* L.) gill and its uncoupling by copper and cadmium exposures. Comparative Biochemistry and Physiology 81C(2): 267-272.

Sunila, I., 1984. Copper- and cadmium-induced histological changes in the mantle of *Mytilus edulis* L. (Bivalvia). Limnologica (Berlin) 15: 523-527.

Sunila, I., 1986a. Chronic histopathological effects of short-term copper and cadmium exposure on the gill of the mussel *Mytilus edulis*. Journal of Invertebrate Pathology 47: 125-142.

Sunila, I., 1986b. Histopathological changes in the mussel *Mytilus edulis* L. at the outlet from a titanium dioxide plant in the Northern Baltic. Annales Zoologici Fennici, 23: 61-70..

Sunila, I., 1987. Histopathology of mussels (*Mytilus edulis* L.) from the Tvarminne area, Gulf of Finland (Baltic Sea). Annales Zoologici Fennici, 24: 55-69.

Sutherland, J.P., 1972. Energetics of high and low populations of the limpet *Acmaea scabra* (Gould). Ecology 53: 430-438.

Taylor, L.A., 1986. Marine macrobenthic colonization of mine tailings in Rupert Inlet, British Columbia. M.Sc. Thesis, University of Victoria.

Thorson, G., 1957. Bottom communities (sublittoral or shallow shelf). Memoirs of the Geological Society of America, 67(1): 461-534.

Tsutsumi, H., 1987. Population dynamics of C··nitella capitata (Polychaeta; Capitellidae) in an organically polluted cove. M ine Ecology Progress Series, 36: 139-149.

Vega, M.M., J.A. Marigomez and E. Angulo, 1989. Quantitative alterations in the structure of the digestive cell of Littorina littorea on exposure to cadmium. Marine Biology 103: 547-553.

Vermeij, G.J., 1978. Biogeography and Adaptation: Patterns of Marine Life. Harvard University Press, Cambridge, Mass. 332 pp.

Waldichuk, M. and R.J. Buchanan, 1980. Significance of environmental changes due to mine waste disposal in Rupert Inlet. Fisheries and Oceans Canada and British Columbia Ministry of Environment, unpublished report.

Weibel, E.R. 1979. Stereological Methods. Vol. 1. Academic Press, London.

Widdows, J., T. Bakke, B.L. Bayne, P. Donkin, D.R. Livingstone, D.M. Lowe, M.N. Moore, S.V. Evans and S.L. Moore, 1982. Response of Mytilus edulis L. on exposure to the water accomodated fraction of North Sea oil. Marine Biology 67: 15-31.

Wilkinson, L., 1988. SYSTAT: The System for Statistics. Evanston, IL: Systat, Inc. pg. 709.

Yevich, P.P and C.A. Barszcz, 1976. Mussel Watch 1976. Histopathology Report. Histopathology Unit. Environmental Research Laboratory, Narragansett. Environmental Protection Agency. 42 pp.

Yevich, C.A. and P.P Yevich, 1985. Histopathological effects of cadmium and copper on the sea scallop Placopecten magellanicus. in Marine Pollution and Physiology: Recent Advances. F.J. Vernberg et al, eds. University of South Carolina Press, South Carolina. pp. 187-198.

Zajac, R.N. and R.B. Whitlatch, 1989. Natural and disturbance-induced demographic variations in an infaunal polychaete, Nepthys incisa. Marine Ecology Progress Series 57:89-102.

Zar, J.H., 1984. Biostatistical Analysis. Prentice-Hall, N.J., 718 pp.

Appendix I: Quantitative analysis of tissue variation in the clam, *Axinopsida serricata*: raw data. An explanation of the variables is provided, in part, in Chapter 3. A further explanation follows:

Variable code	Full name and particulars
Code	Randomly assigned code used to score sections in blind study
STN	Station: 11 = SI, 5 = H1, 1 = H2, 3 = H3, 24 = H4, 26 = H5, 50 = QS, 12 = GB (see Table 1. page 23).
YEAR	Year collection was made (1987 or 1989).
REPL	Replicate
MWVM	Clam size as indicated by maximum width of the visceral mass (mm).
SEX	Sex as male (1), female (2) or undeterminable based on histological examination (0).
SRC	Stage of reproductive cycle. -2: reproductive follicles are in post-spawn condition; -1: post-spawn follicles are recovering; 0: gonad is indifferent and sex may not be determinable; 2: gonad is developing; 3: gonad is mature and clam is nearly ready to spawn or spawning.
DTI	Digestive tubule index, ranging from 5 (excellent condition) to 0 (completely necrotic).
DCH	Digestive cell height (m).
PDTD	Proximal digestive duct density.
FTI	Fragmented tubule index: percent frequency of unfragmented digestive tubules.
NVR	Nephrolith volume ratio (percent of total kidney volume).
MAI	Muscle atrophy index.
FLAG	Incidence of flagellates in the ctenidial pouch.*
SEL	Stomach epithelium tertiary lysosomes.*
CTEN	Disruption of ctenidial filaments.*
MUCOHYP	Hyperactivity of ctenidial mucocytes.*
OEDEMA	Oedema of ctenidial lateral cells.*
ID	Cells resembling hyalinocytes in the posterior loop of the intestine.*
OVA	Premature breakdown of oocytes.*
DCHV	Intra-individual variability (standard deviation) of digestive cell height, based on 16 measurements.
PDTDV	Intra-individual variability (standard deviation) of proximal digestive duct density, based on 5 measurements.
MAIV	Intra-individual variability (standard deviation) of the muscle atrophy index based on 5 measurements.

* Present (1) or absent (0).

CASE	CODE	STN	YEAR	REPL	MWVM
1.0,	33.0,	11.0,	87.0,	1.0,	1.6
2.0,	43.0,	11.0,	87.0,	2.0,	1.8
3.0,	60.0,	11.0,	87.0,	3.0,	1.8
4.0,	84.0,	11.0,	87.0,	4.0,	1.3
5.0,	1.0,	11.0,	87.0,	5.0,	1.9
6.0,	55.0,	11.0,	87.0,	6.0,	1.1
7.0,	17.0,	11.0,	87.0,	7.0,	1.5
8.0,	80.0,	11.0,	87.0,	8.0,	0.8
9.0,	8.0,	11.0,	87.0,	9.0,	1.4
10.0,	98.0,	12.0,	87.0,	1.0,	1.7
11.0,	67.0,	12.0,	87.0,	2.0,	1.7
12.0,	72.0,	12.0,	87.0,	3.0,	1.6
13.0,	100.0,	12.0,	87.0,	4.0,	1.6
14.0,	81.0,	12.0,	87.0,	5.0,	1.5
15.0,	86.0,	12.0,	87.0,	6.0,	2.0
16.0,	48.0,	12.0,	87.0,	7.0,	1.9
17.0,	24.0,	12.0,	87.0,	8.0,	1.8
18.0,	28.0,	12.0,	87.0,	9.0,	1.6
19.0,	26.0,	12.0,	87.0,	10.0,	1.8
20.0,	92.0,	5.0,	87.0,	1.0,	2.3
21.0,	42.0,	5.0,	87.0,	2.0,	2.2
22.0,	18.0,	5.0,	87.0,	3.0,	2.2
23.0,	57.0,	5.0,	87.0,	4.0,	1.7
24.0,	99.0,	5.0,	87.0,	5.0,	2.4
25.0,	73.0,	5.0,	87.0,	6.0,	2.2
26.0,	61.0,	5.0,	87.0,	7.0,	2.1
27.0,	76.0,	5.0,	87.0,	8.0,	2.2
28.0,	74.0,	5.0,	87.0,	9.0,	1.7
29.0,	15.0,	5.0,	87.0,	10.0,	1.8
30.0,	51.0,	3.0,	87.0,	1.0,	2.0
31.0,	93.0,	3.0,	87.0,	2.0,	1.3
32.0,	44.0,	3.0,	87.0,	3.0,	1.6
33.0,	23.0,	3.0,	87.0,	4.0,	1.2

CASE	CODE	STN	YEAR	REPL	MWVM
34.0,	5.0,	1.0,	87.0,	1.0,	1.9
35.0,	45.0,	1.0,	87.0,	2.0,	2.0
36.0,	13.0.	1.0,	87.0,	3.0,	2.4
37.0,	7.0,	1.0,	87.0,	4.0,	1.4
38.0,	95.0,	1.0,	87.0,	5.0,	1.4
39.0,	20.0,	1.0,	87.0,	6.0,	2.2
40.0,	47.0,	1.0,	87.0,	7.0,	2.1
41.0,	102.0,	1.0,	87.0,	8.0,	2.0
42.0,	8.0,	1.0,	87.0,	9.0,	2.6
43.0,	1.0,	1.0,	87.0,	10.0,	2.3
44.0,	63.0,	26.0,	87.0,	1.0,	1.5
45.0,	21.0,	26.0,	87.0,	2.0,	1.4
46.0,	66.0,	26.0,	87.0,	3.0,	1.5
47.0,	69.0,	26.0,	87.0,	4.0,	1.1
48.0,	4.0,	26.0,	87.0,	5.0,	1.1
49.0,	91.0,	26.0,	87.0,	6.0,	1.8
50.0,	40.0,	26.0,	87.0,	7.0,	1.3
51.0,	31.0,	26.0,	87.0,	8.0,	1.6
52.0,	68.0,	26.0,	87.0,	9.0,	1.5
53.0,	96.0,	26.0,	87.0,	10.0,	1.2
54.0,	2.0,	50.0,	87.0,	1.0,	1.4
55.0,	59.0,	50.0,	87.0,	2.0,	1.4
56.0,	70.0,	50.0,	87.0,	3.0,	1.4
57.0,	58.0,	50.0,	87.0,	4.0,	1.1
58.0,	27.0,	24.0,	87.0,	1.0,	1.7
59.0,	37.0,	24.0,	87.0,	2.0,	1.3
60.0,	65.0,	24.0,	87.0,	3.0,	1.6
61.0,	77.0,	24.0,	87.0,	4.0,	1.7
62.0,	56.0,	24.0,	87.0,	5.0,	1.5
63.0,	39.0,	24.0,	87.0,	6.0,	1.5
64.0,	50.0,	24.0,	87.0,	7.0,	1.3
65.0,	6.0,	24.0,	87.0,	8.0,	1.3
66.0,	29.0,	24.0,	87.0,	9.0,	1.4
67.0,	85.0,	24.0,	87.0,	10.0,	1.2
68.0,	53.0,	3.0,	89.0,	1.0,	1.6
69.0,	64.0,	3.0,	89.0,	2.0,	1.2

CASE	CODE	STN	YEAR	REPL	MWVM
70.0,	89.0,	3.0,	89.0,	3.0,	1.3
71.0,	71.0,	3.0,	89.0,	4.0,	1.3
72.0,	3.0,	5.0,	89.0,	1.0,	1.2
73.0,	35.0,	5.0,	89.0,	2.0,	2.0
74.0,	62.0,	5.0,	89.0,	3.0,	1.3
75.0,	54.0,	5.0,	89.0,	4.0,	1.5
76.0,	10.0,	5.0,	89.0,	5.0,	1.5
77.0,	38.0,	5.0,	89.0,	6.0,	1.2
78.0,	52.0,	5.0,	89.0,	7.0,	1.6
79.0,	82.0,	5.0,	89.0,	8.0,	1.6
80.0,	46.0,	5.0,	89.0,	9.0,	1.8
81.0,	88.0,	5.0,	89.0,	10.0,	1.1
82.0,	16.0,	1.0,	89.0,	1.0,	1.2
83.0,	19.0,	1.0,	89.0,	2.0,	1.3
84.0,	101.0,	1.0,	89.0,	3.0,	1.5
85.0,	90.0,	1.0,	89.0,	4.0,	1.9
86.0,	1.0,	1.0,	89.0,	5.0,	1.6
87.0,	34.0,	1.0,	89.0,	6.0,	1.3
88.0,	97.0,	1.0,	89.0,	7.0,	1.3
89.0,	9.0,	1.0,	89.0,	8.0,	1.4
90.0,	49.0,	1.0,	89.0,	9.0,	1.5
91.0,	79.0,	1.0,	89.0,	10.0,	1.4
92.0,	87.0,	11.0,	89.0,	1.0,	1.3
93.0,	83.0,	11.0,	89.0,	2.0,	1.3
94.0,	75.0,	11.0,	89.0,	3.0,	1.5
95.0,	30.0,	11.0,	89.0,	4.0,	1.2
96.0,	32.0,	11.0,	89.0,	5.0,	1.4
97.0,	12.0,	11.0,	89.0,	6.0,	1.3
98.0,	25.0,	11.0,	89.0,	7.0,	1.4
99.0,	36.0,	11.0,	89.0,	8.0,	1.6
100.0,	94.0,	11.0,	89.0,	9.0,	1.8
101.0,	22.0,	11.0,	89.0,	10.0,	1.4

CASE	SEX	SRC	DTI	DCH
1.0,	2.0,	3.0,	4.0,	39.0
2.0,	1.0,	3.0,	3.0,	27.0
3.0,	1.0,	3.0,	2.0,	38.0
4.0,	0.0,	0.0,	2.0,	36.0
5.0,	2.0,	2.0,	1.0,	32.0
6.0,	1.0,	2.0,	5.0,	33.0
7.0,	2.0,	3.0,	2.0,	27.0
8.0,	0.0,	0.0,	2.0,	39.0
9.0,	1.0,	3.0,	3.0,	41.0
10.0,	2.0,	3.0,	1.0,	53.0
11.0,	1.0,	3.0,	1.0,	37.0
12.0,	2.0,	3.0,	1.0,	37.0
13.0,	2.0,	3.0,	1.0,	36.0
14.0,	2.0,	3.0,	2.0,	34.0
15.0,	2.0,	3.0,	2.0,	31.0
16.0,	2.0,	2.0,	1.0,	23.0
17.0,	1.0,	3.0,	0.0,	35.0
18.0,	2.0,	2.0,	1.0,	61.0
19.0,	2.0,	2.0,	2.0,	32.0
20.0,	1.0,	3.0,	2.0,	39.0
21.0,	2.0,	3.0,	1.0,	41.0
22.0,	2.0,	2.0,	1.0,	60.0
23.0,	2.0,	2.0,	2.0,	53.0
24.0,	2.0,	3.0,	0.0,	60.0
25.0,	2.0,	3.0,	2.0,	56.0
26.0,	1.0,	3.0,	1.0,	66.0
27.0,	1.0,	3.0,	1.0,	52.0
28.0,	2.0,	2.0,	1.0,	51.0
29.0,	1.0,	3.0,	1.0,	42.0
30.0,	1.0,	3.0,	1.0,	64.0
31.0,	2.0,	3.0,	2.0,	38.0
32.0,	2.0,	-2.0,	1.0,	56.0
33.0,	2.0,	3.0,	2.0,	30.0
34.0,	1.0,	3.0,	0.0,	43.0
35.0,	1.0,	3.0,	1.0,	53.0
36.0,	2.0,	3.0,	2.0,	40.0

CASE	SEX	SRC	DTI	DCH
37.0,	1.0,	3.0,	1.0,	33.0
38.0,	1.0,	3.0,	1.0,	47.0
39.0,	2.0,	2.0,	1.0,	41.0
40.0,	2.0,	2.0,	0.0,	41.0
41.0,	1.0,	3.0,	1.0,	55.0
42.0,	1.0,	3.0,	1.0,	44.0
43.0,	2.0,	2.0,	1.0,	63.0
44.0,	1.0,	3.0,	1.0,	56.0
45.0,	2.0,	3.0,	2.0,	31.0
46.0,	1.0,	3.0,	2.0,	36.0
47.0,	2.0,	3.0,	2.0,	35.0
48.0,	2.0,	2.0,	2.0,	34.0
49.0,	1.0,	3.0,	1.0,	45.0
50.0,	2.0,	3.0,	3.0,	53.0
51.0,	1.0,	3.0,	2.0,	37.0
52.0,	2.0,	3.0,	1.0,	50.0
53.0,	1.0,	3.0,	2.0,	28.0
54.0,	2.0,	2.0,	4.0,	31.0
55.0,	1.0,	3.0,	2.0,	35.0
56.0,	2.0,	3.0,	1.0,	46.0
57.0,	2.0,	-2.0,	1.0,	36.0
58.0,	1.0,	3.0,	2.0,	36.0
59.0,	1.0,	3.0,	1.0,	50.0
60.0,	2.0,	3.0,	1.0,	29.0
61.0,	1.0,	3.0,	2.0,	38.0
62.0,	1.0,	2.0,	1.0,	23.0
63.0,	1.0,	3.0,	4.0,	36.0
64.0,	1.0,	3.0,	3.0,	26.0
65.0,	2.0,	3.0,	4.0,	24.0
66.0,	2.0,	-1.0,	4.0,	39.0
67.0,	1.0,	3.0,	2.0,	29.0
68.0,	1.0,	3.0,	0.0,	27.0
69.0,	0.0,	0.0,	0.0,	21.0
70.0,	2.0,	-2.0,	0.0,	31.0
71.0,	1.0,	-1.0,	1.0,	41.0
72.0,	2.0,	-2.0,	1.0,	40.0

CASE	SEX	SRC	DTI	DCH
73.0,	1.0,	2.0,	0.0,	39.0
74.0,	2.0,	-1.0,	1.0,	32.0
75.0,	1.0,	-2.0,	3.0,	44.0
76.0,	2.0,	-1.0,	1.0,	38.0
77.0,	1.0,	-2.0,	1.0,	56.0
78.0,	0.0,	0.0,	1.0,	38.0
79.0,	1.0,	-1.0,	0.0,	42.0
80.0,	2.0,	-1.0,	1.0,	40.0
81.0,	1.0,	-2.0,	0.0,	39.0
82.0,	2.0,	-1.0,	1.0,	23.0
83.0,	1.0,	-1.0,	0.0,	22.0
84.0,	1.0,	-1.0,	0.0,	36.0
85.0,	1.0,	-1.0,	1.0,	36.0
86.0,	2.0,	-1.0,	1.0,	23.0
87.0,	0.0,	0.0,	1.0,	23.0
88.0,	1.0,	-1.0,	0.0,	47.0
89.0,	1.0,	-1.0,	2.0,	40.0
90.0,	1.0,	-1.0,	1.0,	43.0
91.0,	0.0,	0.0,	0.0,	36.0
92.0,	0.0,	0.0,	2.0,	45.0
93.0,	0.0,	0.0,	3.0,	35.0
94.0,	0.0,	0.0,	1.0,	47.0
95.0,	2.0,	-1.0,	2.0,	44.0
96.0,	2.0,	-2.0,	3.0,	41.0
97.0,	1.0,	-1.0,	1.0,	46.0
98.0,	0.0,	0.0,	2.0,	49.0
99.0,	1.0,	-1.0,	2.0,	46.0
100.0,	2.0,	-1.0,	2.0,	47.0
101.0,	1.0,	-1.0,	3.0,	43.0

CASE	PDTD	FTI	NVR	MAI
1.000,	0.270,	0.940,	0.000,	0.740
2.000,	0.160,	0.930,	0.062,	0.540
3.000,	0.150,	0.880,	0.008,	0.730
4.000,	0.070,	0.630,	0.024,	0.750
5.000,	0.120,	0.880,	0.008,	0.700
6.000,	0.310,	1.000,	0.016,	0.650
7.000,	0.190,	0.860,	0.007,	0.540
8.000,	0.200,	0.940,	0.016,	0.680
9.000,	0.150,	1.000,	0.000,	0.730
10.000,	0.240,	0.830,	0.022,	0.640
11.000,	0.270,	0.500,	0.017,	0.930
12.000,	0.280,	0.380,	0.086,	0.670
13.000,	0.300,	0.750,	0.006,	0.760
14.000,	0.200,	0.940,	0.002,	0.750
15.000,	0.280,	0.730,	0.002,	0.710
16.000,	0.120,	1.000,	0.002,	0.570
17.000,	0.260,	0.710,	0.000,	0.780
18.000,	0.200,	0.500,	0.030,	0.560
19.000,	. ,	0.880,	0.016,	0.750
20.000,	0.369,	0.940,	0.106,	0.680
21.000,	0.204,	0.560,	0.150,	0.690
22.000,	0.370,	0.270,	0.100,	0.790
23.000,	0.450,	0.440,	0.012,	0.760
24.000,	0.410,	0.500,	0.002,	0.700
25.000,	0.370,	0.690,	0.047,	0.690
26.000,	0.390,	0.310,	0.024,	0.610
27.000,	0.370,	0.440,	0.086,	0.710
28.000,	0.440,	0.630,	0.000,	0.640
29.000,	0.300,	0.500,	0.002,	0.710
30.000,	0.300,	0.810,	0.025,	0.540
31.000,	0.190,	0.500,	0.002,	0.690
32.000,	0.068,	0.130,	0.058,	0.530
33.000,	0.230,	1.000,	0.014,	0.740
34.000,	0.330,	0.310,	0.180,	0.670
35.000,	0.280,	0.380,	0.061,	0.740
36.000,	0.170,	0.250,	0.210,	0.760

CASE	PDTD	FTI	NVR	MAI
37.000,	0.430,	0.470,	0.061,	0.760
38.000,	0.430,	0.250,	0.000,	0.740
39.000,	0.380,	0.310,	0.020,	0.710
40.000,	0.340,	0.250,	0.002,	0.640
41.000,	0.540,	0.130,	0.008,	0.760
42.000,	0.440,	0.060,	0.004,	0.690
43.000,	0.490,	0.250,	0.061,	0.680
44.000,	0.460,	0.130,	0.003,	0.560
45.000,	0.320,	0.810,	0.000,	0.670
46.000,	0.190,	0.930,	0.000,	0.730
47.000,	0.270,	0.870,	0.000,	0.700
48.000,	0.120,	1.000,	0.000,	0.700
49.000,	0.230,	0.690,	0.000,	0.780
50.000,	0.300,	0.860,	0.000,	0.690
51.000,	0.180,	0.880,	0.002,	0.520
52.000,	0.360,	0.600,	0.000,	0.700
53.000,	0.160,	0.940,	0.007,	0.790
54.000,	0.130,	1.000,	0.004,	0.730
55.000,	0.140,	0.880,	0.004,	0.700
56.000,	0.220,	1.000,	0.002,	0.750
57.000,	0.280,	0.750,	0.003,	0.750
58.000,	0.170,	0.810,	0.012,	0.740
59.000,	0.120,	0.440,	0.000,	0.743
60.000,	0.090,	0.570,	0.050,	0.660
61.000,	0.200,	0.810,	0.087,	0.740
62.000,	0.090,	0.880,	0.000,	0.760
63.000,	0.200,	1.000,	0.000,	0.740
64 000,	0.150,	0.870,	0.006,	0.780
65.000,	0.160,	0.940,	0.002,	0.790
66.000,	0.100,	1.000,	0.000,	0.790
67.000,	0.170,	1.000,	0.056,	0.690
68.000,	0.190,	1.000,	0.006,	0.760
69.000,	0.120,	0.380,	0.125,	0.740
70.000,	0.150,	1.000,	0.020,	0.700
71.000,	0.170,	0.440,	0.014,	0.730
72.000,	0.170,	0.440,	0.014,	0.820

CASE	PDTD	FTI	NVR	MAI
73.000,	0.220,	0.930,	0.006,	0.830
74.000,	0.110,	0.810,	0.017,	0.680
75.000,	0.210,	0.810,	0.054,	0.660
76.000,	0.190,	0.810,	0.010,	0.680
77.000,	0.110,	0.690,	0.000,	0.730
78.000,	0.100,	0.810,	0.006,	0.740
79.000,	0.180,	0.690,	0.000,	0.760
80.000,	0.220,	0.750,	0.030,	0.680
81.000,	0.210,	0.770,	0.000,	0.730
82.000,	0.100,	0.560,	0.010,	0.600
83.000,	0.100,	0.400,	0.000,	0.760
84.000,	0.120,	0.750,	0.010,	0.740
85.000,	0.150,	0.690,	0.012,	0.640
86.000,	0.020,	0.300,	0.070,	0.740
87.000,	0.070,	1.000,	0.000,	0.720
88.000,	0.150,	0.830,	0.000,	0.770
89.000,	0.090,	0.810,	0.000,	0.720
90.000,	0.080,	0.880,	0.058,	0.670
91.000,	0.120,	0.170,	0.020,	0.640
92.000,	0.250,	0.690,	0.020,	0.750
93.000,	0.130,	0.930,	0.000,	0.730
94.000,	0.210,	0.690,	0.000,	0.770
95.000,	0.280,	0.940,	0.004,	0.780
96.000,	0.160,	0.940,	0.000,	0.720
97.000,	0.350,	0.800,	0.000,	0.670
98.000,	0.370,	0.370,	0.000,	0.620
99.000,	0.160,	0.940,	0.020,	0.640
100.000,	0.210,	0.830,	0.031,	0.690
101.000,	0.310,	0.440,	0.000,	0.730

CASE	FLAG	SEL	CTEN	MUCOHYP
1.0,	0.0,	0.0,	0.0,	1.0
2.0,	1.0,	1.0,	0.0,	0.0
3.0,	1.0,	1.0,	0.0,	0.0
4.0,	1.0,	0.0,	1.0,	1.0
5.0,	1.0,	1.0,	1.0,	0.0
6.0,	0.0,	0.0,	1.0,	1.0
7.0,	1.0,	1.0,	0.0,	0.0
8.0,	0.0,	0.0,	1.0,	0.0
9.0,	1.0,	.,	0.0,	1.0
10.0,	0.0,	0.0,	1.0,	1.0
11.0,	0.0,	1.0,	1.0,	1.0
12.0,	0.0,	1.0,	1.0,	1.0
13.0,	0.0,	1.0,	1.0,	1.0
14.0,	0.0,	0.0,	1.0,	1.0
15.0,	0.0,	1.0,	1.0,	1.0
16.0,	0.0,	0.0,	1.0,	1.0
17.0,	0.0,	0.0,	1.0,	1.0
18.0,	0.0,	0.0,	1.0,	1.0
19.0,	0.0,	.,	1.0,	0.0
20.0,	1.0,	1.0,	1.0,	1.0
21.0,	1.0,	0.0,	1.0,	1.0
22.0,	1.0,	0.0,	1.0,	1.0
23.0,	1.0,	1.0,	1.0,	1.0
24.0,	1.0,	.,	1.0,	1.0
25.0,	1.0,	0.0,	1.0,	1.0
26.0,	1.0,	0.0,	1.0,	1.0
27.0,	1.0,	1.0,	1.0,	1.0
28.0,	1.0,	1.0,	0.0,	1.0
29.0,	1.0,	0.0,	0.0,	1.0
30.0,	0.0,	0.0,	0.0,	0.0
31.0,	0.0,	1.0,	0.0,	0.0
32.0,	1.0,	1.0,	0.0,	1.0
33.0,	0.0,	1.0,	0.0,	0.0
34.0,	0.0,	1.0,	0.0,	1.0
35.0,	.,	0.0,	.,	.
36.0,	1.0,	0.0,	0.0,	1.0

CASE	FLAG	SEL	CTEN	MUCOHYP
37.0,	1.0,	1.0,	0.0,	1.0
38.0,	1.0,	1.0,	1.0,	1.0
39.0,	1.0,	0.0,	1.0,	0.0
40.0,	0.0,	1.0,	1.0,	0.0
41.0,	0.0,	0.0,	1.0,	1.0
42.0,	1.0,	1.0,	1.0,	1.0
43.0,	0.0,	0.0,	1.0,	0.0
44.0,	0.0,	0.0,	1.0,	1.0
45.0,	1.0,	0.0,	0.0,	0.0
46.0,	0.0,	1.0,	0.0,	0.0
47.0,	0.0,	0.0,	1.0,	1.0
48.0,	0.0,	0.0,	1.0,	0.0
49.0,	1.0,	1.0,	0.0,	1.0
50.0,	1.0,	0.0,	1.0,	1.0
51.0,	0.0,	0.0,	0.0,	0.0
52.0,	1.0,	0.0,	0.0,	0.0
53.0,	0.0,	1.0,	0.0,	1.0
54.0,	0.0,	1.0,	1.0,	0.0
55.0,	0.0,	0.0,	0.0,	1.0
56.0,	1.0,	0.0,	0.0,	1.0
57.0,	0.0,	0.0,	0.0,	0.0
58.0,	1.0,	1.0,	1.0,	0.0
59.0,	1.0,	0.0,	1.0,	1.0
60.0,	1.0,	1.0,	0.0,	1.0
61.0,	1.0,	1.0,	1.0,	0.0
62.0,	0.0,	1.0,	1.0,	0.0
63.0,	0.0,	1.0,	1.0,	1.0
64.0,	1.0,	1.0,	1.0,	0.0
65.0,	0.0,	0.0,	0.0,	0.0
66.0,	1.0,	1.0,	0.0,	1.0
67.0,	0.0,	1.0,	1.0,	0.0
68.0,	1.0,	1.0,	0.0,	1.0
69.0,	1.0,	0.0,	0.0,	0.0
70.0,	0.0,	1.0,	0.0,	1.0
71.0,	1.0,	1.0,	0.0,	1.0
72.0,	1.0,	1.0,	0.0,	1.0

CASE	FLAG	SEL	CTEN	MUCOHYP
73.0,	1.0,	1.0,	1.0,	1.0
74.0,	1.0,	1.0,	0.0,	1.0
75.0,	1.0,	1.0,	1.0,	1.0
76.0,	1.0,	1.0,	0.0,	1.0
77.0,	1.0,	0.0,	0.0,	1.0
78.0,	1.0,	1.0,	0.0,	1.0
79.0,	1.0,	1.0,	1.0,	1.0
80.0,	1.0,	1.0,	1.0,	1.0
81.0,	1.0,	1.0,	0.0,	1.0
82.0,	0.0,	1.0,	1.0,	0.0
83.0,	1.0,	0.0,	.,	0.0
84.0,	1.0,	1.0,	0.0,	1.0
85.0,	1.0,	1.0,	0.0,	1.0
86.0.	1.0,	1.0,	0.0,	0.0
87.0,	1.0,	1.0,	0.0,	1.0
88.0,	1.0,	0.0,	1.0,	1.0
89.0,	1.0,	1.0,	1.0,	0.0
90.0,	1.0,	1.0,	0.0,	1.0
91.0,	1.0,	1.0,	0.0,	1.0
92.0,	1.0,	0.0,	0.0,	1.0
93.0,	0.0,	0.0,	0.0,	0.0
94.0,	1.0,	1.0,	0.0,	0.0
95.0,	0.0,	0.0,	0.0,	0.0
96.0,	0.0,	0.0,	0.0,	1.0
97.0,	1.0,	0.0,	0.0,	0.0
98.0,	0.0,	0.0,	0.0,	1.0
99.0,	0.0,	0.0,	0.0,	0.0
100.0,	0.0,	0.0,	0.0,	0.0
101.0,	0.0,	0.0,	0.0,	0.0

CASE	OEDEMA	ID	OVA
1.0,	0.0,	0.0,	0.0
2.0,	0.0,	0.0,	.
3.0,	0.0,	0.0,	.
4.0,	0.0,	1.0,	.
5.0,	0.0,	0.0,	1.0
6.0,	1.0,	1.0,	.
7.0,	0.0,	0.0,	0.0
8.0,	0.0,	0.0,	.
9.0,	0.0,	1.0,	.
10.0,	1.0,	0.0,	1.0
11.0,	1.0,	0.0,	.
12.0,	1.0,	0.0,	0.0
13.0,	1.0,	0.0,	1.0
14.0,	1.0,	1.0,	1.0
15.0,	1.0,	1.0,	1.0
16.0,	0.0,	0.0,	1.0
17.0,	1.0,	0.0,	.
18.0,	1.0,	0.0,	1.0
19.0,	1.0,	0.0,	0.0
20.0,	1.0,	0.0,	.
21.0,	0.0,	0.0,	1.0
22.0,	1.0,	0.0,	0.0
23.0,	1.0,	1.0,	1.0
24.0,	1.0,	1.0,	0.0
25.0,	1.0,	1.0,	0.0
26.0,	1.0,	1.0,	.
27.0,	1.0,	.,	.
28.0,	0.0,	0.0,	0.0
29.0,	0.0,	0.0,	.
30.0,	0.0,	0.0,	.
31.0,	0.0,	1.0,	1.0
32.0,	0.0,	0.0,	0.0
33.0,	0.0,	0.0,	1.0
34.0,	0.0,	0.0,	.
35.0,	.,	0.0,	.
36.0,	0.0,	0.0,	1.0

CASE	OEDEMA	ID	OVA
37.0,	0.0,	.,	.
38.0,	0.0,	1.0,	.
39.0,	0.0,	0.0,	0.0
40.0,	0.0,	1.0,	1.0
41.0,	1.0,	0.0,	.
42.0,	1.0,	0.0,	.
43.0,	0.0,	0.0,	0.0
44.0,	0.0,	0.0,	.
45.0,	0.0,	0.0,	1.0
46.0,	0.0,	0.0,	.
47.0,	0.0,	0.0,	0.0
48.0,	0.0,	0.0,	0.0
49.0,	0.0,	1.0,	.
50.0,	1.0,	1.0,	0.0
51.0,	0.0,	0.0,	.
52.0,	0.0,	0.0,	0.0
53.0,	0.0,	0.0,	.
54.0,	0.0,	0.0,	1.0
55.0,	0.0,	0.0,	.
56.0,	0.0,	0.0,	0.0
57.0,	0.0,	0.0,	1.0
58.0,	0.0,	1.0,	.
59.0,	0.0,	1.0,	.
60.0,	0.0,	1.0,	0.0
61.0,	0.0,	1.0,	.
62.0,	0.0,	1.0,	.
63.0,	1.0,	1.0,	.
64.0,	0.0,	0.0,	.
65.0,	0.0,	0.0,	1.0
66.0,	0.0,	0.0,	0.0
67.0,	0.0,	0.0,	.
68.0,	0.0,	0.0,	.
69.0,	0.0,	.,	.
70.0,	0.0,	1.0,	0.0
71.0,	0.0,	0.0,	.
72.0,	0.0,	0.0,	0.0

CASE	OEDEMA	ID	OVA
73.0,	0.0,	0.0,	.
74.0,	0.0,	0.0,	0.0
75.0,	1.0,	0.0,	.
76.0,	0.0,	0.0,	0.0
77.0,	0.0,	0.0,	.
78.0,	0.0,	0.0,	.
79.0,	1.0,	0.0,	.
80.0,	1.0,	0.0,	0.0
81.0,	0.0,	1.0,	.
82.0,	0.0,	0.0,	0.0
83.0,	.,	0.0,	.
84.0,	0.0,	1.0,	.
85.0,	0.0,	0.0,	.
86.0,	0.0,	.,	0.0
87.0,	0.0,	0.0,	.
88.0,	1.0,	0.0,	.
89.0,	0.0,	0.0,	.
90.0,	0.0,	0.0,	.
91.0,	0.0,	1.0,	.
92.0,	0.0,	1.0,	.
93.0,	0.0,	1.0,	.
94.0,	0.0,	1.0,	.
95.0,	0.0,	0.0,	0.0
96.0,	0.0,	1.0,	0.0
97.0,	0.0,	1.0,	.
98.0,	0.0,	1.0,	.
99.0,	0.0,	1.0,	.
100.0,	0.0,	1.0,	0.0
101.0,	0.0,	1.0,	.

CASE	DCHV	PDTDV	MAIV
1.000,	2.870,	0.101,	0.068
2.000,	2.100,	0.044,	0.080
3.000,	3.070,	0.065,	0.024
4.000,	4.710,	0.055,	0.079
5.000,	3.310,	0.016,	0.050
6.000,	3.280,	0.113,	0.070
7.000,	3.000,	0.068,	0.068
8.000,	3.150,	0.052,	0.098
9.000,	2.700,	0.050,	0.031
10.000,	5.660,	0.085,	0.093
11.000,	4.430,	0.074,	0.000
12.000,	3.510,	0.057,	0.085
13.000,	6.100,	0.057,	0.052
14.000,	4.440,	0.014,	0.097
15.000,	3.380,	0.106,	0.054
16.000,	2.210,	0.093,	0.087
17.000,	2.690,	0.052,	0.051
18.000,	5.940,	0.084,	0.107
19.000,	2.860,	. ,	0.080
20.000,	2.760,	0.101,	0.065
21.000,	4.270,	0.082,	0.096
22.000,	11.050,	0.042,	0.019
23.000,	5.930,	0.116,	0.060
24.000,	5.060,	0.082,	0.041
25.000,	8.650,	0.085,	0.118
26.000,	9.350,	0.054,	0.107
27.000,	8.810,	0.083,	0.100
28.000,	5.950,	0.083,	0.070
29.000,	6.010,	0.011,	0.043
30.000,	5.130,	0.102,	0.044
31.000,	7.000,	0.051,	0.058
32.000,	3.900,	0.061,	0.073
33.000,	1.940,	0.105,	0.068
34.000,	8.770,	0.066,	0.065
35.000,	5.180,	0.023,	0.043
36.000,	2.250,	0.096,	0.048

CASE	DCHV	PDTDV	MAIV
37.000,	4.270,	0.087,	0.044
38.000,	11.500,	0.137,	0.093
39.000,	4.240,	0.048,	0.106
40.000,	1.250,	0.061,	0.085
41.000,	7.780,	0.101,	0.036
42.000,	. ,	0.082,	0.076
43.000,	14.050,	0.080,	0.096
44.000,	6.870,	0.052,	0.042
45.000,	2.380,	0.086,	0.061
46.000,	2.540,	0.054,	0.062
47.000,	4.350,	0.042,	0.038
48.000,	3.200,	0.034,	0.047
49.000,	6.060,	0.086,	0.066
50.000,	3.950,	0.087,	0.048
51.000,	5.160,	0.068,	0.069
52.000,	6.370,	0.125,	0.093
53.000,	1.980,	0.081,	0.065
54.000,	3.980,	0.058,	0.065
55.000,	3.340,	0.050,	0.115
56.000,	3.100,	0.076,	0.098
57.000,	3.030,	0.087,	0.047
58.000,	3.660,	0.087,	0.081
59.000,	5.290,	0.072,	0.057
60.000,	1.700,	0.011,	0.070
61.000,	5.960,	0.042,	0.040
62.000,	2.860,	0.049,	0.072
63.000,	2.750,	0.084,	0.078
64.000,	3.240,	0.036,	0.080
65.000,	1.450,	0.041,	0.023
66.000,	3.200,	0.066,	0.033
67.000,	2.500,	0.043,	0.034
68.000,	2.700,	0.029,	0.079
69.000,	5.410,	0.065,	0.016
70.000,	3.130,	0.068,	0.040
71.000,	3.470,	0.074,	0.073
72.000,	5.330,	0.054,	0.074

CASE	DCHV	PDTDV	MAIV
73.000,	3.150,	0.097,	0.046
74.000,	2.270,	0.068,	0.075
75.000,	6.270,	0.089,	0.074
76.000,	4.940,	0.086,	0.060
77.000,	7.600,	0.041,	0.067
78.000,	3.970,	0.024,	0.101
79.000,	4.880,	0.039,	0.050
80.000,	3.440,	0.057,	0.085
81.000,	4.110,	0.024,	0.091
82.000,	2.980,	0.039,	0.057
83.000,	1.450,	0.030,	0.061
84.000,	2.230,	0.070,	0.070
85.000,	3.590,	0.035,	0.056
86.000,	2.500,	0.008,	0.048
87.000,	1.970,	0.049,	0.050
88.000,	5.060,	0.071,	0.063
89.000,	2.320,	0.057,	0.077
90.000,	4.570,	0.059,	0.051
91.000,	5.200,	0.049,	0.026
92.000,	5.250,	0.097,	0.079
93.000,	3.260,	0.037,	0.040
94.000,	4.070,	0.148,	0.069
95.000,	3.500,	0.114,	0.116
96.000,	3.250,	0.131,	0.100
97.000,	2.720,	0.099,	0.062
98.000,	3.590,	0.096,	0.086
99.000,	4.080,	0.086,	0.056
100.000,	3.050,	0.084,	0.056
101.000,	4.720,	0.098,	0.097