EXFOLIATION OF EPITHELIAL CELLS IN THE SCALLOP, PLACOPECTEN MAGELLANICUS (GMELIN)- SEASONAL VARIATION AND THE EFFECTS OF ELEVATED WATER TEMPERATURES

TRACY MARIE POTTER

EXFOLIATION OF EPITHELIAL CELLS IN THE SCALLOP, *PLACOPECTEN MAGELLANICUS* (GMELIN)- SEASONAL VARIATION AND THE EFFECTS OF ELEVATED WATER TEMPERATURES

BY

TRACY MARIE POTTER

A thesis submitted to the School of Graduate
Studies in partial fulfilment of the
requirements for the degree of
Master of Science

Department of Biology
Memorial University of Newfoundland
May 1995

St John's Newfoundland

ISBN 0-612-13921-2

ABSTRACT

EXFOLIATION OF EPITHELIAL CELLS IN *PLACOPECTEN MAGELLANICUS* (GMELIN)- SEASONAL VARIATION AND THE EFFECTS OF ELEVATED WATER TEMPERATURES

It has previously been shown that large numbers of ciliated and non ciliated cells (6-15μm) were released by adult sea scallops, *Placopecten magellanicus*, during summer months in Newfoundland. Proportions of scallops exfoliating cells was closely correlated with maximum water temperatures and peak gametogenic activity in this area. These results suggested that epithelial sloughing could be a response to stresses associated with elevated water temperatures and/or spawning activity. Scanning electron microscopy (SEM) was used to illustrate the damage to the pallial structures, particularly the disappearance of ciliated cells from the gills, mantle and gonad in specimens known to have sloughed cells.

In the first component of this study, juvenile and adult sea scallops were monitored during 1992-93 to establish whether or not this species exfoliated epithelial cells throughout the year. An electronic particle counter was used in this seasonal study to distinguish epithelial cells from natural particulate matter and instantly determine which scallops were exfoliating cells. Both juvenile and adult scallops released relatively small amounts of epithelial cells during most months of the year. This observation for juveniles and for adults monitored during months when

gametogenic activity was minimal, indicated that exfoliation does not occur as a result of reproductive activity alone. SEM analysis revealed little difference in surface characteristics of the gills, mantle and gonad from scallops observed to have sloughed small numbers of cells when compared to tissues from scallops that had apparently not sloughed any cells at all. These low rates of epithelial exfoliation throughout the entire year and lack of damage to the pallial tissues were considered to be consistent with cellular turnover and normal physiological function.

In the second component of this study, adult scallops were monitored to determine the effects of elevated water temperatures (14-21.0^{0}C) on the frequency (proportion) of scallops sloughing cells and the rates of cell release. A baseline sloughing rate of approximately 5,300 cells minute^{-1} was established for scallops held at ambient temperatures of approximately 8.0 to 10.0^{0}C. Sloughing rates greater than this were considered to be a measurable response to stress associated with elevated water temperatures. Only at the highest experimental temperature (21.0^{0}C) were sloughing rates significantly higher than baseline levels. SEM analyses revealed some damage to gills, mantle and gonad tissue when scallops were exposed to relatively high temperatures for short periods of time, less than five days.

This is the first study to establish that a species of marine bivalve is exfoliating cells throughout most of the year and that an environmental stressor, such as elevated water temperatures, can produce a detectable increase in sloughing rates. The determination of exfoliation rates using this technique may prove to be a useful

quantitative of environmental stress, including anthropogenic pollutants, not only for this species but possibly for many others.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

The undertaking of this thesis was not only an important academic experience but offered lessons in professional, social and political interactions. I am grateful to have been given the opportunity to venture into such a project and for that, I extend my sincere appreciation and gratitude to Dr. B.A. MacDonald. As with any learning experience, in order to reap the full benefits, it is essential to have the expertise, guidance and support from a variety of individuals that might be directly and indirectly involved with the project on hand. For this, I am indebted to my supervisory committee which included Dr. B.A. MacDonald, Dr. R.J. Thompson and Dr. D. Innes. To Dr. J.E. Ward, I wish to offer my deepest appreciation as the guidance provided by you along with your experience in statistical analyses, proved to be invaluable. Also, many heartfelt thanks for the energy expended by you so that I might keep the "gazillion controls" straight in my mind.

I would like to express gratitude to D.Decker for her countless hours of advice on many subjects and to the technical staff of MUN, UNBSJ & HMSL for their assistance with the technical components of experimentation including the construction of experimental apparatus, maintenance of instrumentation and the inventive suggestions for critically required odds n'ends. To B. Hatfield & the MUN diving team, my extreme appreciation for the organization of the specimen collection, the collection itself and shipment of scallops from Newfie waters to NB. Heartfelt thanks to W. Morris in recognition of his perserverence despite frustrating rounds with

photography equipment and computer sessions.

Expressions of love and thanks to my family whose support, love and reassurance gave me the confidence to initiate this project, kept me going through the less glamorous times and made the end result appear attainable. To C. Maillet, S. Richardson, L.Leader, G. Bacon, T.Gallant and E. Garnier, my warmest appreciation for your support and words of encouragement when times got tough and more importantly, for making me laugh when there wasn't anything to laugh about. To all of you, who contributed to the production of this manuscript by listening to my ideas, reading sections of the draft copies and offering constructive criticisms, I wish to thank you. Last, but not least, to the scallops of the Atlantic region..... thanks so much for your "undying" cooperation and loyalty!

1.0 INTRODUCTION

1.1 Epithelial cells

Some multicellular organisms, (i.e. mammalian), consist of approximately 200 different cell types each having a specific role(s) to play (Hole, 1987). Together these cells function inter-dependently to maintain the structural integrity and physiological function of the organism. Cells are continuously replaced by new cells for general maintenance, growth and repair processes that are inherent within a living organism (Hole, 1987). As a vital component of normal development in living organisms, cells senesce, die, eventually are sloughed off the tissue and are then replaced by fresh cells (Clark, 1985; Holtzman, 1975; Ericcson, 1969). This is not to imply that all senescent cells are sloughed off, some cellular material can also be resorbed or lysed. Numerous cells of similar character become organized into tissues and each tissue type is specialized for a particular function(s).

One such tissue type, epithelial tissue, lines the external and internal surfaces of body tissues and cavities and constitutes the major tissue of glands. Epithelial tissues form protective coverings and function in secretion, absorption, excretion, sensory reception and contraction (Junqueira et al., 1992; Holtzman, 1975). Epithelial tissues always have a free surface, exposed either internally or externally to an open space. The innermost boundary of the epithelium, referred to as the basement (or basal) membrane, secures the epithelium to the underlying connective tissue. Epithelial tissues lack blood vessels and therefore obtain nourishment via metabolites

which diffuse across the basement membrane from heavily supplied connective tissue. Growth and healing processes are quite fast within epithelia as a result of continual cellular turnover. However, the renewal rate for epithelial cells can vary. The closely aggregated polyhedral epithelial cells, bound to one another by means of specialized junctions called desmosomes, have little intracellular material between them and can easily be separated by comparatively mild disturbances (Junqueira et al., 1992; Hole, 1987; Weinstein & Pauli, 1981).

Epithelial tissues can be either simple (one cell layer) or stratified (multiple cell layers) and composed of either squamous, cuboidal or columnar cell types. Simple squamous epithelium exists as flattened cells which fit together like tiles of a floor allowing substances to quickly pass through the epithelium. As a result this epithelial tissue type lines the blood and lymph vessels, including portions of the respiratory tract where diffusion, filtration and osmosis are the primary functions of the tissue. The single layer of cube like cells found in simple cuboidal epithelium provides cover and lining for tissues such as gonads, kidney tubules and ducts of glands. The functions of this epithelium include secretion (i.e. in glands) and absorption (i.e. in kidneys). The simple columnar epithelium is thick in comparison to the simple cuboid epithelium. The elongated columnar cells line organs of the digestive tract, thereby protecting underlying tissues, as well as functioning in secretion and, to a greater extent, absorption. For this reason, microvilli, which extend upward from the cell surface, greatly increasing the surface area of the tissue,

are found in association with this epithelial type. Flask-like goblet cells, which are distributed among the columnar cells, secrete mucus and are characteristic of columnar epithelium. Pseudostratified columnar epithelia, which appear layered but are not, often possess ciliary structures that extend out from the free surface of the epithelium. Goblet cells are scattered throughout this tissue. This epithelium is found lining the tissues of the respiratory and reproductive systems where its functions include protection, secretion and mucus dispersion. Stratified squamous epithelium is many cell layers thick. The surface layer consists of flattened squamous cells, whereas the deeper layers are cuboidal or columnar. With the production of fresh cells, older ones get pushed forward and in the process become flattened. This protective epithelium forms the epidermis and lines the mouth cavity and internal surfaces of reproductive organs (Junqueira et al., 1992; Hole, 1987).

Molluscan epithelium shares many of the same functions and characteristics as its mammalian counterpart, previously described. Epithelial attributes distinctive of molluscs include pigment and tendon cells, repugnatorial, calcareous boring, and byssus glands, operculum, radula, and beak formation (Bubel, 1984). The primary roles of molluscan epithelium are protection, secretion, absorption, transport of particulates and sensory reception (Bubel, 1984). The epithelial categories best describing the epithelial surfaces, for members of the molluscan phylum, are simple cuboidal or columnar and pseudostratified columnar (depending on the region of the body) (Newell, 1977). The epithelium, specifically of the mantle, gill, and labial

palps of various bivalves species, including the scallops, *Placopecten magellanicus* and *Chlamys varia*, can be ciliated or non ciliated and well supplied with mucus secreting cells (mucocytes) (Beninger et al., 1990a&b; Zylstra, 1972a). The pallial organs in bivalves, including the labial palps and gills, which are primarily involved with feeding activities, are well ciliated (Beninger et al., 1990a&b, 1988; Bubel, 1984; Tamarin et al. 1976). In this case, the ciliated epithelium generates water currents which bring suspended particulates into the mantle cavity. The epithelium then transports the material dorsally toward the mouth or ventrally for cleansing (Beninger et al., 1992, 1990a&b, 1988). Ciliation is less frequent in general surface epithelium and that of nonspecialized organs because its principal function is mucus distribution (Bubel, 1984). The non ciliated portions of the epithelia are often microvilliated, which serves to increase the surface area of the tissue for such processes as respiration and digestion in the gills and digestive diverticula, respectively. Microvilli also help maintain a layer of mucus above the external surface of the epithelium, such as in the oral regions of gastropods and cephalopods (Beninger, 1988; Emery, 1975a; Zylstra, 1972a). To a lesser extent, microvilli exist between adjacent cilia of ciliated gill epithelium of freshwater mussels, but still maintain the same function (Reed et al., 1981). The basal membrane of general epithelium is usually undulated with occasional basal folds, although, epithelia such as that of the shell- secreting mantle possess numerous folds (Tsujii, 1976; Bubel, 1973b; Zylstra, 1972a).

In addition to the physiological turnover of cellular constituents described earlier, cellular sloughing may play a protective role in which the animal is able to rid itself of potentially harmful organisms or toxins. Also, epithelial sloughing has been described as a response to stress associated with increased concentrations of heavy metals, anthropogenic pollution, dredge spoil dumpsites, and temperature increase (Marigomez et al., 1990; Sunila, 1986, 1981; Couch, 1984; Simkiss & Mason, 1984; Arimoto & Feng,1983; Cunningham, 1979; Baker, 1969; Ericsson, 1969). Here, the stress response can be described as the reaction of an organism to an environmental stimulus which upsets normal animal function by exceeding a threshold value (Bayne, 1985). Examples of cellular exfoliation in response to stress include the thinning of digestive gland epithelium in molluscs, as a result of enhanced secretion and cellular autolysis caused by detoxification processes and as a general response to stress, eventually leading to the formation of atrophic diverticula (Marigomez et al., 1990). According to Sunila (1986), histopathological changes in the blue mussel, *Mytlius edulis* which resulted from 24 hour exposure to copper include the complete detachment of some abfrontal cells from gill tissue and loss of some ciliated structures from intact gill epithelium, although new abfrontal cells form and cilia regenerate. Sunila (1981) found that siphon epithelium in *Mytilus edulis* broke into large sections or single cells as a result of short-term exposure to 10 ppm cadmium and the interfilamentar junctions and epithelial cells of the gill tissue broke and detached respectively, after short-term exposure to 0.5ppm copper. One year after exposure to

0.1mgL^{-1} copper, the abfrontal section of the gill epithelium of *Mytilus edulis* had atrophied. Couch (1984) found a positive association between epithelial atrophy of the digestive diverticulum in the oyster, *Crassostrea virginica* and contamination by base neutral organic pollutants. Simkiss and Mason (1984) suggest that there is reliable evidence to show the ill effects of metal toxicity on digestive gland epithelium in molluscs. Arimoto and Feng (1983) reported extensive epithelial necrosis and cell sloughing from the gills of *Mytilus edulis* growing in the New Haven dredge spoil disposal area. It was noted that in some cases the epithelium had detached from the chitinous rod of the gill filament. Ericsson (1969) concluded that in addition to the process of cellular autophagy, which is involved in the normal physiological turnover of cellular constituents, cellular sloughing could also be a physiological survival mechanism and a stress response. Histological and electron microscopical observations on copper poisoning in the winter flounder, *Pseudopleuronectes americanus*, illustrated gill epithelium detachment from the corresponding lamellae and subsequent disintegration after exposure to 1000mgL^{-1} copper. Furthermore, exposure to 3200 mgL^{-1} copper resulted in the total cellular destruction of gill epithelium (Baker, 1969).

MacDonald et al. (1995) previously described the release of large numbers of ciliated and non-ciliated epithelial cells (6-12 μm), from the mantle cavity of adult *Placopecten magellanicus* from Newfoundland, Canada. In this preliminary study they sampled between the months of April and October (1990-1992) and suggested that the greater numbers of scallops sloughing cells in August may have been a result of the

higher summer water temperatures, the peak in gametogenic activity or a combination of both factors. The percentage of individuals sloughing cells ranged from zero in April, when the water temperature was 2°C, to a maximum of 50-70% in August when the animals were gravid and the water temperatures were 9-15°C. Scanning electron micrographs revealed that cells were being released from at least three tissues: gill, mantle, and gonad. Damage was very extensive in some of these tissues, particularly the gill, where the lateral, laterofrontal and frontal tracts were missing most of the cilia. This extensive damage has obvious implications for the animals' ability to capture and transport food particles.

The present study was conducted on adult and juvenile sea scallops, *Placopecten magellanicus*, which belong to the class Bivalvia in the phylum Mollusca. This cold water suspension-feeding bivalve is one of the largest of the scallop species and can attain shell heights of 200 mm. The geographical distribution of this species ranges from the north shore of the Gulf of St.Lawrence to Cape Hatteras, North Carolina (Rehder, 1981; Gosner, 1978). The temperature range to which the scallops can be exposed varies from -1.5°C in the more northerly locations to 21°C in the more southerly locations. Water depths at which *P. magellanicus* can be found are often less than 18m towards the northern end of its range, and 54m or more towards the southern end of the range (Rehder, 1981).

1.2 Objectives

Objectives of the present study are:

1. To determine whether or not the sloughing of epithelial cells in juvenile and adult *Placopecten magellanicus* occurs during all seasons of the year and to determine seasonal changes in the frequency of cellular exfoliation in these scallops.

2. To identify which tissues in the pallial cavity are sloughing epithelial cells and whether or not a qualitative relationship exists between the rate of sloughing and the tissue ultrastructure.

3. To experimentally determine whether or not a potential environmental stressor, such as elevated water temperature, will induce cellular sloughing in this species.

2.0 METHODS

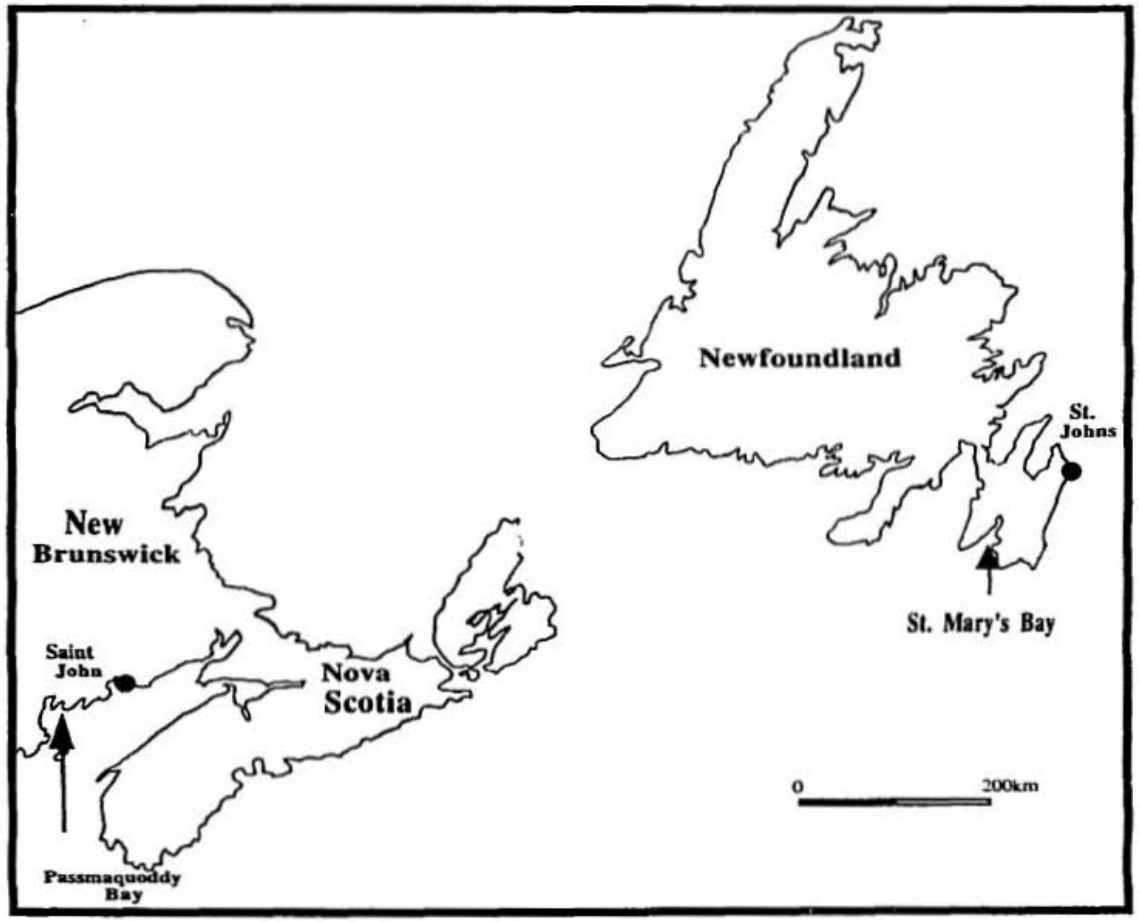
2.1 Collection Site

Animals for the seasonal study were randomly collected by SCUBA from St. Mary's Bay, Newfoundland whereas those for the temperature study were randomly collected from Passamaquoddy Bay, New Brunswick (Figure 1) at depths ranging from 10-20m. Because the seasonal study was designed to investigate epithelial sloughing in juvenile as well as adult sacllops, St. Mary's Bay was chosen as a sampling site because juvenile scallops were readily available. As well, locations in both New Brunswick and Newfoundland were chosen because an historical data base for *Placopecten magellanicus* exists from both areas. The use of scallops from two separate geographic locations was advantageous as it allowed me to determine if cellular exfoliation in *Placopecten magellanicus* was restricted to scallops from one area. After the animals were collected they were placed into coolers and transported to the Ocean Sciences Centre in Logy Bay, St.John's, Newfoundland or to the Huntsman Marine Science Centre in St.Andrew's, New Brunswick. Only scallops that immediately opened their valves and responded to direct agitation by closing their valves were used throughout this project.

2.2 Seasonal Variation

To determine whether or not epithelial cells were being sloughed from scallops throughout the year as a result of "normal" physiological cellular renewal, 25-30

Figure 1. Geographical location of the collection sites for *Placopecten magellanicus* in Newfoundland & New Brunswick, eastern Canada.


Newfoundland
St.
Johns
St. Mary's Bay
New
Brunswick
Saint
John
Nova
Scotia
Passmaquoddy
Bay
0
200km

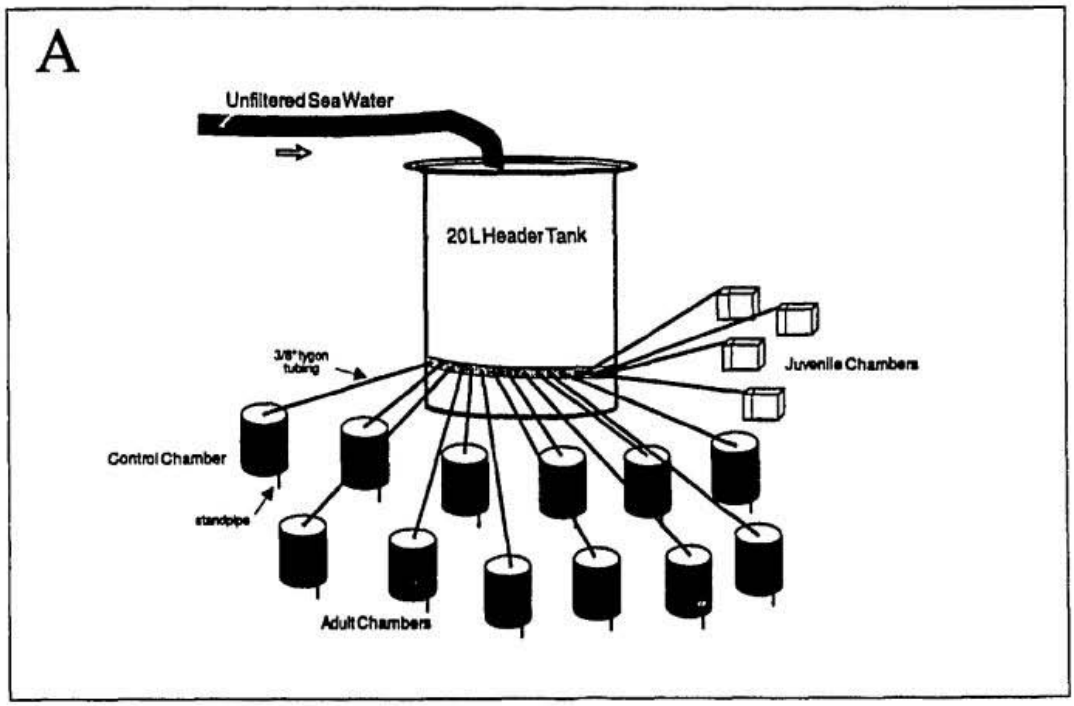
scallops, both juvenile (less than 35mm shell height) and adult (greater than 110mm shell height), were collected approximately monthly between March 1992 and April, 1993. To determine whether or not sloughing is related to reproductive activity, juvenile scallops (i.e. non reproductive animals) were studied. This project was transferred from Newfoundland to New Brunswick in September 1992, however on five occassions (December 1992, January, February, April and October 1993) the animals were collected in Newfoundland and shipped to New Brunswick for completion of the study. An electronic particle counter/ sizer was unavailable from September until the beginning of December 1992, resulting in the absence of data for the three month period. However, scallops were collected in October 1993 to determine whether or not cells were also being released during this time of the year. Upon arrival at the laboratory, the scallops were transferred to, and held in, a tank which received a continuous supply of unfiltered free-flowing seawater. Each animal was cleaned of debris, labelled, and its shell height recorded.

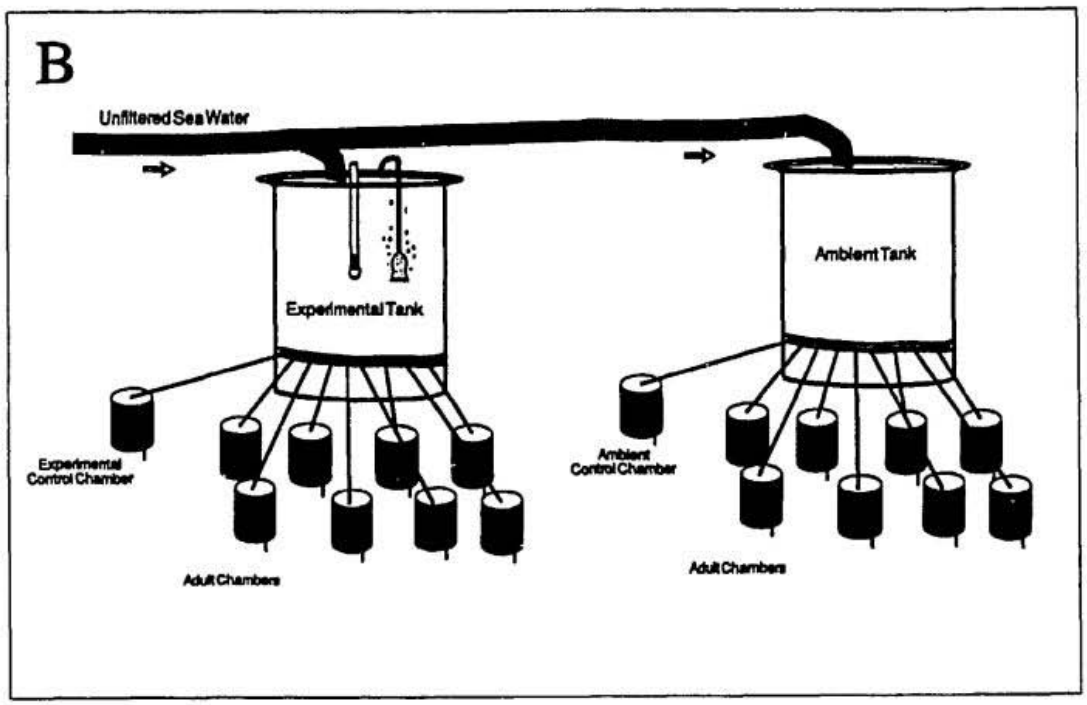
The experimental apparatus used to determine feeding and sloughing activities and rates of the animals was slightly modified from that used by MacDonald (1984) and consisted of a 20L header tank supplied with free-flowing unfiltered sea water through a garden hose. From the header tank, ambient seawater was gravity fed in equal volumes into individual feeding chambers by means of 3/8" tygon tubing. Each scallop was exposed to an independent seawater source containing natural suspended particulate food. Flow restrictors were placed in each tygon delivery tube to regulate

water flow independently for each feeding chamber (250-300mls/min for adults & 160-170mls/min for juveniles). The flow rates for each restrictor were established by collecting all the water passing through the chamber in a graduated cylinder for a known period of time, usually one minute. The bottom of each chamber was fitted with a plastic standpipe to maintain the desired water level within the chambers. Water leaving the chamber through the standpipe and was collected for particle analysis. This system was designed to study activity for a maximum of 16 scallops simultaneously. All but one chamber contained a scallop, leaving a single chamber empty to serve as a control for the natural suspended particles in the seawater which the animals are filtering. In addition to the control, 11 chambers were used for adults and four for the juveniles (Figure 2).

For each month throughout the seasonal study, freshly collected scallops were placed individually into the chambers of the feeding apparatus and allowed to acclimate for 12-24 hours. Replicate water samples (250 mls) were collected from each standpipe approximately 12 to 18 times throughout the month from each of the 16 chambers to determine the concentration and size range of particles. This schedule maximized the probability of recording animals sloughing epithelial cells sometime throughout the month. Samples (0.5ml) were immediately analyzed for particle count and size distribution using a Coulter Multisizer equipped with a 100μm aperture glass tube. If the particle concentration in the water sample from the experimental feeding chamber was less than that measured for the control chamber, the animal was feeding.

Figure 2. Experimental apparatus used to determine feeding & sloughing activities and rates of the scallops. Figure 2A illustrates the apparatus used to study the seasonal variation of cellular exfoliation of the scallops and 2B represents that used to study the effects of elevated water temperatures on cellular exfoliation.


A
Unfiltered Sea Water
20 L Header Tank
3/8" tygon tubing
Juvenile Chambers
Control Chamber
standpipe
Adult Chambers


B
Unfiltered Sea Water
Experimental Tank
Ambient Tank
Experimental Control Chamber
Adult Chambers
Ambient Control Chamber
Adult Chambers

If however, the particle concentration of the water sample collected from the feeding chamber was greater than that for the control chamber, the scallops were considered to be sloughing epithelial cells. This technique provides an instantaneous indication of which animals are sloughing, based on the analysis of the particle concentration and size distribution. If the particle data for a scallop indicated sloughing activity, then a more thorough water analysis was conducted. For example, all particles in approximately 4.5ml of water sample were counted, sized and the data transferred to a computer program (Coulter Multisizer, Accucomp Color Software, 1989) for later analysis. In order to compare these results with the concentration and size distribution of the natural particles available, corresponding water samples were also collected for the same time intervals from the control chamber. At the end of each month, tissue samples were excised from each scallop and fixed in preparation for SEM (scanning electron microscopy) as described below.

2.2.1 Seasonal Incidence of Cellular Exfoliation

The frequency, expressed as a proportion, of scallops sloughing epithelial cells, in terms of total scallops and adults versus juveniles, were calculated for each month. The standard errors of the proportion data were calculated according to formulation presented by Zar (1984). Binomial tests were applied to the frequency data of combined scallops (adults and juveniles together) to determine whether or not sloughing was significantly different from expected (i.e., 50:50 distribution, 50% of

the scallops will slough cells and other 50% will not) within each monthly sampling period.

2.3 Tissue Analysis Using Scanning Electron Microscopy

Small sections of the gill, mantle, gonad, and labial palp were excised from both the adult and juvenile scallops. To account for potential misinterpretation of the micrographs due to the small fraction of organ which was sampled for SEM analyses and a possible lack of structural homogeneity of the tissue surface, several sections excised from different areas of the tissue were examined for a number of individuals. Tissue preparation outlined in Beninger et al. (1988) was modified for the study of scallop tissues throughout this research project. Sucrose was added to the primary and secondary fixatives and the buffer solution to attain an osmolarity of approximately 1000 milliosmoles (C. Powell, Saint John Regional Hospital, pers. comm.). The tissue samples were first fixed in a 2% glutaraldehyde solution buffered with 0.1M Sorensens phosphate buffer at pH 7.4 (1000 mOsm)(Glauert, 1980). The tissue samples were rinsed with this buffer three times to remove any precipitate or debris prior to secondary fixing. Tissue samples were then osmicated with 1% osmium tetroxide buffered with 0.1M Sorensens phosphate buffer at pH 7.4 (1000mOsm)(Glauert, 1980).

After fixation, the tissue samples were dehydrated in a graded ethanol series followed by a CO_2 mediated critical point drying. The samples were immediately

mounted on aluminum stubs and sputter coated with gold. Specimens were observed using either the JEOL JSM 6400 or Hitachi S 570 scanning electron microscope.

2.4 Effects of Elevated Water Temperature on Cellular Exfoliation

In order to determine whether or not the stress associated with elevated water temperatures would induce epithelial sloughing in scallops, the following manipulative experiments were performed. These studies were conducted at the Huntsman Marine Science Centre, St. Andrews, New Brunswick. The laboratory facility is supplied with unfiltered, flowing seawater pumped from Passamaquoddy Bay.

Water temperatures in June and July normally range from 8-11^{0}C but can get as high as 18^{0}C during the summer months. To investigate the potential effects of warmer water on sloughing activity, ambient seawater (8.5^{0}C) was increased to temperatures of 14.7 and 21.0^{0}C. The latter temperature was chosen as it represents the maximum temperature experienced by *Placopecten magellanicus* within its geographical range. To accomplish this it was necessary to use two parallel flow through feeding apparatus identical in design to that used in the previous seasonal study. One system functioned as the experimental apparatus (system A) in which water temperature was increased to 14.7 and 21.0^{0}C, while the second served as the ambient control system (system B), in which temperature slightly increased during the experimental period (8.5, 9.9 & 10.8^{0}C).

Each of the two feeding apparatuses was modified to hold 9 experimental

chambers (eight chambers for the scallops and one to remain empty serving as a control for the group being tested). System A was subjected to three different experimental temperature regimes (8.5°C, 14.7°C & 21.0°C), and system B remained at ambient water temperatures (8.5°C, 9.9°C & 10.8°C). A continuous supply of flowing seawater was delivered to each system by means of separate garden hoses supplied from the same seawater line to ensure that each system received an identical food supply. The seawater in system A was heated to the desired experimental temperatures by means of submersible aquarium heaters placed in the header tank. To ensure adequate mixing of the heated water, an air stone was placed into the systems' header tank (Figure 2). Eight animals of similar size (130-150mm) were placed individually in the feeding chambers of each feeding apparatus (systems A & B- total of 16 animals: A-8 experimental, B-8 control). For the initial experiments, scallops from both groups (experimental & control) were exposed to ambient water temperatures of approximately 8.5°C for four consecutive days, the first of which served as an acclimation period of 12-24 hours. Water samples were then simultaneously collected from each chamber of both systems A and B at 0900, 1200 and 1600 hours for the remaining three consecutive days. At the end of the fourth day of the initial experiment (after the final collection of water samples), the initial water temperature in system A was increased from 8.5 to 14.7°C while the animals in system B were held at slightly elevated ambient temperatures of approximately 9.9°C. The scallops in both systems were given an acclimation period of 12-24 hours after

which water samples were concurrently collected from all chambers of both systems at 0900, 1200 and 1600 hours for three consecutive days. At the end of the third day, the water temperature flowing through the experimental system was raised from 14.7 to 21.0^{0}C while the control apparatus was held at ambient temperature of roughly 10.8^{0}C. As above, there was a 12-24 hour acclimation period for the scallops post temperature increase. Water samples were collected from the feeding chambers of both systems at 0900, 1200 and 1600 hours for four consecutive days. The same 16 scallops were used for each temperature study, facilitating a repeated measures experimental design and analysis. The water samples were analyzed for particle concentration and size distribution with the electronic multisizer and data downloaded to disk for further analyses as described above.

The control system (system B) served a dual purpose; (1) To allow for the determination of the baseline sloughing rate for scallops at ambient conditions against which the sloughing rates from the experimental group (exposed to 8.5, 14.7 &21.0^{0}C) were compared to determine the effects of elevated water temperature, if any, on the sloughing frequency and sloughing rate and (2) To control for time, i.e., to ensure that any change in sloughing frequency and rate of the experimental scallops beyond that of "baseline" levels, was not due to time spent in the feeding chambers but rather a reflection of the elevated water temperatures. Scallops placed into this control system were referred to as the "master control group" to avoid confusion regarding the different controls.

To determine the effects of elevated water temperature on the scallops' frequency of sloughing epithelial cells and the rates of exfoliation as a result of temperature stress: (1) **Individual sloughing rates** (cells min^{-1}) for both the experimental and control scallops were determined at each sampling period (i.e., 0900, 1200 & 1600 hrs) within the separate temperature regimes (i.e., at 8.5, 14.7 & 21.0°C for the experimental scallops and 8.5, 9.9 & 10.8°C for the control group) by calculating the number of cells sloughed from each scallop during these times. To calculate the approximate numbers of cells sloughed per minute from individual scallops: (i) the concentration of particles between 6-20μm diameter was estimated by determining the size distribution of a water sample analyzed for two minutes from a chamber containing a scallop. The particle concentration between 6-20μm recorded for the corresponding empty control chamber was subtracted from the number of same size particles recorded for chamber containing the scallop. The resulting value was then divided in half to obtain the approximate number of cells sloughed per minute (X cells min^{-1}). (ii) The value for X was multiplied by a 1 : 2.25 ratio (2.25 mls of water sample were analyzed per one minute of analysis) to determine the number of cells sloughed per millilitre of water sample collected from the chambers containing a scallop (Y cells ml^{-1}). (iii) The value for Y was then multiplied by the flow rate (ml min^{-1}) of sea water into the individual chambers containing a scallop in order to obtain a **final sloughing rate** (cells min^{-1}) for the scallops (Appendix 1 contains the formulae for the calculations described above). A 6-20μm size range was

selected because epithelial cells have been found to be 7-15μm in size. The particles remaining within the 4-20μm range, after subtracting that of the "reference" chamber, indicated the presence of ciliated and non ciliated epithelial cells. (2) The **maximum number of epithelial cells released** min^{-1} from a scallop of the "master control group", at any time throughout the sampling period while exposed to the initial ambient 8.5°C water temperature, was taken as the scallops' "baseline sloughing rate" for the scallops. The maximum number was chosen as the conservative baseline value in order to be sure that animals sloughing at rates above this value were the result of some factor other than "normal" physiologically induced cellular turnover, in this case, temperature stress. (3) Individual sloughing rates of the experimental scallops were compared to the baseline sloughing rate. Sloughing was considered a result of "normal" physiologically induced cellular turnover if it was at or below the baseline value; however, above this level, sloughing was considered a result of the elevated water temperatures. To determine the effects of elevated water temperatures on the frequency of scallops sloughing cells, the numbers of experimental scallops with sloughing rates above baseline were tabulated and statistically analyzed as described below.

2.4.1 Incidence of Exfoliation at Elevated Water Temperatures

The frequency, in terms of proportion, of scallops sloughing epithelial cells above the baseline rate at least once during the sampling period at each experimental

temperature (8.5, 14.7 & 21.0°C) was established. The standard errors for the proportions were calculated using standard formulations (Zar, 1984). To determine the effects of time spent in the feeding chambers (a total of 13 days, i.e. 4 days at 8.5°C, 4 days at 9.9°C & 5 days at 10.8°C) on the frequency (proportions) of scallops sloughing cells, the proportions of control scallops releasing cells above the baseline level were statistically analyzed by Cochrans' Q (Sokal & Rohlf, 1981). The proportions were examined across the first 4 days, the second 4 days and the third 5 days spent in the chambers while exposed to each temperature (8.5, 9.9 and 10.8°C). This was done to obtain an unbiased effect of elevated water temperatures on the frequency (proportions) of experimental scallops sloughing cells. Both Cochran's Q and the Binomial test were then performed on the data collected from the experimental group to determine the effect of increasing water temperatures on the frequency (proportions) of scallops releasing cells (Zar, 1984).

2.4.2 Exfoliation Rates at Elevated Water Temperatures

There was no noticeable pattern of sloughing activity of the individual scallops of either group with respect to sampling time (0900, 1200, 1600 HRS) or day (day 1-day 4, 5) within a particular temperature (i.e., the presence and rate of cellular sloughing varied extremely within sampling periods). For these reasons, the sloughing rate data accumulated over 13 days for each group was pooled across sampling times and days and compared between temperatures (8.5, 14.7 & 21.0°C for the

experimental group and 8.5, 9.9 &10.8^{0}C for the control group). Mean rates (# epithelial cells · minute^{-1}) for sloughing activity above baseline cellular release (at any one time throughout the sampling period), were calculated for the 8 experimental scallops at each sampling period for each temperature (8.5, 14.7 & 21.0^{0}C. The standard errors for the sloughing rates which were calculated for the scallops sampled in this study, were calculated using standard formulations (Zar, 1984). Mean sloughing rates for scallops of both groups at each temperature regime were transformed using the following transformation, $y = \log(X+1)$, (where y is the tranformed value and x is the original value before transformation), in order to normalise the data set and ensure that the data set meet the distribution requirements of the test (SAS Institute Inc., 1990). A repeated measures MANOVA (GLM) described by the SAS/STAT procedures guide was initially performed on the rate data of the control group to determine whether or not time spent in the feeding chambers influenced the sloughing rates of the scallops. The sloughing rate data for the control group was manipulated in order to test for the effect of time with respect to time spent at each temperature (8.5, 9.9 & 10.8^{0}C). The same statistical test was then performed on the experimental group to determine the effect of the temperature increase on the sloughing rates of the experimental scallops (SAS Institute Inc., 1990).

At the end of the manipulative temperature experiments, scallops were promptly dissected and tissue samples of the gill, mantle, gonad and labial palps were

removed and fixed in preparation for SEM analyses. SEM protocol used throughout this latter study followed that previously outlined in section 2.3 of the methods. Due to experimental design (repeated measures) and the fact that obtaining tissue samples for SEM examination involved sacrificing the animal, it was not possible with this study to investigate the effects, if any, of elevated water temperatures on tissue ultrastructure after exposure to the 14.7°C temperature regime. Any effect to tissue ultrastructure is therefore a result of exposure to the warmest water temperature (21.0°C) or a cumulative result of the two elevated water temperature treatments (14.7 & 21.0°C). One may not expect damage to tissue ultrastructure after short-term exposure to 14.7°C water because *Placopecten magellanicus* may encounter such temperatures in the Atlantic region. It is less likely for the scallops to be exposed to water temperatures of 21.0°C, which may change tissue ultrastructure as a result of temperature stress.

To confirm that the increase in particle concentration, observed for animals apparently releasing cells, was due to the actual release of epithelial cells, water samples were collected from animals assumed to be releasing cells. Cells were preserved in 5% formaldeyde solution which was buffered with marble chalk. The preserved water sample was filtered under vacuum through a 1μm nuclepore filter pretreated with polylysine (Marchant & Thomas, 1983). The filter and adhered cells

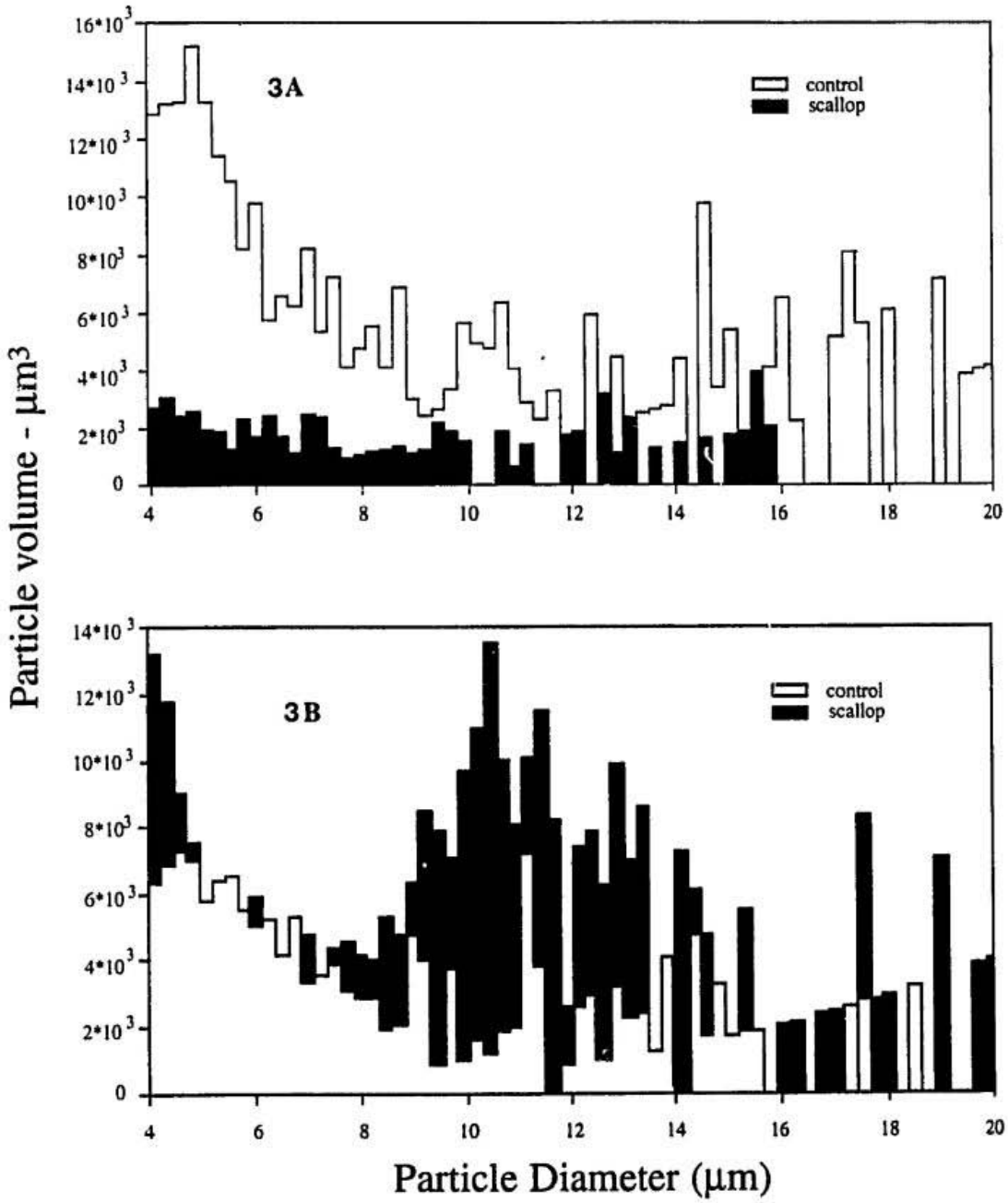
were then dehydrated in an ethanol series, after which the filters were critical point dried, mounted on an aluminum stub, gold coated and observed with the Jeol JSM 6400 scanning microscope.

3.0 RESULTS

3.1 Seasonal Variation

It is possible to determine whether or not a scallop was feeding or sloughing epithelial cells by analyzing the concentration and size distribution of particles from the empty control chamber and comparing it to the data collected from the experimental scallop chamber. If the particle concentration, expressed in number of particles per millilitre, of the water sample from the feeding is less than that for the control chamber, it indicates that the scallop was feeding. Figure 3A illustrates a particle size distribution typical for an animal which is clearing suspended particulates from the water. If however, the particle concentration of the water sample collected from the feeding chamber is greater than that for the control chamber, the scallops were considered to be sloughing epithelial cells. Figure 3B illustrates a particle size distribution typical of a scallop sloughing epithelial cells. A distinct peak exists in the 7-15μm particle size range that is not observed in the control sample. This peak corresponds to the occurrence of epithelial cells that were sloughed from the scallop. As well, there appears to be a peak in the 4-5μm size range which could be related to the release of male gametes during the spawning season. There is a high variability in sizes of these cells and this peak may simply be small epithelial cells or fragments of cells. Note that the particle data for analyses were based on particle concentration in terms of particle number however, the size distributions are expressed in terms of particle volume in order to see the epithelial peak in the 7-15μm size range. The

Figure 3. Particle size distributions of water samples collected from a control chamber and that containing a scallop. Figure 3A illustrates a scallop that was clearing particles from the water for feeding purpose. Figure 3B is indicative of a scallop found to be sloughing epithelial cells.


3A
control
scallop
16*10^3
14*10^3
12*10^3
10*10^3
8*10^3
6*10^3
4*10^3
2*10^3
0
4
6
8
10
12
14
16
18
20
3B
control
scallop
14*10^3
12*10^3
10*10^3
8*10^3
6*10^3
4*10^3
2*10^3
0
4
6
8
10
12
14
16
18
20
Particle volume - µm3
Particle Diameter (µm)

criterion established throughout the seasonal studies which classified a scallop as a slougher required the water sample collected from a feeding chamber containing an animal to have a particle concentration (in term of numbers) greater than that for the "empty" control water sample. It was later discovered (i.e., after completion of the seasonal studies but prior to the temperature manipulative studies) that particle concentration above that for the control, is not an ideal indicator for distinguishing which scallops are releasing cells. This is because they may be both releasing cells and consuming particles simultaneously, resulting in a concentration lower than the control (refer to section two of the results for a more detailed explanation). The use of the criterion mentioned above, resulted in very conservative estimates for percent frequencies of scallops sloughing epithelial cells throughout the seasonal studies, thus representing the minimum number of scallops sloughing. For these reasons, a revised, more precise criterion was used throughout the manipulative temperature experiments and is described in greater detail in section 3.2 of the results.

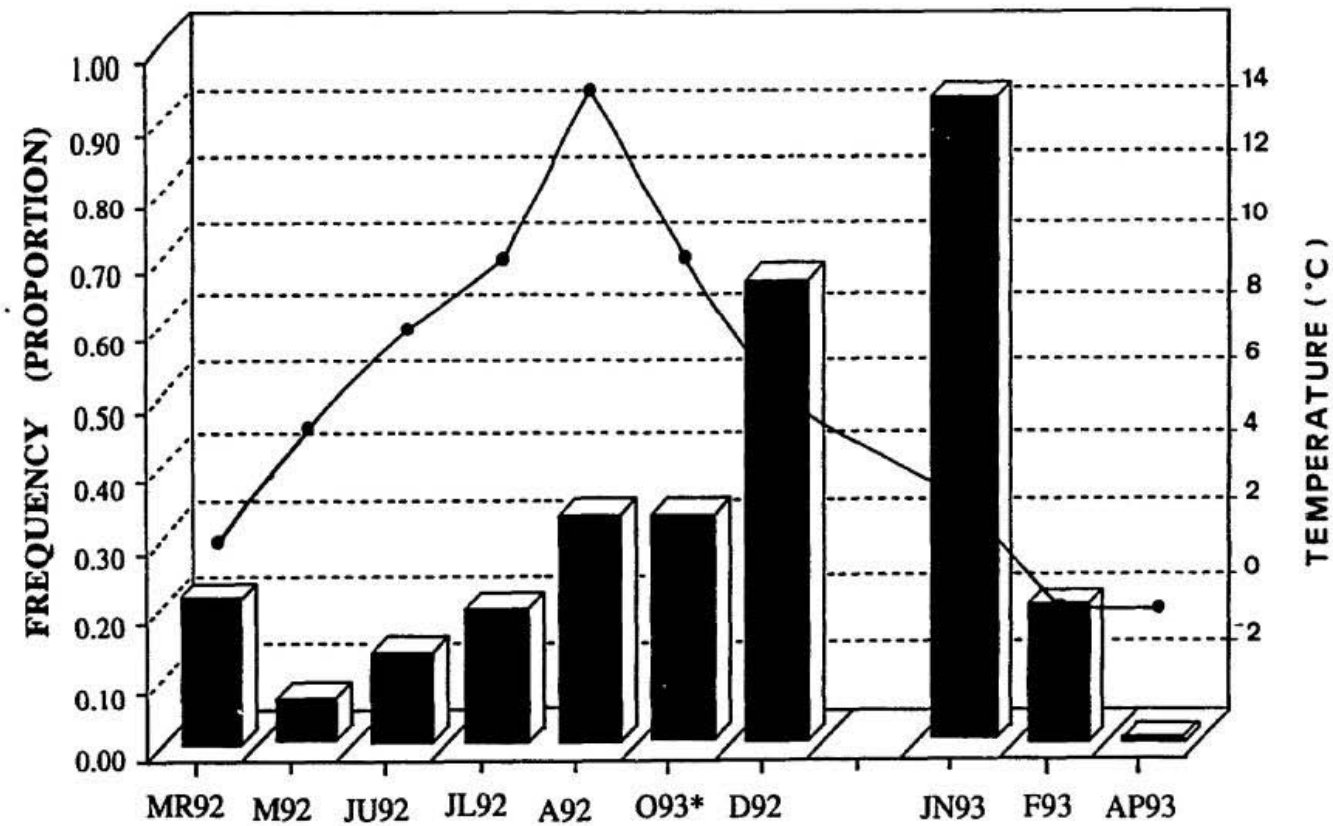
3.1.1 Seasonal Incidence of Cellular Exfoliation

Seasonal frequencies in terms of proportions and variation of sloughing activity in adult and juvenile scallops combined between March 1992 and April 1993 and for October 1993 are summarized in Table 1 & Figure 4. Evidence for epithelial sloughing exists in all but one month (April 1993). As many as 93.3% of all scallops observed (adults and juveniles) were found to be shedding epithelial cells at least once

Table 1: Seasonal frequencies (proportions) of all scallops (both adults and juveniles) that were found to be releasing epithelial cells during the 1992-1993 year in relation to monthly mean water temperatures. Frequency (proportion) = Freq (P), standard error = STD ER, sample size = N, mean temperature (^{0}C) = T & standard error for mean temperature = T ERR. "*" indicates that samples were collected out of sequence.

MONTH/ YEAR	FREQ (P)	STD ER	N	T	T ERR
MARCH 1992	0.214	0.114	14	1.500	0.118
MAY 1992	0.067	0.067	15	4.200	0.100
JUNE 1992	0.133	0.091	15	7.200	0.264
JULY 1992	0.200	0.107	15	9.100	0.239
AUGUST 1992	0.333	0.126	15	13.900	0.214
OCTOBER 1993*	0.333	0.126	15	9.200	0.374
DECEMBER 1992	0.667	0.126	15	4.700	0.167
JANUARY 1993	0.933	0.067	15	2.200	0.279
FEBRUARY 1993	0.200	0.107	15	-1.200	0.167
APRIL 1993	0.000	0.000	15	-1.000	0.000

Figure 4. Seasonal frequencies of scallops releasing epithelial cells during the 1992-1993 study in relation to monthly mean water temperatures. The bar graph represents frequencies and the line graph indicates the water temperatures. March = MR, May = M, June = JU, July = JL, August = A, October = O, December = D, January = JN, February = F & April = AP.



* Samples collected out of sequence

during January 1993. Binomial testing of all seasonal frequency data found sloughing activity for the month of January 1993 alone to be significantly different from the expected frequency (i.e., 50:50 distribution), ($p < 0.05$). Sloughing activity throughout the remaining months was not found to be significantly different from expected ($p > 0.05$). The sloughing frequencies of the adult and juvenile groups were calculated separately and are shown in Table 2 & Figure 5. It is important to note that the sloughing of epithelial cells was frequently reported for juveniles. In addition, the size frequency distribution of sloughed cells from the juveniles is similar to that of the adults with the exception that the peak appears to shift from 6-15μm to 4-5μm.

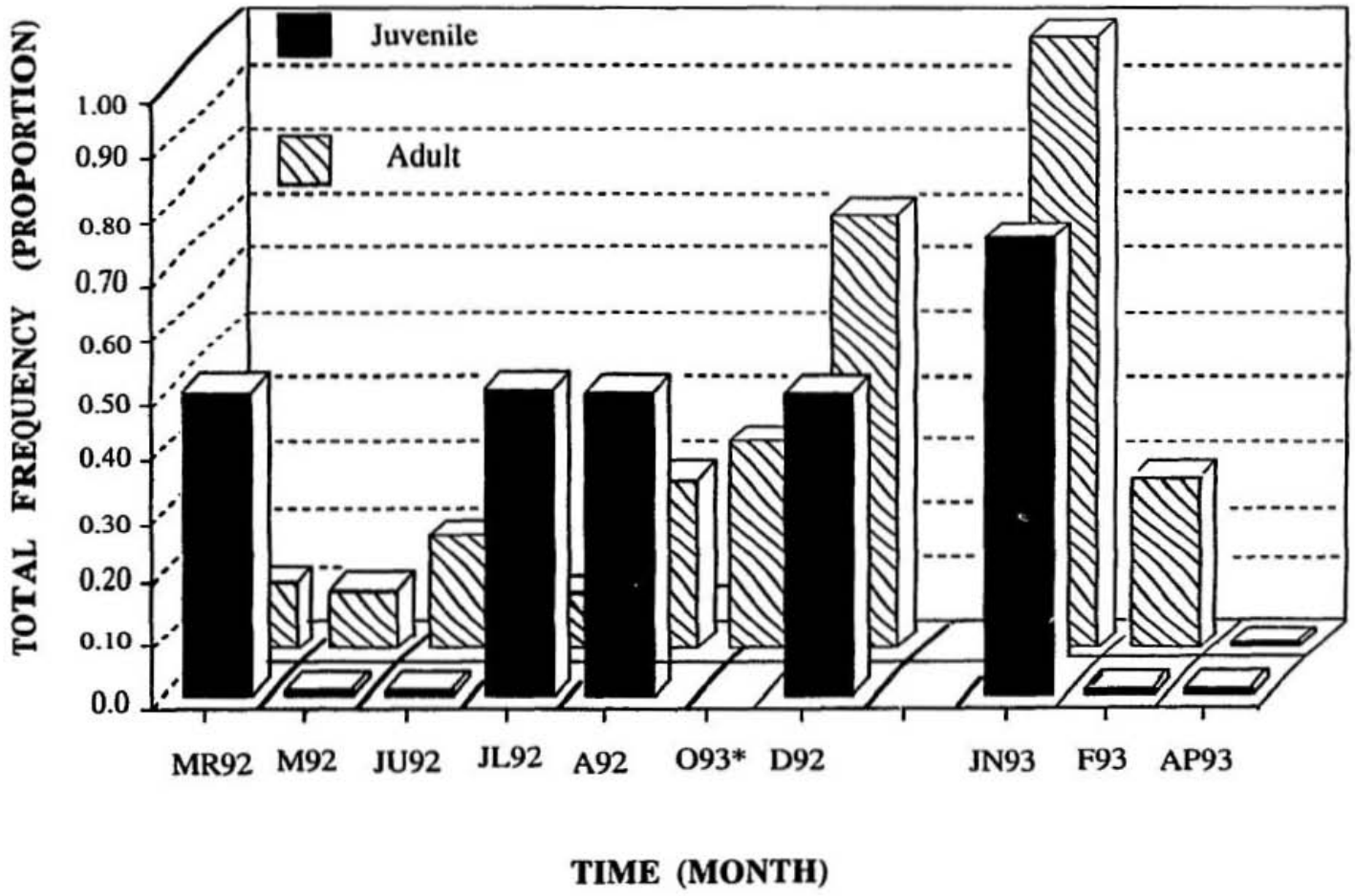
3.1.2 Tissue Analysis using Scanning Electron Microscopy, Part I

Gill epithelia considered to be typical of a "normal, healthy" scallop are very well ciliated with lateral, frontal and laterofrontal cilia found along the gill filaments (Figure 6). The surface microanatomy of the mantle and gonad includes a number of ciliary tufts which interrupt regions of non ciliated, microvilliated epithelia. In general, the surface epithelia of a "normal" are continuous, i.e., the surface is not pitted. For a detailed description of "normal" surface ultrastructure of the gills, mantle and peribuccal organs of *P. magellanicus*, refer to Beninger et al. (1990a&b), (1988); Bubel (1984), (1973b); Tamarin et al. (1976); Zylstra (1972a). Figures 6A-C are representative micrographs of the tissues dissected from approximately 75 animals that apparently had not released cells. Tissue ultrastructure of the gill, mantle and

Table 2: Seasonal frequencies of adult versus juvenile scallops that were found to be sloughing epithelial cells throughout 1992-1993. Frequency (proportion) = Freq (P), standard error = STD ER & sample size = N. "*" indicates that samples were collected out of sequence. N/A signifies that juveniles were not used during the October 93 study.

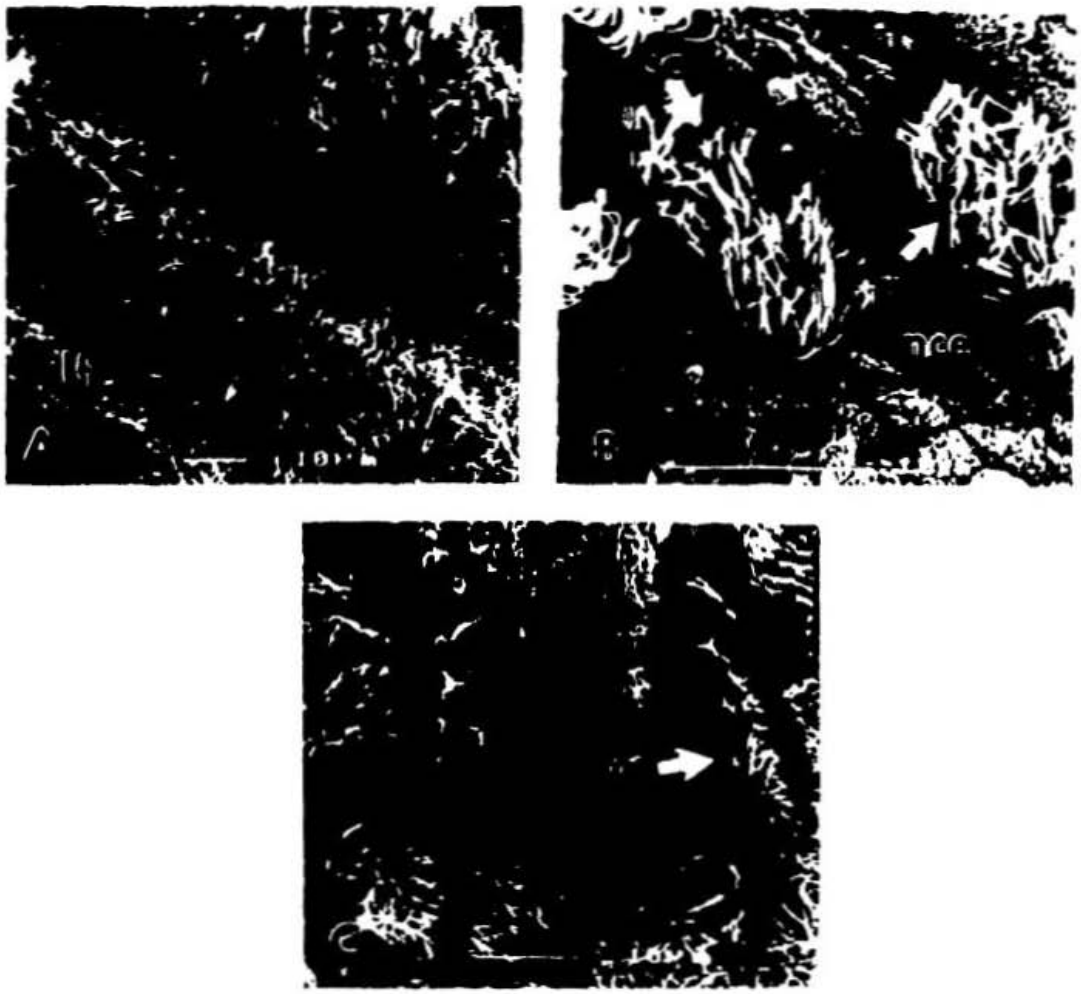
MONTH/ YEAR	ADULTS			JUVENILES		
	FREQ (P)	STD ER	N	FREQ (P)	STD ER	N
MARCH 92	0.100	0.100	10	0.500	0.287	4
MAY 92	0.091	0.091	11	0.000	0.000	4
JUNE 92	0.182	0.122	11	0.000	0.000	4
JULY 92	0.091	0.091	11	0.500	0.287	4
AUGUST 92	0.273	0.141	11	0.500	0.287	4
OCTOBER 93*	0.333	0.126	15	N/A	N/A	N/A
DECEMBER 92	0.723	0.141	11	0.500	0.287	4
JANUARY 93	1.000	0.000	11	0.750	0.250	4
FEBRUARY 93	0.273	0.141	11	0.000	0.000	4
APRIL 93	0.000	0.000	11	0.000	0.000	4

Figure 5. Seasonal sloughing frequencies of adult versus juvenile scallops during the 1992-1993 study. March = MR, May = M, June = JU, July = JL, August = A, October = O, December = D, January = JN, February = F & April = AP.



* Samples collected out of sequence

Figure 6. Scanning electron micrographs of the gill, mantle and gonad of *Placopecten magellanicus* which illustrate characteristics of "normal" epithelium. This animal was not known to have released epithelial cells. A, piece of gill; note the abundance of cilia on each the lateral (l), laterofrontal (lf) & frontal (f) tracts. B, mantle tissue & C, section of gonad. Both the mantle and gonad surface are continuous (i.e. not pitted) and abundant in regions of ciliated epithelium (ce), (as indicated by the arrows), which frequently interrupt regions of non ciliated epithelium (nce).



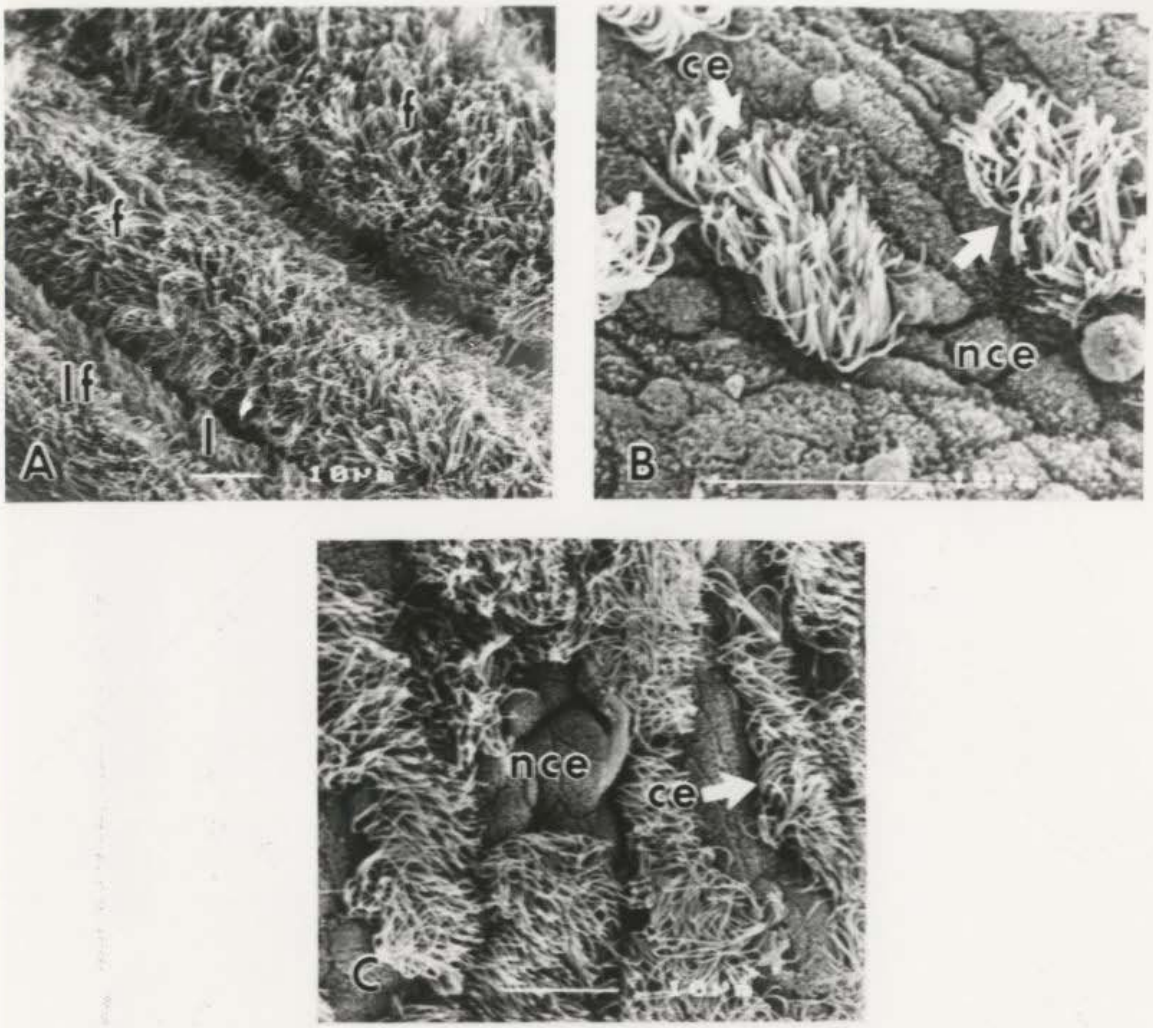
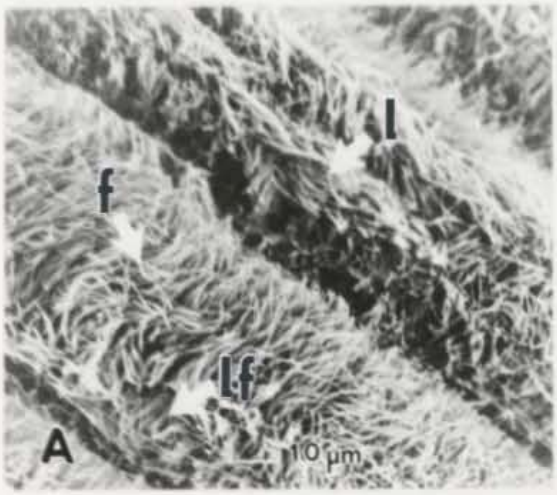

f
f
lf
l
A
ce
nce
B
nce
ce
C

Figure 7. Scanning electron micrographs of the gill, mantle and gonad of *Placopecten magellanicus* sloughing epithelial cells. The low degree of sloughing had no obvious detrimental effects on the tissues examined. A, section of gill, note the abundance of cilia on all 3 ciliated tracts as indicated by the arrows (l, lateral cilia; lf, laterofrontal cilia & f, frontal cilia). B, mantle tissue & C, section of gonad, both of which are continuous and abundant in ciliary tufts as indicated by the arrows.


l
f
l.f
A
10 µm


B

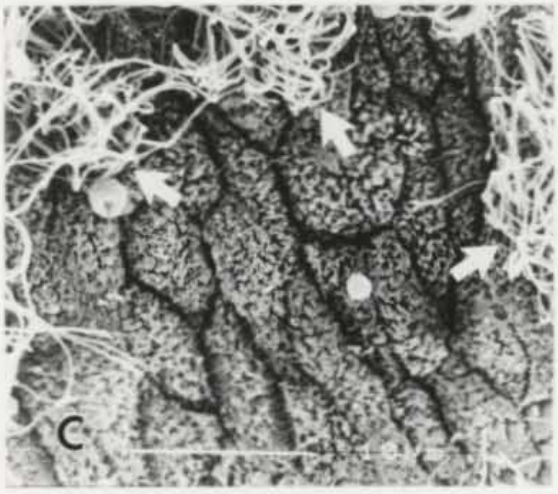
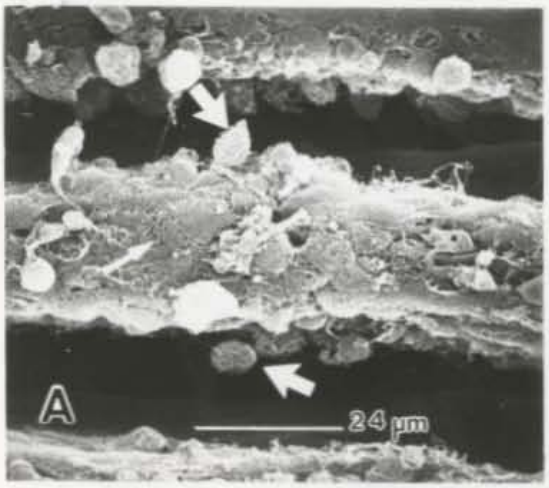
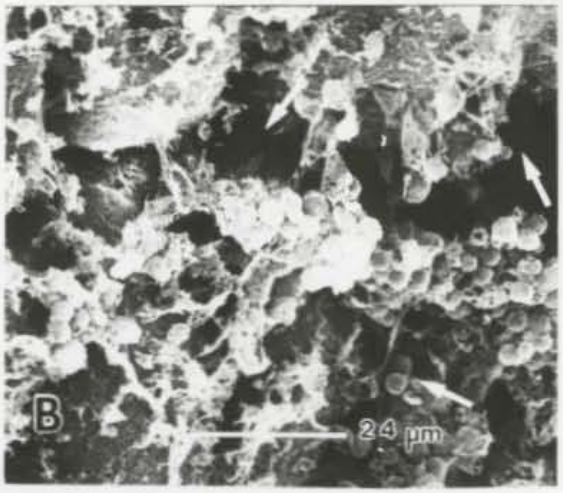
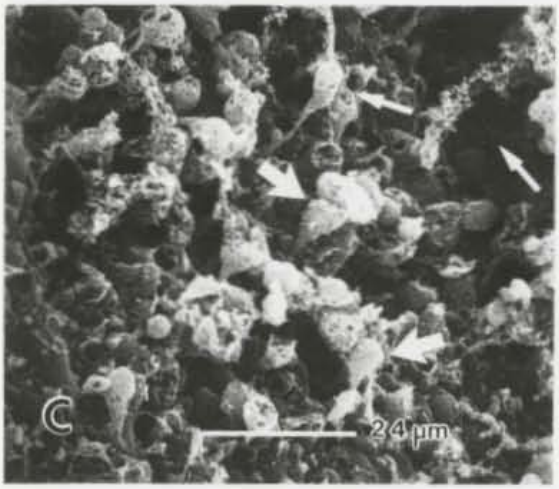

C

Figure 8. Scanning electron micrographs of the gill, mantle and gonad of *Placopecten magellanicus* found to have released large numbers of epithelial cells (photos provided by MacDonald et al., 1995). A, section of gill. B, section of scallop mantle. C, section of scallop gonad. These tissues are lacking in ciliary structures and show signs of atrophy as indicated by the arrows.

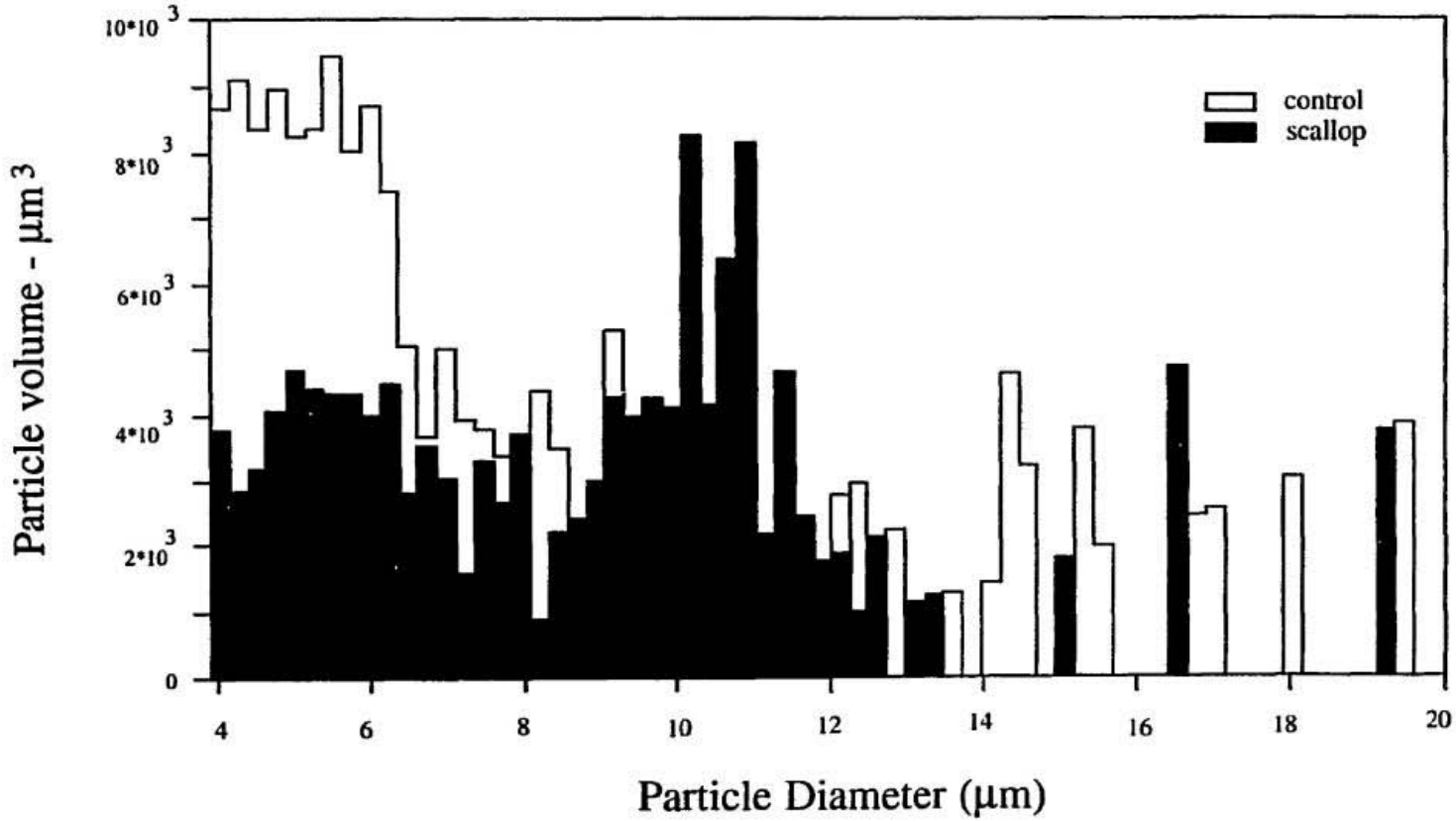

A
24 µm


B
24 µm


C
24 µm

proportionately more particles through feeding than it is adding through sloughing, resulting in a particle concentration lower than the control (Figure 9). In this example, the scallop was sloughing epithelialcells as evidenced by the peak in particle concentration between 8 and 13μm. However, it was simultaneously filtering particles as indicated by the drop in concentration, particularly in the 4 to 8μm size range. Given this information, it was necessary to alter the initial criterion used to identify which scallops were shedding epithelial cells (refer to section 1 of the methods). This was done by subtracting the number of particles between 6 and 20μm recorded for each empty "reference" chamber from the number of the same size particles recorded for each feeding chamber. The number of particles remaining indicated the number of epithelial cells sloughed per minute from an animal. Individual sloughing rates of scallops from the "master control group" were used to determine the "baseline" level of epithelial sloughing (refer to section 2.4 of the Methods for calculation of the baseline sloughing rate). This level reflects the number of cells lost as a result of "normal" cellular regeneration. To determine the effects of elevated water temperatures on the percentage of scallops sloughing and the degree of cellular release, the sloughing rates of the experimental scallops were compared with normal baseline rates. The baseline sloughing rate of epithelial cells released from adult *Placopecten magellanicus* was calculated to be approximately 5300 cells min^{-1}. In order to determine whether or not *Placopecten magellanicus* is sloughing epithelial cells as a result of some external factor, in this case temperature stress, it was

Figure 9. Particle size distributions from both a control water sample and that from a chamber containing a scallop found to be sloughing epithelial cells (ca. 8-13μm) while simultaneously filtering particles (4-8μm) from the water.


Particle volume - µm 3
10*10 3
8*10 3
6*10 3
4*10 3
2*10 3
0
4
6
8
10
12
14
16
18
20
Particle Diameter (µm)
control
scallop

necessary to take the baseline sloughing rate into consideration. If a scallop released more than 5300 cells min^{-1} within the 4-20μm size range, it was considered to be affected by temperature.

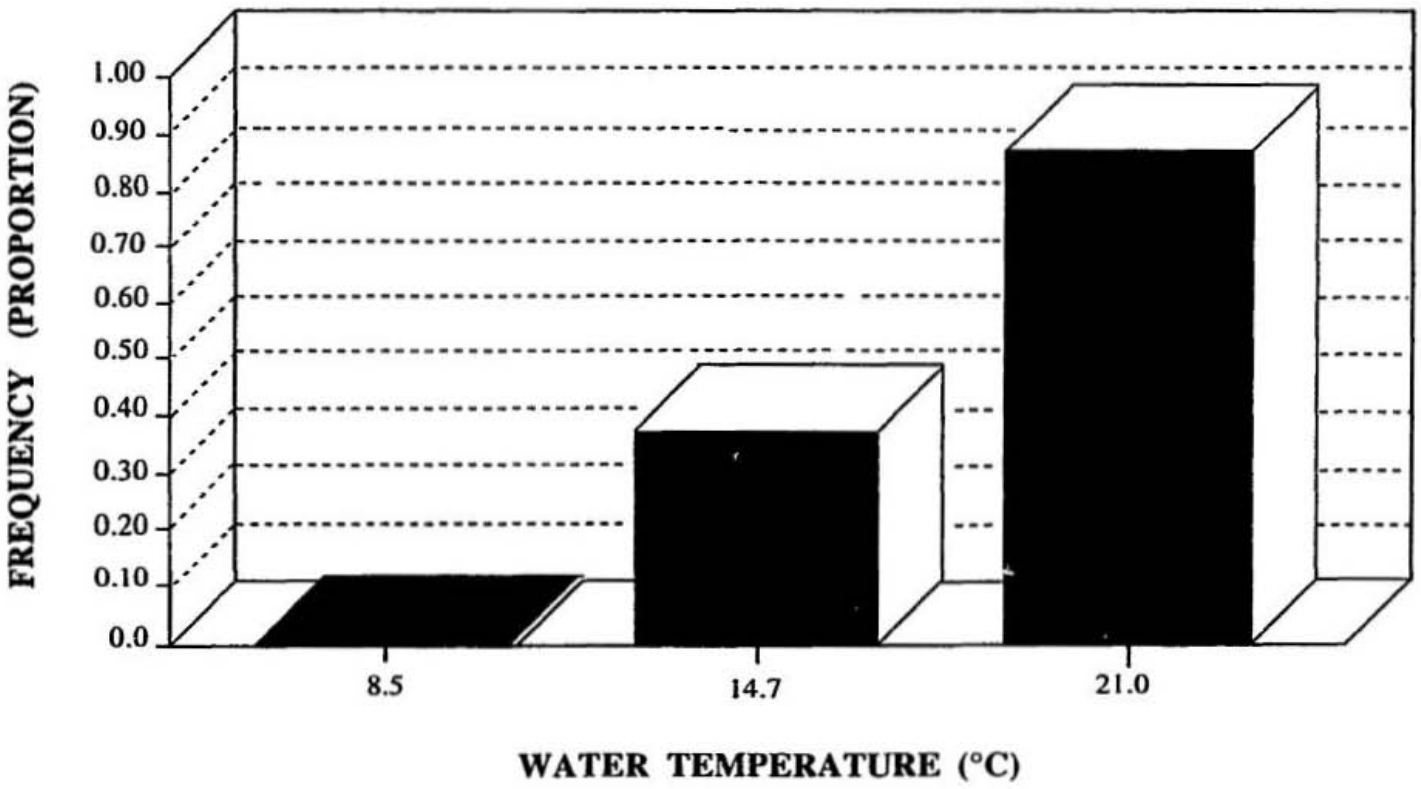
3.2.1 Incidence of Exfoliation at Elevated Water Temperatures

Statistical analysis of the proportion of control scallops releasing cells above the baseline level with respect to time, showed that the time spent in the chambers (total of 13 days) did not significantly affect the frequency of scallops sloughing (Cochrans' Q, $\chi^2 = 3$, $P > .05$, $df = 2$, $N = 8$). The proportion of experimental scallops releasing cells above baseline levels was calculated at the end of each temperature experiment. There was an overall increase in the frequency of scallops sloughing as temperature increased: 0/8 scallops sloughed at 8.5°C, 3/8 scallops sloughed at 14.7°C & 7/8 scallops sloughed at 21.0°C (Table 3 & Figure 10). Cochrans' Q test statistic indicated that the increased water temperature had a significant effect on the sloughing frequency ($\chi^2 = 9.25$, $P < 0.005$, $df = 2$, $N = 8$). Binomial tests of this same data set demonstrate that at 8.5°C and 14.7°C, sloughing frequency does not differ significantly from expected (i.e., 50:50 distribution), ($p > 0.05$) however, at 21.0°C, sloughing frequency was found to differ significantly from the expected value ($p < 0.05$).

Table 3. Frequencies (proportions) of adult experimental scallops sloughing epithelial cells upon exposure to three different temperature regimes. Frequency (proportion) = Freq (P), standard error = STD ER & sample size = N.

TEMP EX (°C)	EXPERIMENTAL		N
	FREQ (P)	STD ER	
8.5	0.000	0.000	8
14.7	0.375	0.183	8
21.0	0.875	0.125	8

Figure 10. Frequencies (proportions) of experimental scallops releasing epithelial cells while exposed to experimental water temperatures (8.5^{0}C, 14.7^{0}C & 21.0^{0}C).


FREQUENCY (PROPORTION)
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.0
8.5
14.7
21.0
WATER TEMPERATURE (°C)

3.2.2 Exfoliation Rates at Elevated Water Temperatures

The repeated measures MANOVA (GLM) of the mean sloughing rates of the control group showed that the time spent in the feeding chambers did not significantly influence the sloughing rates of the scallops. Mean sloughing rates (# epithelial cells min^{-1}) as a measure of sloughing activity were calculated for the 8 individual test scallops after each temperature manipulation (Table 4). Scatterplots of these data showed a positive relationship between sloughing rate and increasing water temperatures (8.5, 14.7 & 21.0°C) (Figure 11). The General Linear Models Procedure (GLM) for a Repeated Measures Analysis of Variance of Contrast Variables demonstrated a significant linear relationship between sloughing rate and temperature (ANOVAR, $F = 45.430$, $P < 0.01$, $df = 1,7$). Increasing water temperatures significantly increased the sloughing rates of the scallops (MANOVAR, Pillai's Trace $= 0.900$, $F = 27.077$, $P < 0.01$, $df = 2,6$). Contrast analyses showed the sloughing rates of scallops at 8.5 and 14.7°C did not differ significantly from one another (MANOVAR, $F = 4.200$, $P > 0.05$, $df = 1,7$), although, there was a significant difference between sloughing rates at 8.5 and 21.0°C (MANOVAR, $F = 45.840$, $P < 0.01$, $df = 1,7$).

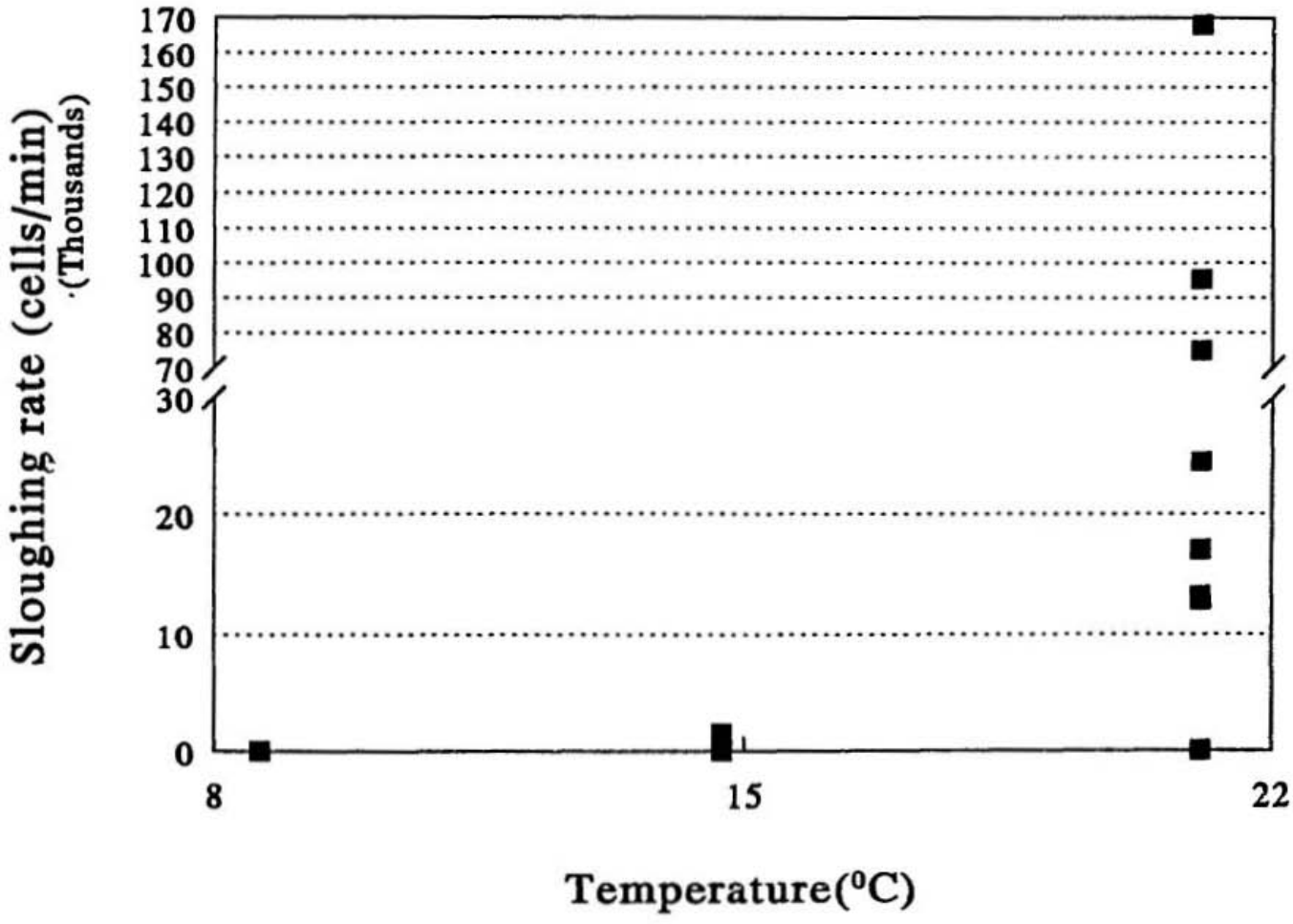
3.2.3 Tissue Analysis using Scanning Electron Microscopy, Part II

SEM analyses of the several tissue sections excised from the experimental animals illustrate that sloughing, to the degree seen throughout the manipulative

Table 4. Mean sloughing rates (cells min^{-1}) and associated standard errors of the eight experimental scallops at each experimental water temperature. Ex = experimental scallops, "*" = standard error.

GROUP	MEAN SLOUGHING RATES (#CELLS/MIN) & STANDARD ERROR					
EX	8.5°C		14.7°C		21.0°C	
1	0.00	0.00*	0.00	0.00*	13127.78	5632.37*
2	0.00	0.00*	0.00	0.00*	17011.11	7784.98*
3	0.00	0.00*	0.00	0.00*	95227.78	40438.39*
4	0.00	0.00*	1337.65	1337.65*	75011.11	38284.29*
5	0.00	0.00*	0.00	0.00*	24555.56	21962.06*
6	0.00	0.00*	1432.72	1432.72*	168222.22	87007.46*
7	0.00	0.00*	1032.10	1032.10*	0.00	0.00*
8	0.00	0.00*	0.00	0.00*	12833.33	6982.36*

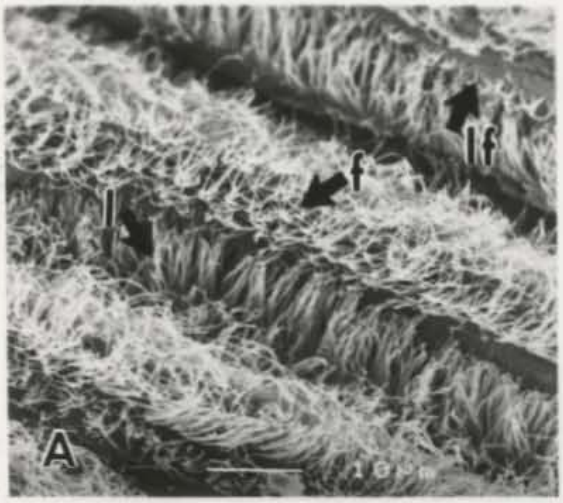
Figure 11. Scatterplot of the individual mean sloughing rates (cells min^{-1}) of the experimental scallops at each experimental water temperature (8.5, 14.7 & 21.0°C).

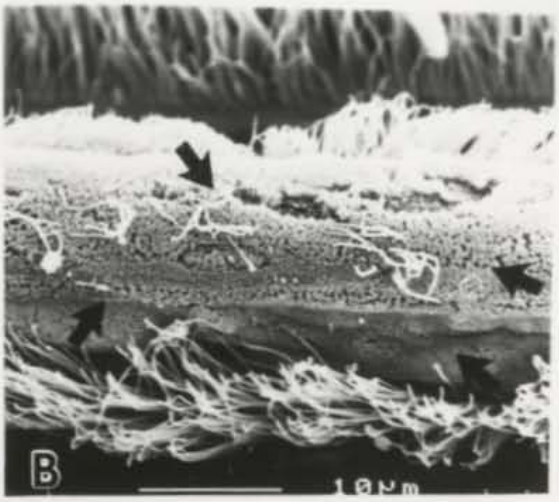

Sloughing rate (cells/min)
(Thousands)
170
160
150
140
130
120
110
100
90
80
70
30
20
10
0
8
15
22
Temperature(°C)

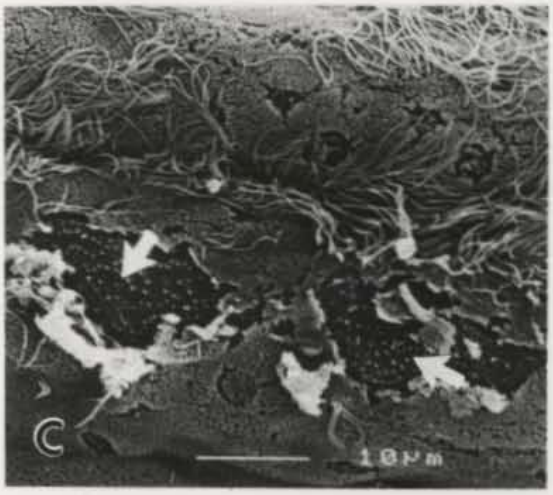
experiments, had a noticeable effect on the tissues (Figures 12A,B,C,D). The figures of scanning micrographs shown here were selected as they best illustrate the overall results of greatly increased sloughing rates on surface ultrastructure of the gill and the mantle. The frontal surfaces of the gill filaments were well ciliated in terms of frontal, lateral & laterofrontal cilia in the dorsal "portion" of the gill (Figure 12A), however, approaching the ventral margin, the filaments became sparsely ciliated until sections of tissue were devoid of cilia (Figure 12B). Sections of epithelium (towards the ventral region) were also missing and/or peeled away from the underlying basement membrane (Figure 12C,D). In a few cases, the ciliary junctions between the ciliated spurs along the abfrontal surface of the filaments had uncoupled. Analyses of the mantle have found random regions of tissue epithelium to be pitted and lacking in any ciliated and non ciliated structures (Figure 12E). The remaining epithelium was composed of both ciliated and non ciliated cells, and appeared similar to that of a "normal" tissue. The degree of sloughing found in this manipulative study appears to represent an intermediate stage of tissue damage, that is, greater than that observed for "normal" sloughing which occurs on a regular basis and would not appear to have any destructive effects on the tissues, and is less than the damage observed when very high numbers of cells were released by adult scallops in a previous study (MacDonald et al., 1995).

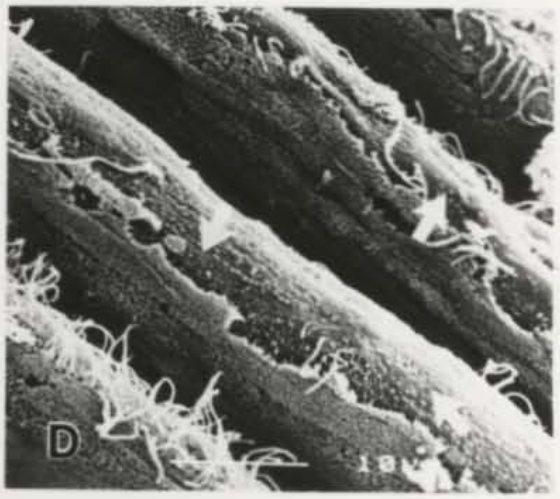
To confirm that the particles being released in this study were actually epithelial cells, SEM analyses was performed on water samples that were collected

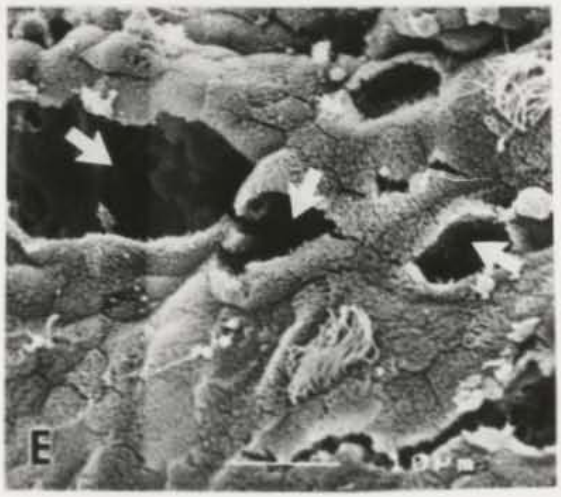
Figure 12. Scanning electron micrographs of scallop tissues excised from experimental animals after exposure to the 21.0°C experimental water temperature. A, ciliated lateral (l), laterofrontal (lf) & frontal (f) tracts of the gill filaments within the dorsal region of the gill. B, sparse ciliation of the gill filament approaching the ventral margin, note the lack of cilia along the three tracts. C&D, gill filaments that have sections of epithelium peeled away from the basement membrane. E, section of mantle tissue which is pitted and lacking in epithelial structures.


A
f
lf
l


B

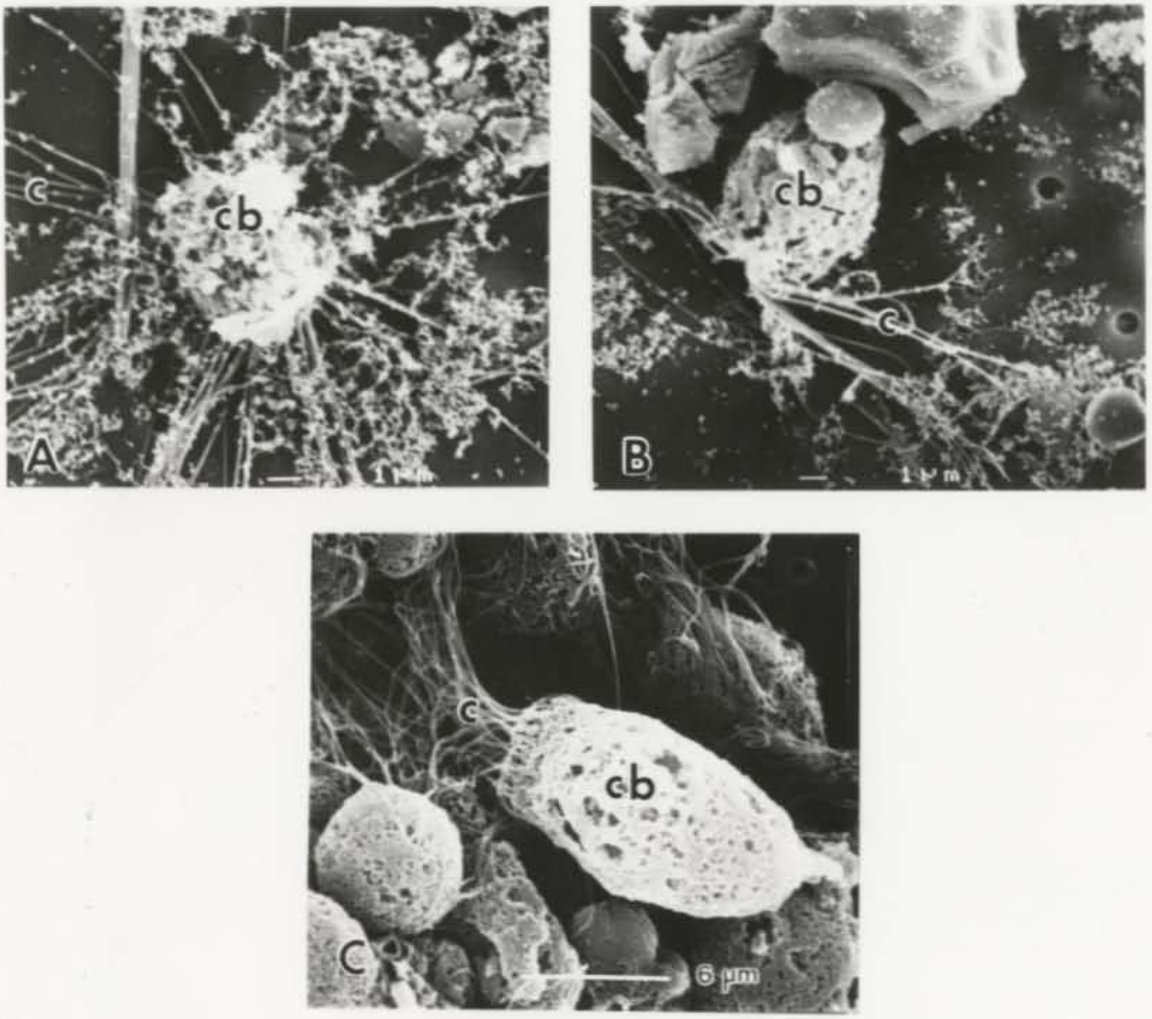

C


D


E

from the experimental group. Figures 13A & B are representative scanning micrographs of typical ciliated epithelial cells that were sloughed from adult scallops post exposure to 21.0°C water temperature. Note the size range of these cells to be approximately 6-15μ. The cellular body is somewhat lacking in cellular detail, but the cilia are quite visible. These micrographs are similar to those published by MacDonald et al. (1995), (Figure 12C).

Figure 13. Representative micrographs of sloughed ciliated epithelial cells. Figures A&B demonstrate ciliated epithelial cells sloughed from scallops exposed to 8.5, 14.7 & 21.0°C water temperatures. Figure C represents one of several ciliated epithelial cells collected by MacDonald et al. from a scallop known to be releasing cells during routine feeding studies (photo provided by MacDonald et al., 1995). Each cell clearly illustrates the cellular body (cb) and attached cilia (c).


c
cb
A
1 µm
cb
c
B
1 µm
c
cb
C
6 µm

4.0 DISCUSSION

4.1 Seasonal Variation

4.1.1 Seasonal Incidence of Cellular Exfoliation

The natural life cycle of a cell involves cellular production via continual cell division, aging, and mortality. Dead cells are perpetually sloughed from the tissues and replaced with fresh ones at a variable rate, thereby facilitating the growth, maintenance and repair processes of normal development (Hole, 1987; Clark (ed.), 1985; Holtzman, 1975). This "normal" physiological turnover of cellular constituents within an organism is controlled by cellular metabolism which simultaneously maintains the internal equilibrium (Cunningham, 1979; Ericcson, 1969). Through compensating for changes in the environment, both internal and external, the metabolic system can preserve the homeostatic state within an organism (Koehn & Bayne, 1989).

The present study demonstrates that relatively low, but detectable numbers of epithelial cells are sloughed from adult and juvenile *Placopecten magellanicus*, on both a regular and sporadic basis respectively, throughout the year (Figures 4 & 5). The frequency of sloughing in the adult scallops is comparable to the values reported for adults from a site in Sunnyside, NF (MacDonald et al., 1995). However, the number of cells being released in this study (less than 5300 cells min^{-1}) is much lower than those recorded in their study (approximately 613333 cells min^{-1}). The regular release of cells from the adult scallops in this study is not restricted to months

exhibiting warm seasonal temperatures or months when gametogenic or spawning activity is occurring (i.e., July-August) but rather, seems to be characteristic of the process of natural physiological turnover of cellular components described above. There appears to be a pattern of increasing frequency of adult scallops sloughing cells from May 1992 until the end of January 1993. However, we should remember that the technique used in the seasonal study (i.e., concentration of particles in outflow water sample greater than that for the control water sample indicated a scallop that was sloughing cells) may underestimate the frequency of scallops (both adults and juveniles) sloughing. Had the technique been more sensitive, like that used in the manipulative temperature study, the frequency of scallops found to be releasing epithelial cells would have been greater and possibly have reduced the variation in the results from one month to another. Interestingly, the size frequency data of cells sloughed from juvenile scallops throughout the seasonal study illustrate the presence of epithelial cells between the 4-15μm size range with a peak in the 4-5μm size range. This represents a shift in the peak to the 4-5μm range from the 7-15μm size range that was observed in the adult scallops. This might suggest that cells which are sloughed from the juvenile scallops, are of slightly different size than those sloughed from the adults or that there is greater variability in size between non ciliated and ciliated epithelial cells.

Epithelial sloughing in *Placopecten magellanicus* during routine field feeding studies has been reported by MacDonald et al. (1995). Others have reported an

increase in concentration of particles, 2-3μm in diameter, during retention efficiency experiments of 6 species of suspension feeding bivalves. This increase resulted in "negative retention" or production of particles (Riisgard, 1988; Mohlenberg & Riisgard, 1978; Vahl, 1972). They believed these particles of unknown composition to be produced by the bivalve and excluded individuals displaying the "negative retention" from the study. The release of particles has also been reported for the hard clam, *Mercenaria mercenaria*, (M. Bricelj, State University of New York, pers. comm.) and the chilean oyster, *Ostrea chilensis*, (R.J. Thompson, Memorial University of Newfoundland, pers. comm.). It is possible that these instances of cellular release occurred as a result of cellular sloughing, either routine or resulting from environmental stress. Therefore, epithelial sloughing in some instances could seriously compromise clearance rate determinations made via use of the electronic particle counter if not taken into consideration.

Juvenile scallops were used in the seasonal study to determine whether or not sloughing was related to reproductive activity, as suggested by MacDonald et al. (1995). If sloughing is exclusively caused by such activity, then sloughing would be restricted to the adults during the months when the scallops undergo gametogenesis and spawning (i.e., April to late August, MacDonald, 1984) and not observed in the juveniles. However, low levels of epithelial cells were released by both adults and juveniles, indicating that reproductive activity was not the sole cause. This does not rule out the possibility that reproductive activity during the months of April until late

August could increase the frequency or rate of sloughing observed in adults as a result of reproductive stress.

The metabolic rate of a bivalve, which presumably regulates sloughing activity, is dependent on body size and age (Bayne and Newell, 1983). For this reason, Bayne and Newell (1983) have suggested that for comparisons between animals of differing sizes, a weight correction would be necessary. The greater the metabolic rate, the more quickly cellular constituents are turned over and vice versa; thus, a smaller/ younger organism would have a metabolic rate, per unit weight, greater than that of a larger individual. With increased cellular turnover, it would follow that the ability to identify individuals sloughing cells should increase and become more consistent. In this study, when the juveniles were found to be sloughing, the proportion of juveniles sloughing cells was higher in all but two months than that observed for the adults. Furthermore, the proportions for the juveniles remained more constant across time. However, sample sizes for juveniles (N=4) were smaller than those for adults (N=10-15). Juveniles might prove to be more useful than adults in monitoring epithelial sloughing resulting from normal physiological processes because of their greater speed of cellular turnover and resulting increased ability to detect "baseline" sloughing.

4.1.2 Tissue Analysis using Scanning Electron Microscopy, Part I

Scanning electron microscopy (SEM) of sections of gill, mantle, gonad and

labial palp tissues removed from adults and juveniles throughout the seasonal study were found to be consistent, in terms of tissue ultrastructure and cellular detail, with those dissected from animals that apparently had not sloughed cells, and with the findings by Beninger (1990a&b, 1988), Bubel (1984, 1973b), Tamarin et al. (1976) and Zylstra (1972a). There was no obvious tissue damage as a result of the low level of sloughing occurring throughout the seasons. If the extent of sloughing seen throughout the seasonal study represents "normal" growth, maintenance and repair processes, one would not expect to see signs of epithelial damage of the gill and mantle as was illustrated by MacDonald et al. (1995). Epithelial cells that are sloughed are continuously replaced by new ones and cilia or microvilli are generated upon formation of these new formed cells (Holtzman, 1975; C. Powell, Saint John Regional Hospital, pers. comm.). Evidence of cilia genesis on the tissue surfaces of both adult and juvenile scallops may represent the "normal" cyclical events of cellular renewal. The tissues do not appear to suffer damage from epithelial sloughing when these cells are released as a result of "normal" cellular renewal.

4.2 Effects of Elevated Water Temperature on Cellular Exfoliation

4.2.1 Incidence of Exfoliation & Rate at Elevated Water Temperatures

It has been previously suggested that stress associated with elevated water temperatures may have been at least partially responsible for the high frequency of epithelial sloughing observed in sea scallops from Newfoundland (MacDonald et al.,

1995). Cellular sloughing associated with epithelium atrophy and necrosis has been well documented in response to other stressors such as heavy metals and anthropogenic pollution (Marigomez et al., 1990; Sunila, 1986 & 1981; Couch, 1984; Simkiss & Mason, 1984; Arimoto & Feng,1983; Ericsson, 1969). Temperature increase has been cited as a potential cause of epithelial sloughing as it can affect many physiological processes in molluscs, but temperature effects on sloughing in bivalves are not well recorded (Cunningham; 1979).

One of the objectives of this study was to determine whether or not unseasonably warm water temperatures would cause the sloughing of epithelial cells in *Placopecten magellanicus*. It is evident from these experiments that elevated water temperatures will significantly increase sloughing activity in *P. magellanicus*. The techniques used in this study were sensitive enough to distinguish between baseline sloughing rates and those produced by temperature stress. Analyses of the frequency and rates of sloughing recorded during the manipulative temperature study have demonstrated significant increases at 21 ^{0}C but not at 8.5 or 14.7^{0}C. Ambient and intermediate temperatures (8.5^{0}C & 14.7^{0}C) did not significantly increase cellular sloughing above normal baseline sloughing rates. Therefore, it can be concluded that rates of cellular release at these temperatures are not different from "normal" physiological functioning. This does not seem unreasonable when considering that *Placopecten magellanicus* can be exposed to such temperatures in Newfoundland and New Brunswick, and may not find these temperatures stressful. The increase in

frequency of scallops sloughing epithelial cells at 21.0°C was obvious and significantly different from the expected distribution. This increase may represent a stress response to elevated water temperatures at a time of the year when scallops are acclimatized to much cooler temperatures. *Placopecten magellanicus* of more northerly geographic locations such as Passamaquoddy Bay, thrive in cold water thus it is not unreasonable to expect this cold water species to respond to the stress associated with warm water temperatures, especially those which it is unlikely to experience. An increase in temperature increases basal metabolic rate, which in turn increases the rate of cellular autophagy or the cellular turnover (Cunningham, 1979; Moore et al., 1979). Under conditions of metabolic stress, sloughing rates of cellular material can increase profoundly (Holtzman, 1975). In fact, the temperature differential between 14.7 and 21.0°C was sufficient to generate sloughing rates beyond those typical of "normal" conditions, implying that sloughing occurred as a result of temperature stress.

Elevated experimental water temperature has been shown to significantly increase the incidence and rate of sloughing in the adult scallops, however the seasonal data showed a higher incidence of scallops sloughing low levels of cells during the winter months. Food availability for scallops is low during the winter months in the Passamaquoddy Bay region (MacDonald, 1984). This could represent a stress to the animals causing them to slough epithelial cells during times of low food availability. It has been suggested that epithelial sloughing during the seasonal cycle

might be related to the "normal" physiological turnover of cellular constituents. The observed peak within the seasonal exfoliation cycle in adult scallops occuring during the winter months may be related to stressful conditions associated with low food availability and/or extremely low water temperatures.

4.2.2 Tissue Analysis using Scanning Electron Microscopy, Part II

Tissues from scallops that released epithelial cells in the seasonal study exhibited no apparent structural differences from scallops not known to have released cells. However, there were obvious differences in tissues from animals sloughing cells when exposed to the highest experimental water temperature. This included damage to the gill and mantle tissues in the form of missing cilia and absence of sections of surface membrane which lie above the epithelial cells. The level of sloughing observed during exposure to the highest experimental water temperature (21.0°C) appears to produce an intermediate level of damage to the tissue. This damage is greater than that observed for "normal" sloughing, which occurs on a regular basis and would not appear to have any destructive effects on the tissues, but less than that resulting in the complete breakdown of the tissue epithelia reported by MacDonald et al. (1995). This intermediate degree of sloughing resulted in "minor" tissue atrophy in terms of area damaged and the animals did not show any physical signs of distress such as gaping valves as a result of such damage. Furthermore, the animals were only subjected to a temperature of 21.0°C for four consecutive days and therefore may not

have been exposed long enough for extreme damage to occur.

Wound repair is a critical epithelial function in all animals whereby blood amoebocytes initially infiltrate the wound site to form a plug. An increase in cellular division at the place of injury, results in the production of squamous epithelial cells, which cover the plug and eventually differentiate into normal epithelia for that particular region (Sminia et al., 1973; Spencer, 1972; Ruddell, 1971). There have been indications of the restoration of uncoupled ciliated interfilamentar junctions and cilia regeneration along the gill filaments of *Mytilus edulis* after short-term exposure to copper and cadmium (Sunila, 1986), and cilia are capable of regenerating from the epithelial cells (Holtzman, 1975). Given this information, it is probable that the scallops could have compensated for the "minor" tissue damage by means of growth and repair processes, although there was no evidence of cilia genesis on the tissue surfaces in this situation. Wound repair within an organism may occur quickly, but the animals in this study were sacrificed immediately post experimentation and might not have had enough time to undergo significant repair (Hole, 1987). Should epithelial sloughing occur at a rate which exceeds compensatory processes, such as when scallops are exposed to high water temperatures, the resulting epithelial distress, on the gill for example, might compromise the scallops' ability to capture, transport and ingest particles, thereby jeopardizing their survival.

5.0 CONCLUSIONS

5.1 Seasonal Variation

Analyses of particle concentration, using an electronic particle counter, throughout 1992 and 1993, revealed that epithelial cells were sloughed by both adult and juvenile *Placopecten magellanicus*. Cells were released at low levels throughout the year, which might be related to "normal" cellular turnover and does not appear to be directly related to reproductive activity. Scanning microscopical analyses of numerous tissue samples collected during this study has illustrated that this low level of sloughing produced no obvious damage to tissue ultrastructure.

5.2 Effects of Elevated Water Temperature on Cellular Exfoliation

Results of the particle analyses for the control group of scallops exposed to ambient temperatures during the manipulative experiments revealed a baseline sloughing rate of approximately 5300 cells min^{-1} $individual^{-1}$ for *Placopecten magellanicus*. Sloughing rates similar to or below this level may represent sloughing as a result of "normal" physiological cellular turnover at this time of the year. Rates above this baseline level resulted from temperature stress. Exposure to water temperatures of 8.5 and 14.7°C produced no significant increase in the epithelial release of the scallops. Exposure to elevated water temperatures (21.0°C) significantly increased the sloughing activity in sea scallops in terms of the incidence of animals releasing cells and the rate of cellular release. Microscopical tissue analyses illustrated

that sloughing induced by short-term exposure to high temperatures resulted in minor to moderate tissue damage to the gills and mantle tissues, including presence of sections devoid of cilia and completely lacking in cellular structures. It was interesting to note that the scallops were found to be sloughing epithelial cells in both New Bruswick and Newfoundland suggesting that cellular exfoliation is not limited to any one particular area.

5.3 Recommendations

The use of juveniles rather than adults, for reasons of faster metabolic activity, might facilitate the identification of epithelial cell release for future studies with this species. In order to confirm, the co-ocurrence of feeding and epithelial sloughing, it would be valuable to calculate separate individual clearance rates for particles between 4-6μm, and between 6-15μm. The former would represent true particle clearance (feeding) whereas the latter would indicate feeding and sloughing. The relative magnitude of these two processes could then be examined for each individual assuming that clearance rate is independent of particle size within the 4-15μm size range. As a result of epithelial sloughing and the co-occurence of feeding and sloughing activities, clearance rate determinations made via the use of the electronic particle counter could be critically jeopardised if the sloughing of cells is not taken into account. This could be minimized by analyzing the particle concentration of the water sample via the particle counter and multisizer outside of the range of the

epithelial cells (i.e., 4-5μm or greater than 15μm). It would be useful to determine the sloughing rates for juveniles in addition to adults, in order to determine whether or not a relationship between body size and rate of exfoliation exists. In this study, animals were sacrificed after exposure to the highest water temperature in order to excise tissue samples for SEM. For this reason, there is no information available on the time required to recover from stress-induced tissue damage resulting from sloughing. It would be worthwhile, should sloughing be used as an index of environmental stress, to study the animals for potential recovery processes. The electronic particle counter/sizer can provide instantaneous quantitative results on sloughing rates. One disadvantage is that this method does not provide any information on the effects of sloughing on tissue ultrastructure. For this reason, it might be useful to employ light microscopical, stereometric, histological and/or scanning electron microscopy techniques together with the electronic particle counter/sizer. Microscopic measurements of the sloughed cells could determine mean sizes and ranges that would allow comparison with those determined by the particle counter. Stereometric techniques could be applied to various tissues in order to quantify the extent of tissue damage as a result of cellular exfoliation. Histological and scanning electron microscopical techniques provide detailed qualitative information of the internal structure and external surface of damaged tissues respectively. The disadvantages to these techniques are that they can be expensive and time consuming. Nonetheless, quantitative procedures together with any combination

of qualitative techniques may provide reliable information on the effects of stress on a filter feeding organism.

LITERATURE CITED

Arimoto, R. and S.Y. Feng. 1983. Histological studies on mussels from dredge spoil dumpsites. Estuar. Coast. Shelf Sci. 17: 535-546.

Baker, J.T.P. 1969. Histological and electron microscopical observations on copper poisoning in the winter flounder (*Pseudopleuronectes americanus*). J. Fish. Res. Bd. Canada 26:2785-2793.

Bayne, B.L. 1985. Responses to environmental stress: tolerance, resistance and adaptation. In: Marine Biology of polar regions and the effects on marine organisms. J.S. Gray & M.E. Christiansen (eds.). Wiley & Sons, London. pp 331-349.

Bayne, B.L. and R.C. Newell. 1983. Physiological energetics of marine molluscs. In: The mollusca, Vol. 4, Physiology, Part 1. A.S.M. Saleuddin and K.M. Wilbur (eds.). Academic Press Inc. New York. pp 407-499.

Beninger, P.G., M. Le Pennec & M. Salaun. 1988. New observations of the gills of *Placopecten magellanicus* (Mollusca: Bivalvia), and implications for nutrition. I. General anatomy and surface micro anatomy. Mar. Biol. 98: 61-70.

Beninger, P.G., M. Auffret & M. Le Pennec. 1990a. Peribuccal organs of *Placopecten magellanicus* and *Chlamys varia* (Mollusca: Bivalvia): structure, ultrastructure and implications for feeding. I. The labial palps. Mar. Biol. 107: 215-223.

Beninger, P.G., M. Le Pennec & M. Auffret. 1990b. Peribuccal organs of *Placopecten magellanicus* and *Chlamys varia* (Mollusca: Bivalvia): structure, ultrastructure and implications for feeding. II. The lips. Mar. Biol. 107(2): 225-233.

Beninger, P.G., J.E.Ward, B.A. MacDonald & R.J. Thompson. 1992. Gill function and particle transport in *Placopecten magellanicus* (Mollusca: Bivalvia) as revealed using video endoscopy. Mar. Biol. 114: 281-288.

Beninger, P.G., S. St-Jean, Y. Poussart & J.E. Ward. 1993. Gill function and mucocyte distribution in *Placopecten magellanicus* and *Mytlius edulis* (Mollusca: Bivalvia): the role of mucus in particle transport. Mar. Ecol. Prog. Ser. 98: 275-282.

Bubel, A. 1984. Mollusca: epidermal cells. In: Biology of the integument. I. Invertebrates. J. Bereiter-Hahn, A.B. Matoltsy & K.S. Richards (eds.). Springer-Verlag. Berlin. pp. 400-447.

Bubel, A. 1973b. An electron microscope investigation of the cells lining the outer surface of the mantle in some marine molluscs. Mar. Biol. 21: 245-234.

Clark, J. (ed.). 1985. The human body, the cell: a small wonder. Torstar Books. New York. 160 p.

Couch, J.A. 1984. Atrophy of diverticular epithelium as an indicator of environmental irritants in the oyster, *Crassostrea virginica*. Mar. Environ. Res. 14: 525-526.

Coulter Multisizer, Accucomp Color Software. 1989. Reference Manual. Corporate

Communications. Hialeah, Florida.

Cunningham, P.A. 1979. Use of bivalve molluscs in heavy metal research. In: Marine pollution: functional responses. W.B. Vernberg et al., (eds.). Academic Press, Inc. pp. 183-221.

Emery, D.G. 1975. Ciliated sensory neurons in the lip of the squid *Lolliguncula brevis*. Cell Tissue Res. 157: 323-329.

Ericsson, J.L.E. 1969. Mechanisms of cellular autophagy. In: Lysosomes in biology and pathology, Vol. 2. J.T. Dingle & H.B. Fell, (eds.). Elsevier/ North Holland, Amsterdam. pp. 345-394.

Glauert, A.M., (ed.). 1980. Fixation, dehydration and embedding of biological specimens, Volume 3, part I. North Holland Publishing Co., Amsterdam. 207 p.

Gosner, K.L. 1978. A field guide to the atlantic seashore. Houghton Mifflin Company, Boston. 329 p.

Hole, J.W. (ed.). 1987. Human anatomy and physiology, (4th Edition). Wm. C. Brown Publishers. Dubuque, Iowa. 966 p.

Holtzman, E. 1975. The biogenesis of organelles. In: Cell membranes, biochemistry, cell biology & pathology. G. Weissmann & R. Caiborne, (eds.). HP Publishing Co., Inc. New York. pp. 153-166.

Junqueira, L.C., J. Carneiro & R. O. Kelley. 1992. Basic histology, (7th Edition). Appleton & Lange. Norwalk, Conneticut. 518 p.

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

MacDonald, B.A. 1984. The partitioning of energy between growth and reproduction in the giant scallop: *Placopecten magellanicus* (Gmelin). Ph.D. thesis. Memorial University of Newfoundland, St.John's, Newfoundland. 202 p.

MacDonald, B.A., J.E. Ward & C. MacKenzie. 1995. Exfoliation of epithelial cells from the pallial organs of the sea scallop, *Placopecten magellanicus*. J. Exp. Mar. Biol. & Ecol. (submitted).

Marchant, H.J. and D.P. Thomas. 1983. Polylysine as an adhesive for the attachment of nanoplankton to substrates for electron microscopy. J. Microsc. (Oxford) 131: 127- 129.

Marigomez, J.A., V. Saez, M.P. Cajaraville & E. Angulo. 1990. A planimetric study of the mean epithelial thickness (MET) of the molluscan digestive gland over the tidal cycle and under environmental stress conditions. Helgolander Meeresunters 44: 81-94.

Mohlenberg, F. and H.U. Riisgard. 1978. Efficiency of particle retention in 13 species of suspension feeding bivalves. Ophelia 17: 239- 246.

Moore, M.N., D.M. Lowe & S.L. Moore. 1979. Induction of lysosomal destabilisation in marine bivalve molluscs exposed to air. Mar. Biol. Lett. 1: 47-57.

Newell, P.F. 1977. The structure and enzyme histochemistry of slug skin. Malacologia 16: 183-195.

Reed, W., J.A. Avolio & P. Satir. 1981. Structural and functional integration of cilia & microvilli in ciliated epithelial cells. J. Cell Bio. 91(2): 295a.

Rehder, H.A. 1981. The Audubon Society field guide to north american seashells. Alfred A. Knopf, Inc., New York. 894 p.

Riisgard, H.U. 1988. Efficiency of particle retention and filtration rate in 6 species of Northeast American bivalves. Mar. Ecol. Prog. Ser. 45: 217-223.

Ruddell, C.L. 1971. Elucidation of the nature and function of the granular oyster amoebocyte through histochemical studies of normal and traumatised oyster tissues. Histochemie 26: 98-112.

SAS Institute Inc. 1990. SAS/STAT User's Guide. Volume 2, GLM-VARCOMP. Cary, North Carolina.

Simkiss, K. and A.Z. Mason. 1984. Cellular responses of molluscan tissues to environmental metals. Mar. Environ. Res. 14: 103-118.

Sminia T., K. Preterson & J.E.M. Scheerboom. 1973. Histological and ultrastructural observations on wound healing in the fresh water pulmonate *Lymnaea stagnalis*. Z. Zellforsch Mikrosk Anat. 141: 561-573.

Sokal, R. and F.J.Rohlf. 1981. Biometry, (2nd Edition). W.H. Freeman and Co., San Francisco. 859 p.

Spencer, F. (ed.). 1972. Aspects of human biology: Theory relevant to medical

laboratory sciences. Butterworths & Co., Ltd. Toronto, Ontario.

Sunila, I. 1986. Chronic histopathological effects of short-term copper and cadmium exposure on the gill of the mussel, *Mytilus edulis*. J. Inverte. Path. 47: 125-142.

Sunila, I. 1981. Toxicity of copper and cadmium to *Mytilus edulis* L. (Bivalvia) in brackish water. Ann. Zool. Fennici. 18: 213-223.

Tamarin A., P.Lewis & J Askey. 1976. The structure and formation of the byssus attachment plaque in *Mytilus*. J. Morphol. 149: 199-222.

Tsujii, T. 1976. An electron microscopic study of the mantle epithelial cells of *Anodonta* sp. during cell regeneration. In: Belle W. Baruch library in marine science, No. 5. The mechanisms of mineralization in the invertebrates and plants. Watabe, Norimitsu & K.M. Wilbur, (eds.). Symposium. Georgetown, South Carolina, Oct., 1974. University of South Carolina Press: Columbia, S.C. pp. 339- 353.

Vahl, O. 1972. Efficiency of particle retention in *Mytilus edulis* L. Ophelia 10: 67-74.

Weinstein, R.S. & B.U. Pauli. 1981. Cell relationships in epithelia. In: Advances in clinical cytology. L.G. Koss & D.V. Coleman, (eds.). Butterworths. Toronto, Ontario. pp. 160-200.

Zar, J.H. 1984. Biostatistical analysis, (2nd Edition). Prentice-Hall, Inc., Englewood Cliffs, New Jersey. 718 p.

Zylstra, U. 1972a. Histochemistry and ultrastructure of the epidermis of the sub-epidermal cells of the fresh water snails *Lymnaea stagnalis* and *Biomphalaria pfeifferi*. Z. Zellforsch Mikrosk Anat. 130: 93-131.

PERSONAL COMMUNICATIONS

Name	Affiliation
Dr. Catherine Powell	Microscopy Unit, Saint John Regional Hospital, Saint John, NB.
Dr. Monica Bricelj	Marine Science Center, State University of New York at Stony Brook, New York.
Dr. Ray Thompson	Ocean Sciences Center, Memorial University of Newfoundland, St. John's, NF.

APPENDIX

FORMULAE USED TO CALCULATE SLOUGHING RATES

Individual sloughing rates (SR) (cells min^{-1}) for both the experimental & control scallops were determined at each sampling period within the separate temperature regimes by calculating the approximate number of cells sloughed per minute from each scallop:

EC sloughed · minute $^{-1}$ = [FP] - [RP] (cells) / 2 (min) → **(X cells min^{-1})**

X * 1 min / 2.25 (ml) → **(Y cells ml^{-1})**

Y * FR (ml min^{-1}) → **(Z cells min^{-1})**

where
- FP = particle concentration (numbers of cells) of feeding chamber
- RP = particle concentration (numbers of cells) of reference chamber, (i.e. the chamber without a scallop)
- FR = flow rate (ml min^{-1}) of sea water into the chambers
- **Z = final sloughing rate**