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ONTOGENY OF SUSPENSION FEEDING IN POST-METAMORPHIC JAPANESE SCALLOPS, *PATINOPECTEN YESSOENSIS* (JAY)

BY

BRIAN CHARLES KINGZETT

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Examining Committee:

Chair: Dr. L. Dill, Professor

Dr. B. Hartwick, Associate Professor, Senior Supervisor,
Department of Biological Sciences, SFU

Dr. L. Druehl, Professor,
Department of Biological Sciences, SFU

Dr. N. Bourne, Research Scientist,
Department of Fisheries and Ocean, Nanaimo, B.C.

Dr. P.J. Harrison, Professor,
Department of Botany, UBC
Public Examiner

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Ontogeny of suspension feeding in post-metamorphic Japanese scallops Patinopecten yessoensis (Jay)

Author: _____
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Brian Kingzett
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ABSTRACT

The development of feeding structures, feeding mechanisms and associated changes in the filter feeding ability were investigated for early juvenile Japanese scallops *Patinopecten yessoensis* (Jay) from the time of metamorphosis until the juveniles measured two mm shell height.

The ability of developing juveniles to filter eight species of cultured phytoplankton from suspension was measured with the use of a flow-through measuring apparatus. Clearance rates were extremely low from the time of metamorphosis through until juveniles measured 400 µm shell height. When juveniles were approximately 600 µm shell height there was a sudden increase in clearance ability at all cell densities. Clearance rates increased slightly until juveniles were 1000 µm shell height and logarithmically between 1000 and 2000 µm shell heights. Particles as small a 2 µm were ingested by post-metamorphic scallops but particles greater than 25 µm were not ingested.

The ontogeny of feeding structures (ctenidia, peribuccal organs and the foot) were described. Juvenile ctenidia are filibranchiate, homorhabdic and non-plicate, arising from primordia present in the larvae. Initial ctenidial growth is in the number and length of filaments which possess lateral and frontal tracts of cilia. Maximum filament length prior to reflection occurs when juveniles are approximately 600 µm shell height. The labial palps arise as extensions of the larval mouth apparatus and extend into the mantle cavity. The highly mobile foot reaches its greatest allometric size during post-metamorphic growth.

Particle capture was examined with video microscopy in live tethered specimens at the stage when juveniles were capable of particle capture. Juvenile scallops were observed to utilize a unique hydrodynamic mode of suspension feeding which serves to bridge pedal feeding immediately after metamorphosis with the development of more efficient adult feeding structures.

Morphological descriptions, particle clearance data and behavioural observations suggest that until formation of a simple functional gill apparatus at approximately 600 µm shell height, *P. yessoensis* juveniles are unable to capture phytoplankton efficiently. Observed increases in particle clearance ability were associated with initial coordination and function of the 8 - 9 ctendial filaments present. This is a critical developmental stage in post-metamorphic growth and a period of high mortality in the nursery stage of culture rearing.

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LIST OF ABBREVIATIONS

a:	adductor muscle
ac:	algal cell
al:	ascending lamellae
b:	ethylcellulose bead
bg:	byssal groove
bn:	byssal notch
c:	cilia
ca:	capitula
cj:	ciliary junction
cs:	coverslip
ct:	ciliated tract
dl:	descending lamellae
dg:	digestive gland
ds:	dissoconch shell
e:	eye
es:	eosophagus
f:	foot
ff:	fused filament
ga:	gill axis
gf:	gill filaments
gp:	gill primordia
ic:	infrabranchial chamber
id:	inner demibranch
if:	interfilamentary space
in:	inlet
ip:	inner palp surface
j:	juvenile scallop
lc:	lateral cilia
lv:	left valve
m:	mouth
ma:	mantle
mc:	mantle curtain
ms:	microscope stage
od:	outer demibranch
op:	outer palp surface
ol:	objective lens
ou:	outlet
p:	labial palps
pp:	pressure plate
pr:	propodium
ps:	prodissoconch shell
rv:	right valve
s:	stomach
sc:	suprabranchial cavity
sl:	objective lens sleeve
sw:	seawater
t:	tentacle
v:	velum

CHAPTER 1. GENERAL INTRODUCTION AND RESEARCH OBJECTIVES

Little is known about the ecology of early juvenile scallops in the natural environment (Motavkin, 1990; Minchin, 1992). Post-metamorphic growth of bivalves relies initially on lipid energy reserves sequestered during larval growth (Holland and Spencer, 1973). Subsequent growth and survival is dependant upon the efficient acquisition of energy before energy reserves are depleted. Suspension feeding is believed to commence early in post-metamorphic oysters, whereas other groups of bivalves are known to utilize transitory phases of pedal feeding (King, 1986; Reid *et al.*, 1992). The uptake of dissolved organic matter (DOM) in seawater may also assist in energy acquisition during this phase (Manahan and Crisp, 1983). Development of ctenidial feeding structures and initiation of efficient suspension feeding are necessary for continued juvenile growth and survival.

The gills of adult scallops differ from many other bivalves in that the latero-frontal cirri, the structures which are believed to be most used to filter particulates (phytoplankton) out of seawater, are highly reduced (Atkins, 1937a, b, 1938; Owen and McCrae, 1976; Beninger, 1991; Beninger and Le Pennec, 1991). As a result, it is believed that the scallop gill is not as efficient at capturing smaller particles as gills of other lamellibranchs such as oysters and mussels (Owen and McCrae, 1976; Møhlenberg and Riisgård, 1978; Palmer and Williams, 1980). It is not known if the reduced filtration ability of adult scallops is magnified in juveniles in which the functional gill apparatus is in an early stage of development.

Typically, only a small percentage (< 2 - 6%) of Japanese scallop larvae which were induced to settle, survive to a size of a few a millimetres (Ó Foighil *et al.*, 1990; Bourne and Hodgson, 1991). High mortalities during post-metamorphic growth have been reported for most species of commercially cultivated bivalves including clams, oysters and geoducks (Castagna and Krauter, 1981; Nosho and Chew, 1991). Preliminary investigations of growth and survival through the post-metamorhic phase showed that use of small species of cultured phytoplankton (< 10 µm diameter) as food resulted in inferior growth rates in juvenile scallops when compared those fed assemblages of natural phytoplankton and indicated that mortality may be due to an inadequate diet (Ó Foighil *et al.*, 1990). Presumably, the natural assemblages contained a number of phytoplankton species which were quantitatively or qualitatively superior to cultured phytoplankton. Many previous studies of feeding of juvenile bivalves have examined the nutritional qualities of cultured phytoplankton diets (Rodde *et al.*, 1976; Cary, 1982; Ukeles *et al.*, 1984; Wikfors *et al.*, 1984; Enright *et al.*, 1986a, b; Laing and Millican, 1986; Utting, 1986; Walsh *et al.*, 1987; Whyte, 1987; Laing and Verdugo, 1991). Algal species deemed nutritive will only be of use if they are readily captured, ingested and assimilated by juvenile scallops. Insufficiencies in the diet may be related to development of the juvenile gill (ctenidia) formed during metamorphosis and its ability to filter properly.

The objectives of this research were to investigate the development of feeding structures and associated changes in the filter feeding ability of early juvenile Japanese

scallops *Patinopecten yessoensis* (Jay) from the time of metamorphosis until the juveniles measured two mm shell height. This research was conducted in three parts;

1) Measurement of the ability of post-metamorphic scallops to remove various species of phytoplankton from suspension and to ingest a variety of particle sizes.

2) A description of the ontogeny of the feeding structures with emphasis on development of the ctenidia.

3) Observations of the transport and capture of suspended particles by the filter-feeding structures within the mantle cavity.

Information from this research may assist in understanding and overcoming mortalities during post-metamorphic (nursery) rearing. Increasing survival during the early juvenile stage has important commercial implications. The Japanese scallop is a potential candidate for commercial aquaculture in British Columbia and, as with other cultivated bivalve species, successful commercial production is dependant on an economical supply of seedstock (juveniles). Small increases in the percent survival through the early nursery stage could increase the economic viability of commercial production of juvenile scallops in British Columbia.

CHAPTER 2. DEVELOPMENT OF FILTRATION ABILITY IN POST-METAMORPHIC *PATINOPECTEN YESSOENSIS*

INTRODUCTION

The rate and ability of adult scallops to selectively filter suspended materials from seawater has been investigated for a number of species (Vahl, 1972; Møhlenberg and Riisgård, 1978, 1979; Palmer and Williams, 1980; Peirson, 1983; Shumway *et al.*, 1987; Grant and Cranford, 1989; Cranford and Grant, 1990). Energy acquisition in scallops has been the subject of a recent review (Bricelj and Shumway, 1991). Several investigations examined the filtration capabilities of juvenile scallops; however, in most cases the juvenile scallops were actually several centimetres shell height (Kean-Howie *et al.*, 1991; Lesser *et al.*, 1991). Shumway *et al.* (in prep, pers. comm.) investigated particle selection and clearance rate in three species of juvenile scallops approximately two mm shell height.

Information on suspension feeding ability is lacking for early juvenile scallops from metamorphosis until they reach two mm shell height. This is a period of morphogenesis of the ctenidia and a critical stage in development. (Hodgson and Burke, 1988; Ó Foighil *et al.*, 1990; Bourne and Hodgson, 1991). Reid *et al.* (1992) stated that effective suspension feeding commences in post-metamorphic *P. yessoensis* one week after metamorphosis when juveniles exceeded 400 µm shell height, although quantitative evidence was not provided. Pedal feeding is known to occur in post-metamorphic bivalves when the foot reaches its greatest allometric size in the juvenile

(Lesser *et al.*, 1991; Reid *et al.*, 1992). Preliminary investigations into the nutritional significance of pedal feeding during this phase in *P. yessoensis* have been inconclusive (Ó Foighil *et al.*, 1990). Quantitative analyses of the development of filtration ability for other post-metamorphic bivalve species are lacking.

The objective of this study was to examine the ability of juvenile *P. yessoensis* to filter phytoplankton from the time of post-metamorphosis through to a size of two mm shell height. This was performed by measuring filtration rates on a number of species of phytoplankton currently or readily available for the culture of bivalves. Particles removed from suspension by filter feeding bivalves are not necessarily ingested and may be rejected in the form of pseudofeces (Tenore and Dunstan, 1973). A second series of experiments attempted to determine the size specific ability of post-metamorphic juveniles to ingest particles over a size range of 2-50 µm.

Although many different experimental techniques have been used to investigate rates of filter feeding in invertebrates, these usually take one of two forms, *indirect* or *direct* methods of measurement. Filtration rate is defined as the volume of water filtered completely free of particles per unit of time; pumping rate is defined as the volume of water flowing through the gills per unit time (Winter, 1978).

The *indirect* method measures the removal of suspended particles, chlorophyll a, the uptake of P^{32} labelled phytoplankton into tissue or other indicators from a known volume of standing water per unit time (Walne, 1963; Schulte, 1975; Riisgård *et al.*, 1980; Gerdes, 1983; Macdonald, 1988; Ward *et al.*, 1992). The disadvantage of this technique is the continuously changing food concentration in the experimental

medium, which makes it impossible to detect significant correlations between filtration rate and food concentration (Winter, 1978). The theoretical basis of the equation for the calculation of filtration rates using the *indirect* method is based on the assumptions that (a) the animal's pumping rate is constant and that (b) a constant percentage of particles (which may differ from 100% retention efficiency) is retained throughout the experiment (Coughlan, 1969). Winter (1978) stated "that since these equations are not necessarily true, the equation itself is one of the main disadvantages of the indirect method". A further objection to these studies in standing water is that previously filtered material may be resuspended and refiltered (recycled) (Haven and Morales-Alamo, 1970).

The *direct* method of measuring filtration rate involves measuring particle concentration before and after entering a flow-through chamber (Haven and Morales-Alamo, 1970; Bayne *et al.*, 1973; Riisgård, 1977; Wilson, 1979, 1980). The advantages of this system are that constant particle concentrations may be maintained at the inflow and the effect of particle concentration on filtration rate may be tested. Despite this advantage, recirculation of the water within the experimental chambers may result in concentration differences within the experimental chambers. At low flow rates this may result in an underestimation of filtration rate; this error is reduced as flow rates are increased (below a natural limit) (Hildreth and Crisp, 1976; Riisgård, 1977). When the assumption of filtration rate was applied to calculations from flow-through chambers, filtration was found to be independent of flow rate and the quantity actually measured was the rate of particle uptake (Hildreth and Crisp, 1976). In this

study the ability of juvenile scallops to filter phytoplankton cells from suspension was measured as the number of particles removed from suspension per individual per unit time (particle clearance rate).

MATERIALS AND METHODS

Juvenile culture

Larvae and juvenile *Patinopecten yessoensis* were obtained from the Federal Department of Fisheries and Oceans Pacific Biological Station, (Nanaimo, British Columbia) between March and June 1989, and from Island Scallops Ltd. (Qualicum, B.C.) from March 1990, as required. Juveniles at Island Scallops Ltd. were fed and raised using techniques after the method of Bourne *et al.* (1989). At Island Scallops Ltd., scallop larvae and spat are raised in flow-through $5 \cdot 10^4$ L tanks. Japanese style spat bags consisting of 3 mm mesh onion bags stuffed with monofilament mesh were used as juvenile substrate (cultch) material (Ventilla, 1982). Cultured phytoplankton was added to the nursery tanks. Juveniles were transported to the Pacific Biological Station wrapped in moist Nitex® or attached to spat bags in a cooler or in chilled seawater.

At the Pacific Biological Station, scallops were maintained in 350 L flow-through fibreglass tanks. Scallops were either suspended in spat bags in tanks or scallops not attached to cultch were placed on 120 µm Nitex screen in 50 cm diameter upwellers suspended in tanks. Juveniles were fed phytoplankton via a drip system

from a header tank as used for bivalve broodstock conditioning (Bourne *et al.*, 1989). Phytoplankton was pumped at regular intervals from the algal culture facility to the header tank. All species of phytoplankton used in filtration experiments were fed to juveniles in the holding system.

Design of experimental flow-through chambers

To measure feeding rates of post-metamorphic scallops, a series of flow-through chambers were constructed based on a successful design used to study filtration rates of *Ostrea edulis* spat (Wilson 1979, 1980). This design was an inprovement of apparatus used by Winter (1973) who used a flow-through system to perform direct measurements on filtration rate, and that of Hildreth and Crisp (1976), who further modified the apparatus to accommodate bivalve larvae and juveniles.

The experimental chambers consisted of Plexiglass columns, (5 ml volume) held between 180 µm Nitex screens and sealed with rubber O-rings (Fig. 1). Seawater was pumped through the chambers from the base and samples of approximately 20 ml were collected in Coulter Counter® cuvettes for particle analysis. Eight columns were used in two groups each consisting of three replicate columns containing juveniles and one chamber (blank) without juveniles acted as a control.

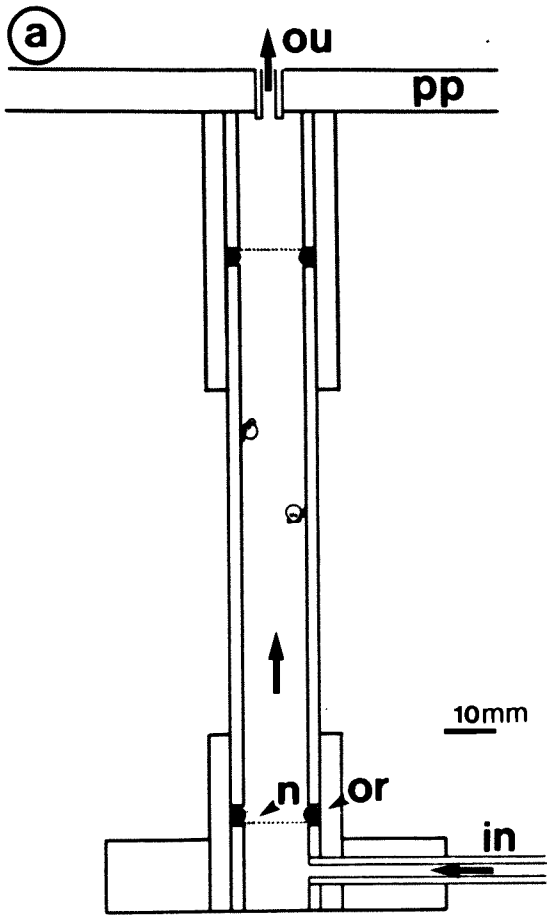
One µm cartridge filtered seawater (FSW) containing phytoplankton at desired cell concentrations was maintained at 15°C in a constant temperature water bath, and kept in uniform suspension with submersible magnetic stirrers.

Figure 1. Design of chambers used to srudy filtration rate of juvenile *P. yessoensis*.

a) Schematic cross-section of chamber construction. Arrows indicate path of water flow through chamber. in, inlet; n, Nitex screen; or, rubber o-ring; ou, outlet; pp, pressure plate.

b) Side view of bank of chambers showing sample cuvettes (left).

c) Front view of bank of chambers.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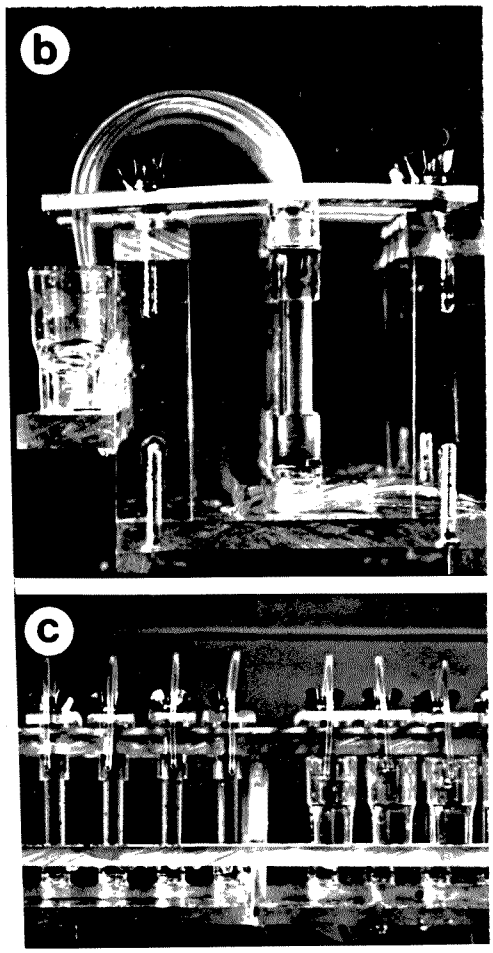
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A variable speed, multichannel peristaltic pump (Cole-Parmer, Chicago, Ill. model #L-07553-30) fitted with a multichannel pump head (model #L-07623-10) was used to produce nearly identical flow rates through the chambers, between 1 to 4 $ml \cdot min^{-1}$ as required. To account for minor differences in flow rate that might exist between chambers, flow rates were calibrated by measuring the time to collect 20 mL of seawater from each chamber for each experiment.

Phytoplankton species and culture

Phytoplankton was cultured in the Pacific Biological Station algal culture facility (Bourne *et al.* 1989). All species used were cultured in batch or semi-continuous culture systems using HESAW culture medium (Harrison *et al.*, 1980).

Phytoplankton used in experiments was harvested during the exponential growth phase from either 20 L carboys or 400 L semi-continuous bag culture. To reduce the number of bacteria which are regularly associated with phytoplankton cultures, algae harvested from culture vessels was centrifuged at 3000 rpm for 10 min in 250 mL vials to concentrate the cells. The major portion of the supernatant was then drawn off and the cells resuspended in FSW. Visual inspection with a compound microscope indicated that this treatment did not affect the structure, movement or size of the cells by breaking spines or causing cells to clump, while substantially reducing the number of bacteria associated with the cultures.

Eight species of phytoplankton were used for analysis (Table 1). These were all unicellular species cultured at the Pacific Biological Station and ranging in size

between 2-11 μm cell diameter. These species were chosen for their availability and their prior use in scallop feeding work (Whyte, 1987; Whyte *et al.*, 1990). Phytoplankton concentrations were determined by a Coulter Counter Multisizer II® (Coulter Corp. Hialeah, FL.) using an orifice diameter of 50 μm. Only those particles which corresponded to the observed size range of the individual phytoplankton species were counted to increase the accuracy of the analysis (Table 1).

Table 1. Species of phytoplankton used in filtration experiments.

Species	Abbrv	Clone*	Size Range (µm)**	Mean Size (µm)**	Taxonomic Class
Nannochloropsis oculata (Droop)	NAN	***	1.8-3.7	2.4	Eustigmatophyceae
Chaetoceros calcitrans (Paulsen)	CC	CCMP1315	2.5-4.8	3.4	Bacillariophyceae
Thalassiosira pseudonana (Hustedt)	3H	CCMP1015	3.2-6.0	4.0	Bacillariophyceae
Chaetoceros gracilis (neogracile) (Schütte)	CG	NRC108	3.5-6.0	4.5	Bacillariophyceae
Isochrysis sp. (galbana) (Parke) Tahitian strain	T-ISO	CCMP13^4	3.8-6.3	4.7	Prymnesiophyceae
Chroomonas salina	3C	CCMP1319	5.7-8.4	6.9	Cryptophyceae
Phaeodactylum tricornutum (Bohlin)	PH	CCMP630	3.7-7.0	4.8	Bacillariophyceae
Rhodomonas lens (Pascher et Ruttner)	RH	NEPCC CR588	7.2-11.3	8.9	Cryptophyceae

* CCMP: Provasoli-Guillard Center for Culture of Marine Phytoplankton West Boothbay Harbor, Maine USA.
NRC: National Research Council of Canada, Halifax
NEPCC: Northeast Pacific Culture Collection, Dept. of Oceanography University of British Columbia, Vancouver, B.C.
** Coulter calculated cell diameters
*** Yoshokuken strain, obtained from Japan 1990 (Whyte, pers. comm.)

Experimental procedures

Between 50 and 12 000 juvenile scallops were pipetted into each chamber as required to produce a measurable reduction in phytoplankton concentration. In the chambers, scallops were allowed to distribute themselves along the sides of the chamber for a minimum of 1 h. Prior to trials with various concentrations of phytoplankton, sand-filtered seawater at 15°C from the Pacific Biological Station's seawater system was pumped through the columns to maintain the juveniles.

At the beginning of each experiment, two-litre reservoirs of the desired species of phytoplankton diluted to the required concentration in 15°C, FSW were placed in the water bath and stirred to maintain the cells in even suspension. These solutions were then pumped through the two banks of experimental chambers at flows ranging from 1 to 4 $mL \cdot min^{-1}$ as appropriate. After 50 min, samples (approximately 20 mL) were collected simultaneously from the outflows of each chamber.

Samples collected from each chamber were analyzed within 5 to 15 min of collection. Three counts were made of each sample to account for errors in counting and the mean value was used in subsequent analyses. Counts of particles in size ranges below those of the phytoplankton peak were also recorded. Print-outs were made of the particle size-frequency histogram for every third count and retained.

Once samples were collected, the two litre reservoirs were replaced with phytoplankton solutions at the next desired concentration, and the procedure was repeated. Phytoplankton concentrations of approximately 10,000, 20,000, 30,000, 40,000 and 50,000 $cells \cdot mL^{-1}$. were used.

Potential sources of experimental error arising from the order in which the algal concentrations were used or the time duration between collecting samples were investigated by randomly repeating samples and repeating or altering the order of phytoplankton concentrations. These factors were not found to influence estimation of clearance rates.

At the end of each experiment, all juveniles were removed from the chambers and preserved for later morphological studies. Prior to fixation all animals were recounted in order that filtration rates could be adjusted for minor differences in number between chambers. Mortality over the period of the experiment was noted and if the number of dead or moribund animals exceeded 10-20%, data from the experiment were discarded.

Calculation of Clearance Rate

Rates of phytoplankton clearance by post-metamorphic scallops in the flow-through chambers were determined using the equation of Hildreth and Crisp (1976) which was subsequently used by Wilson (1980) for calculating the grazing rates of *Ostrea edulis* larvae and spat. The clearance rate (grazing) (**G**) or number of particles consumed by a bivalve per unit time, was calculated using the following formula:

G = Rf·Co = F(C1-C2), where:

Rf = Clearance Rate (filtration cells·individual^{-1})

C1 = Concentration of inflow (cells·mL^{-1})

C2 = Concentration at the outflow (cells·mL^{-1})

F = Flow Rate (mL·min^{-1})

Co = Concentration around the organism (cells·mL^{-1})

At low flow rates, **Co** is less than **C1** owing to particle removal by the bivalve, but as flow rate is increased, the value of **Co** approaches that of **C1**, and **Rf** becomes independent of **F**. Observed clearance and grazing rates above this critical level are designated **Rf"** and **G"**, respectively. Flow rates were maintained at or in excess of this critical point during the investigation, so that only **Rf"** and **G"** were measured.

Ingestion experiments

Two sizes of juvenile scallops (approximately 300 µm and 1000 µm shell height) were examined for their ability to ingest particles of certain diameters. Opacity of the dissoconch shell prevented use of 2000 µm juveniles. Juveniles were exposed to suspensions of fluorescently labelled polystyrene spheres (Fluoresbrite® beads, Analychem Corp. Ltd. 721 Victoria Park Ave. Unit 16, Markham, Ontario L3R 2Z8). Five sizes of beads with mean diameters of: 2.2 µm, 6.49 µm, 9.33 µm, 23.4 µm and 54.9 µm were used. Stock solutions of beads were diluted into chambers containing 15 mL of FSW at 15°C and containing more 50 juveniles. Capillary tubing

was used to bubble air into each chamber to provide mixing. Working concentrations of fluoresbrite beads were approximately 30 000·mL^{-1} for the 2, 6, and 9 µm diameter beads. Concentration of the larger beads was reduced to 12,500·mL^{-1} for 25 µm beads and 1500·mL^{-1} for the 50 µm beads due to the large size of the beads.

After 5 min of exposure to the bead solutions, 100% ethanol was added to each chamber to kill the juveniles. Experimental duration times were extended for the larger bead sizes. The juveniles were then rinsed several times and immersed in a fixative. The number of beads ingested was assessed by squashing the juveniles on a slide and viewing them under a microscope with a fluorescent light source. The number of beads within the stomach region (ingested) was then counted.

RESULTS

Clearance rate experiments

The number of individual juvenile scallops required to produce a measurable reduction in particle concentration at the outflow of the chambers for each size class and the flow rates used for each size class are summarized in Table 2. Calculated clearance rates (cells·min^{-1}·individual^{-1}) for juvenile *P. yessoensis* at each size class versus phytoplankton concentration at the inflow of each chamber for the eight species of algae are shown in Figures 2 - 6.

Table 2. Summary of juvenile scallop sizes (shell height) used, number per chamber and flow rates.

Approximate Size Class µm	Shell Height Mean ± 95%CI	Number of Juveniles	Flow rates $mL \cdot min^{-1}$
300	336 ± 21	951 - 1182	2.13 - 2.54
400	398 ± 28	230 - 447	1.42 - 1.56
600	637 ± 25	379 - 700	2.46 - 3.44
1000	946 ± 62	116 - 250	2.00 - 2.50
2000	1984 ± 79	43 - 67	2.48 - 2.56

At a shell height of 300 µm (Fig. 2), observed clearance rates for all algal species were extremely low at all concentrations with maximum rates between 10 - 20 $cells \cdot min^{-1} \cdot individual^{-1}$. A slight increase in clearance rate with increasing cell density was observed for *C. calcitrans, T. pseudonana* and *P. tricornutum.*

At 400 µm shell height (Fig. 3), clearance rates remained below 20 $cells \cdot min^{-1} \cdot individual^{-1}$ on all species of phytoplankton. Slight increases in clearance rate on *C. calcitrans*, *C. gracilis* and *P. tricornutum* were observed with increasing cell density to 40,000 $cells \cdot mL^{-1}$. Particle clearance was not detected for *C. salina* and *R. lens* at low concentrations or for *N. oculata.*

At 600 µm shell height (Fig. 4), clearance rates increased for all species of phytoplankton except *N. oculata.* Clearance rates for the other species of phytoplankton are similar and a general clearance trend of approximately 10 - 20

cells·min^{-1} at 10,000 cells·mL^{-1}., increasing with concentration to levels between 40 and 80 cells·min^{-1} was observed. Variability within the detected clearance rates increased substantially at this size class.

At 1000 µm shell height (Fig. 5), overall feeding rates remained similar to the previous size class. Variation within the observations remained high. *C. gracilis* and *C. calcitrans* were cleared at the highest rates. Maximum clearance rates were generally observed to occur at densities between 30 - 40,000 cells·mL^{-1}. for all species except *C. calcitrans* where clearance rates continued to increase with cell density and *N. oculata* which was not cleared at any concentration.

At 2000 µm shell height, clearance rates continued to increase for all species of phytoplankton (Fig. 6, note change in scale). Lowest rates were recorded for *N. oculata*. Highest clearance rates were noted for *C. calcitrans*, and they continued to increase with algal density to a mean of approximately 400 cells·min^{-1}·individual^{-1}. All other species showed a general trend of increasing clearance rates with increasing cell density reaching a maximum between 30 - 40,000 cells·mL^{-1}.

Changes in the ability to clear phytoplankton with juvenile growth was summarized by pooling all observations at 30,000 cells·mL^{-1} for all species of phytoplankton and plotting them against shell height (Fig. 7). Clearance rates were extremely low in scallops from metamorphosis until the juveniles measured 400 µm shell height. Between a shell height of 400 and 600 µm there was a sudden rapid increase in clearance ability at all cell densities.

Figure 2. Particle clearance rates for juvenile *P. yessoensis* at approximate 300 μm shell height versus phytoplankton concentration for eight phytoplankton species; a) *Nannochloropsis oculata* (NAN), b) *Chaetoceros calcitrans* (CC), c) *Thalassiosira pseudonana* (3H), d) *Chaetoceros gracilis* (CG), e) Tahitian *Isochrysis sp. (galbana)* (TX), f) *Phaeodactylum tricornutum* (PH), g) *Chroomonas salina* (3C), h) *Rhodomonas lens* (RH). Error bars equal +/- 1 standard deviation, n = 3.

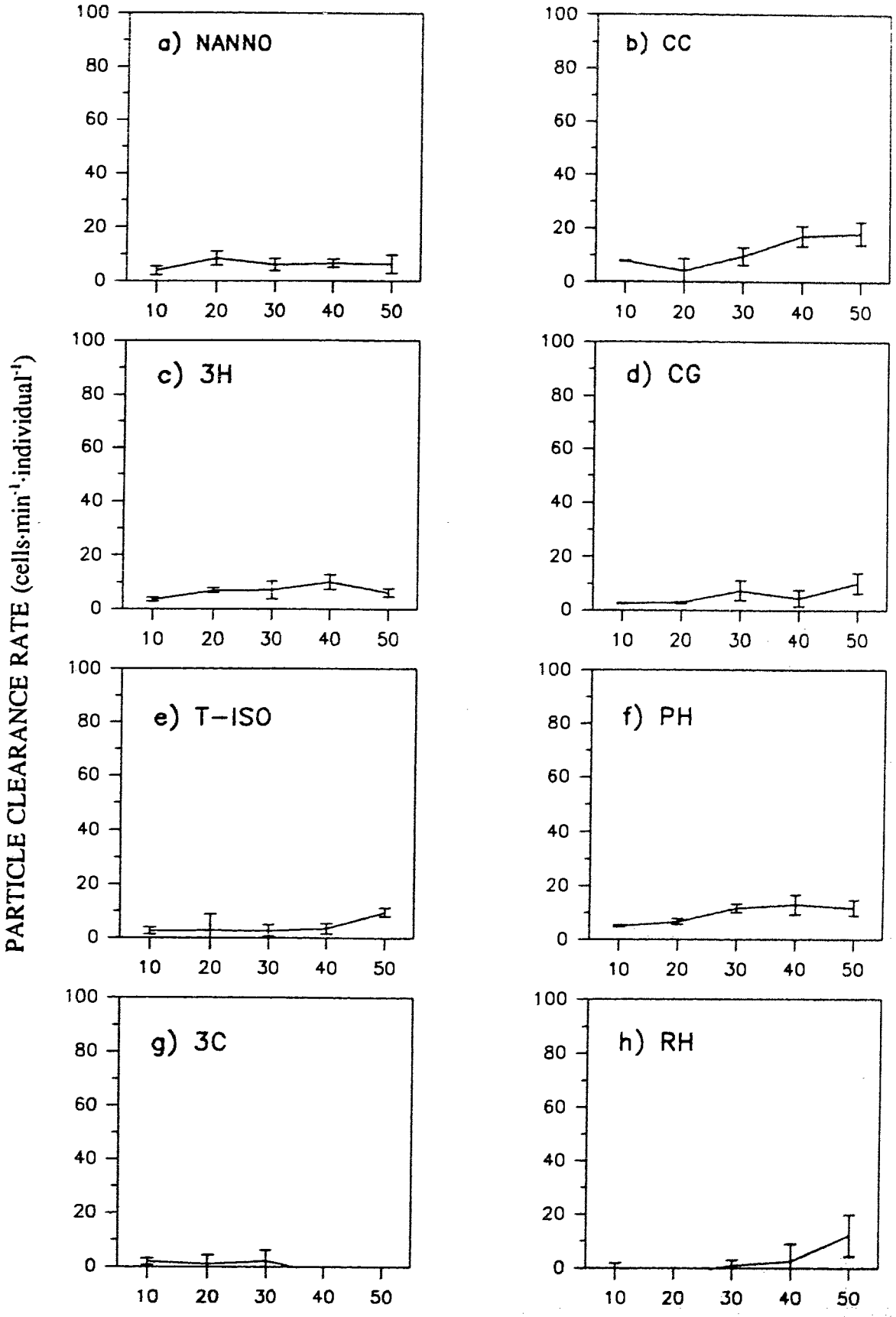

a) NANNO
b) CC
c) 3H
d) CG
e) T-ISO
f) PH
g) 3C
h) RH
PARTICLE CLEARANCE RATE (cells·min⁻¹·individual⁻¹)
PHYTOPLANKTON CONCENTRATION (1000 cells·mL⁻¹)
0
20
40
60
80
100
10
20
30
40
50

Figure 3. Particle clearance rates for juvenile *P. yessoensis* at approximate 400 µm shell height versus phytoplankton concentration for eight phytoplankton species; a) *Nannochloropsis oculata* (NAN), b) *Chaetoceros calcitrans* (CC), c) *Thalassiosira pseudonana* (3H), d) *Chaetoceros gracilis* (CG), e) Tahitian *Isochrysis sp. (galbana)* (TX), f) *Phaeodactylum tricornutum* (PH), g) *Chroomonas salina* (3C), h) *Rhodomonas lens* (RH). Error bars equal +/- 1 standard deviation, n = 3.

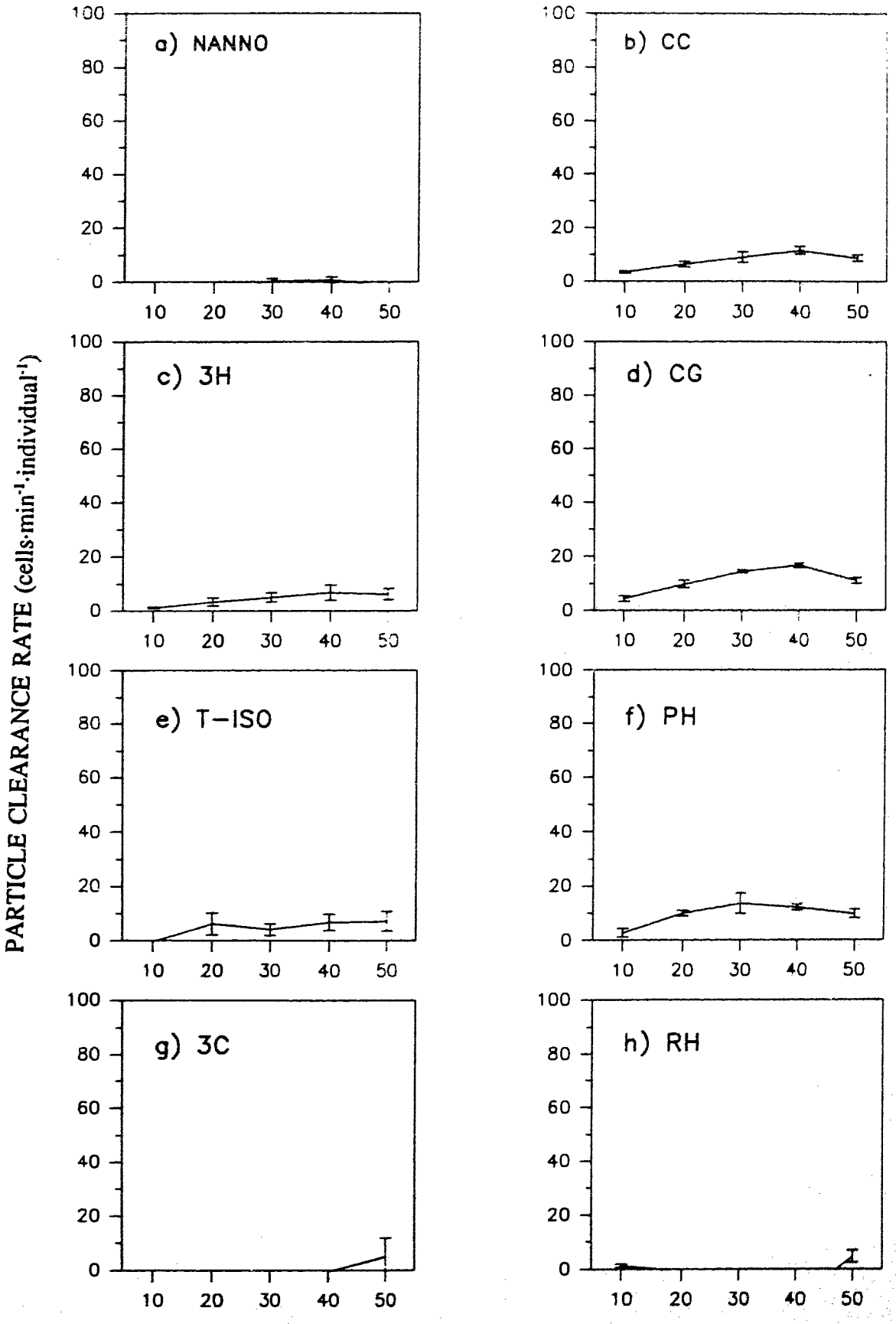

a) NANNO
b) CC
c) 3H
d) CG
e) T-ISO
f) PH
g) 3C
h) RH
PARTICLE CLEARANCE RATE (cells·min⁻¹·individual⁻¹)
PHYTOPLANKTON CONCENTRATION (1000 cells·mL⁻¹)
0
20
40
60
80
100
10
20
30
40
50

Figure 4: Particle clearance rates for juvenile *P. yessoensis* at approximate 600 µm shell height versus phytoplankton concentration for eight phytoplankton species; a) *Nannochloropsis oculata* (NAN), b) *Chaetoceros calcitrans* (CC), c) *Thalassiosira pseudonana* (3H), d) *Chaetoceros gracilis* (CG), e) Tahitian *Isochrysis sp. (galbana)* (TX), f) *Phaeodactylum tricornutum* (PH), g) *Chroomonas salina* (3C), h) *Rhodomonas lens* (RH). Error bars equal +/- 1 standard deviation, n = 3.

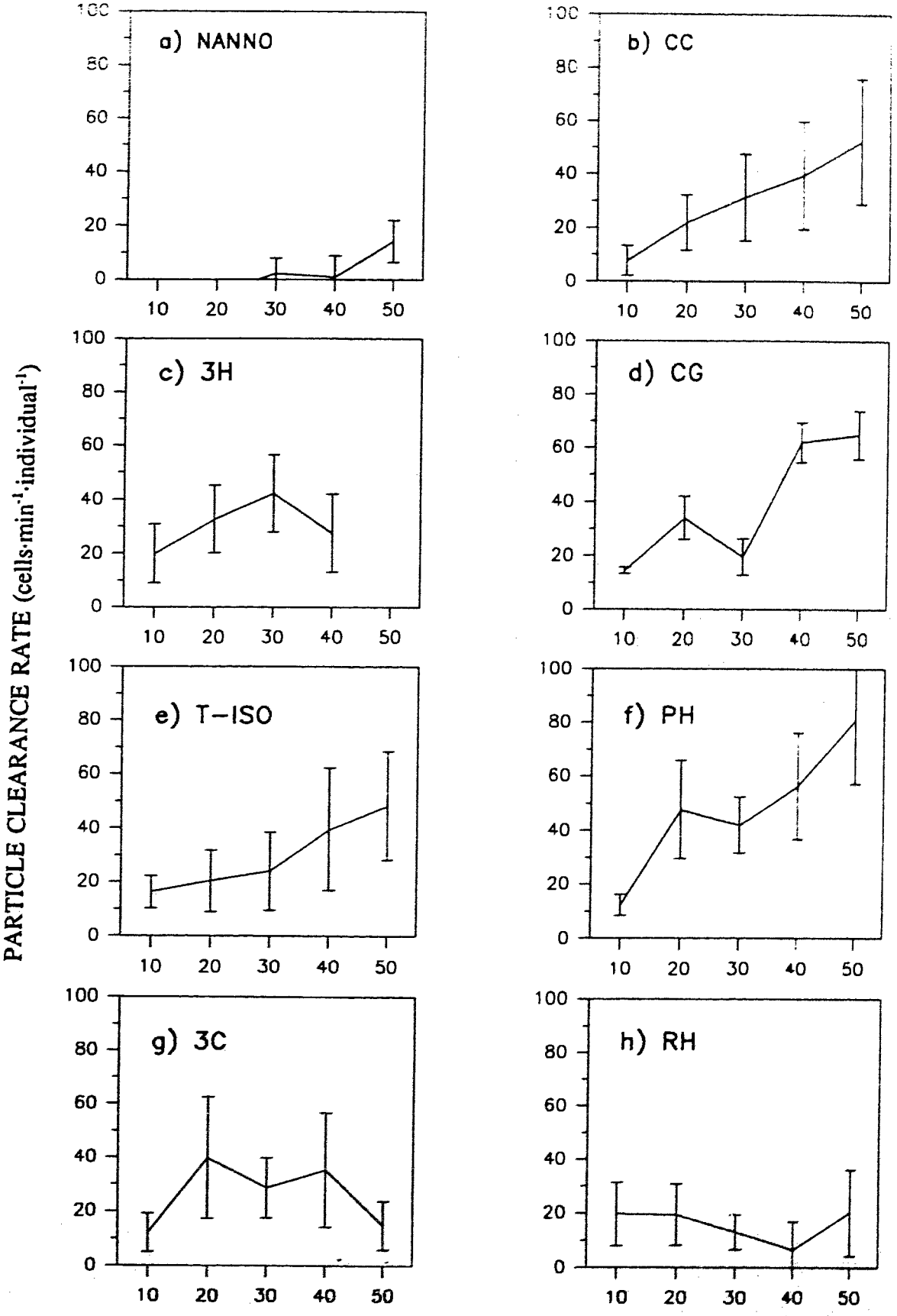

a) NANNO
b) CC
c) 3H
d) CG
e) T-ISO
f) PH
g) 3C
h) RH
PARTICLE CLEARANCE RATE (cells·min⁻¹·individual⁻¹)
PHYTOPLANKTON CONCENTRATION (1000 cells·mL⁻¹)

Figure 5: Particle clearance rates for juvenile *P. yessoensis* at approximate 1000 µm shell height versus phytoplankton concentration for eight phytoplankton species; a) *Nannochloropsis oculata* (NAN), b) *Chaetoceros calcitrans* (CC), c) *Thalassiosira pseudonana* (3H), d) *Chaetoceros gracilis* (CG), e) Tahitian *Isochrysis sp. (galbana)* (TX), f) *Phaeodactylum tricornutum* (PH), g) *Chroomonas salina* (3C), h) *Rhodomonas lens* (RH). Error bars equal +/- 1 standard deviation, n = 3.

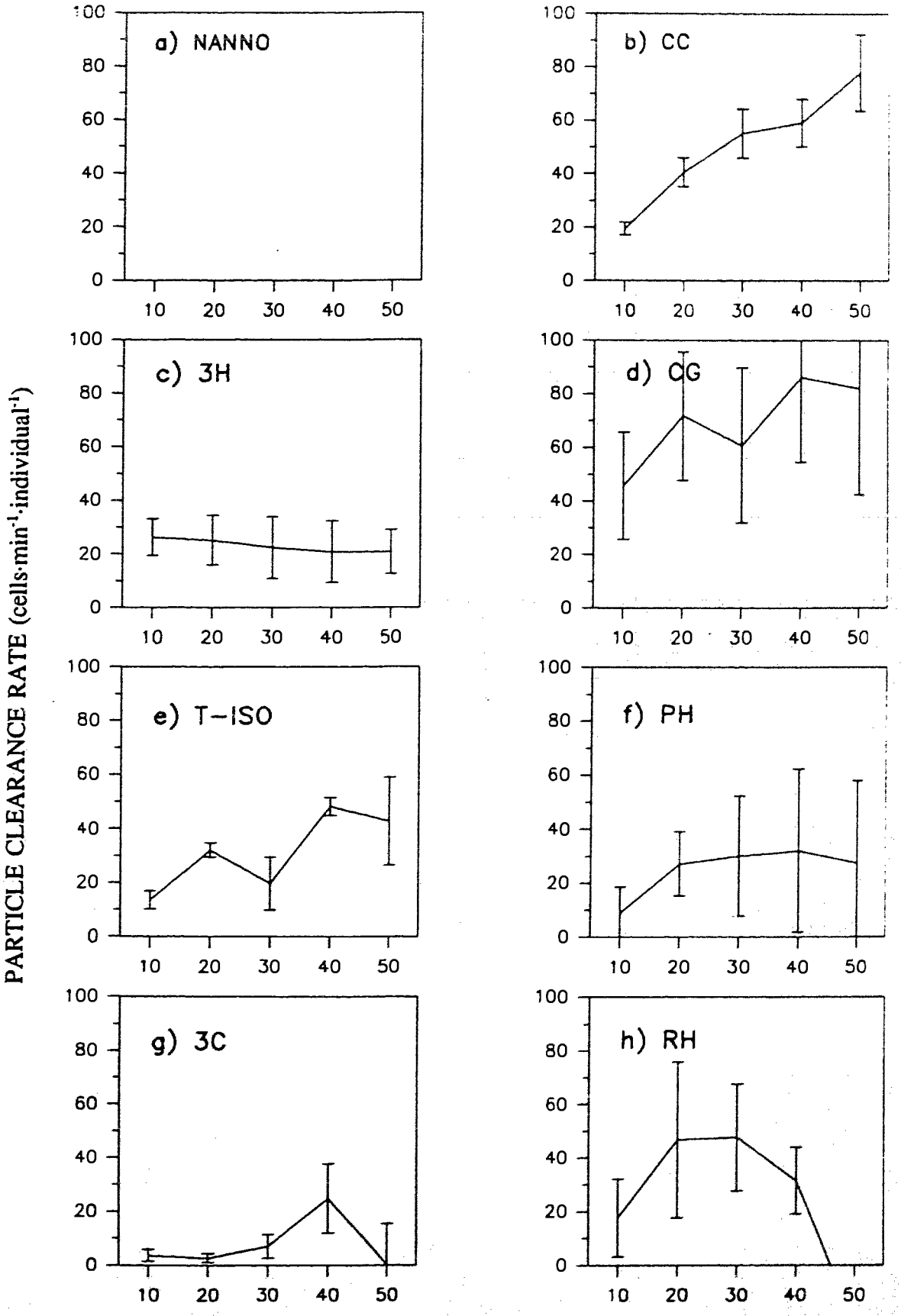

a) NANNO
b) CC
c) 3H
d) CG
e) T-ISO
f) PH
g) 3C
h) RH
PARTICLE CLEARANCE RATE (cells·min⁻¹·individual⁻¹)
PHYTOPLANKTON CONCENTRATION (1000 cells·mL⁻¹)

Figure 6: Particle clearance rates for juvenile *P. yessoensis* at approximate 2000 µm shell height versus phytoplankton concentration for eight phytoplankton species; a) *Nannochloropsis oculata* (NAN), b) *Chaetoceros calcitrans* (CC), c) *Thalassiosira pseudonana* (3H), d) *Chaetoceros gracilis* (CG), e) Tahitian *Isochrysis sp. (galbana)* (TX), f) *Phaeodactylum tricornutum* (PH), g) *Chroomonas salina* (3C), h) *Rhodomonas lens* (RH). Error bars equal +/- 1 standard deviation, n = 3.

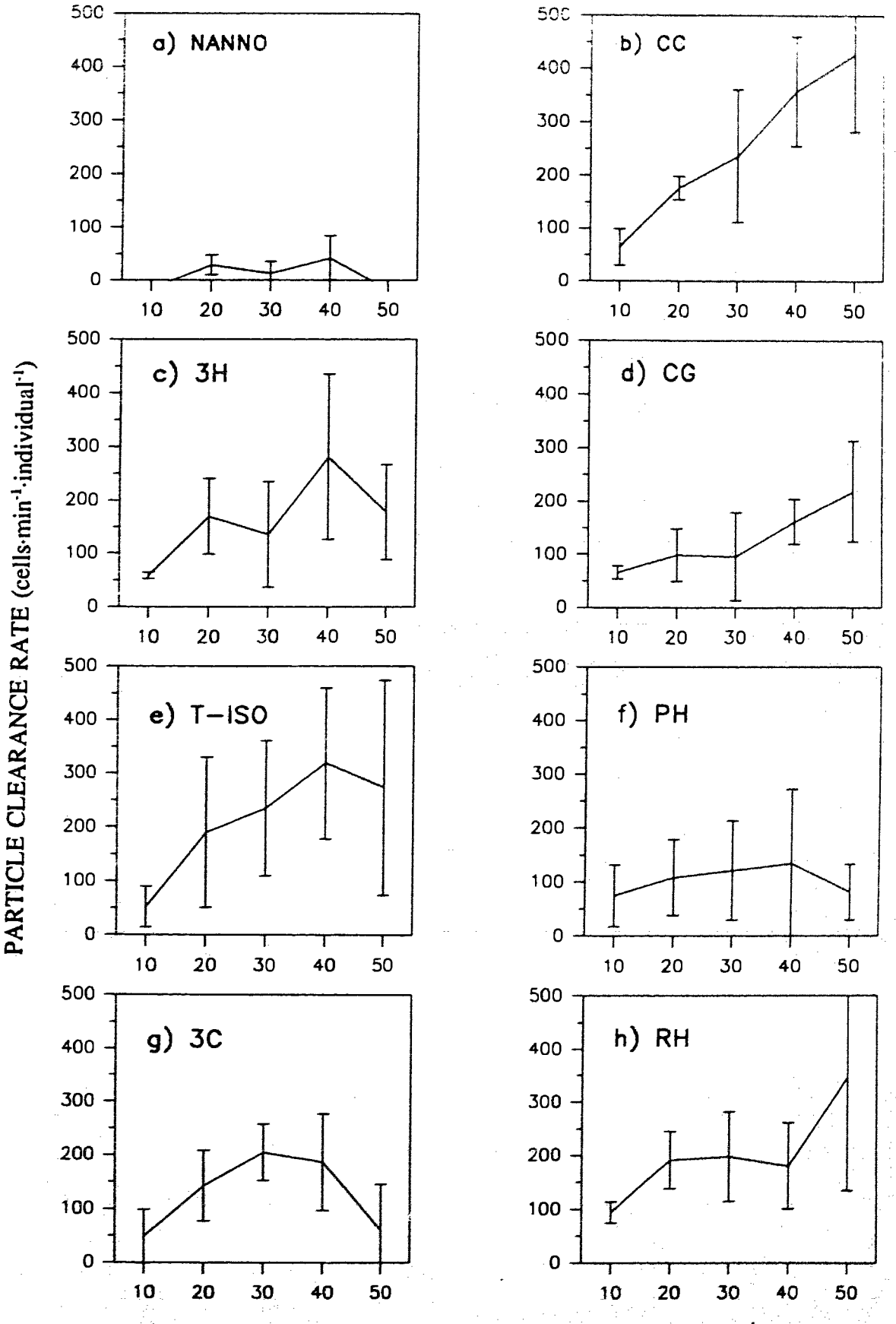
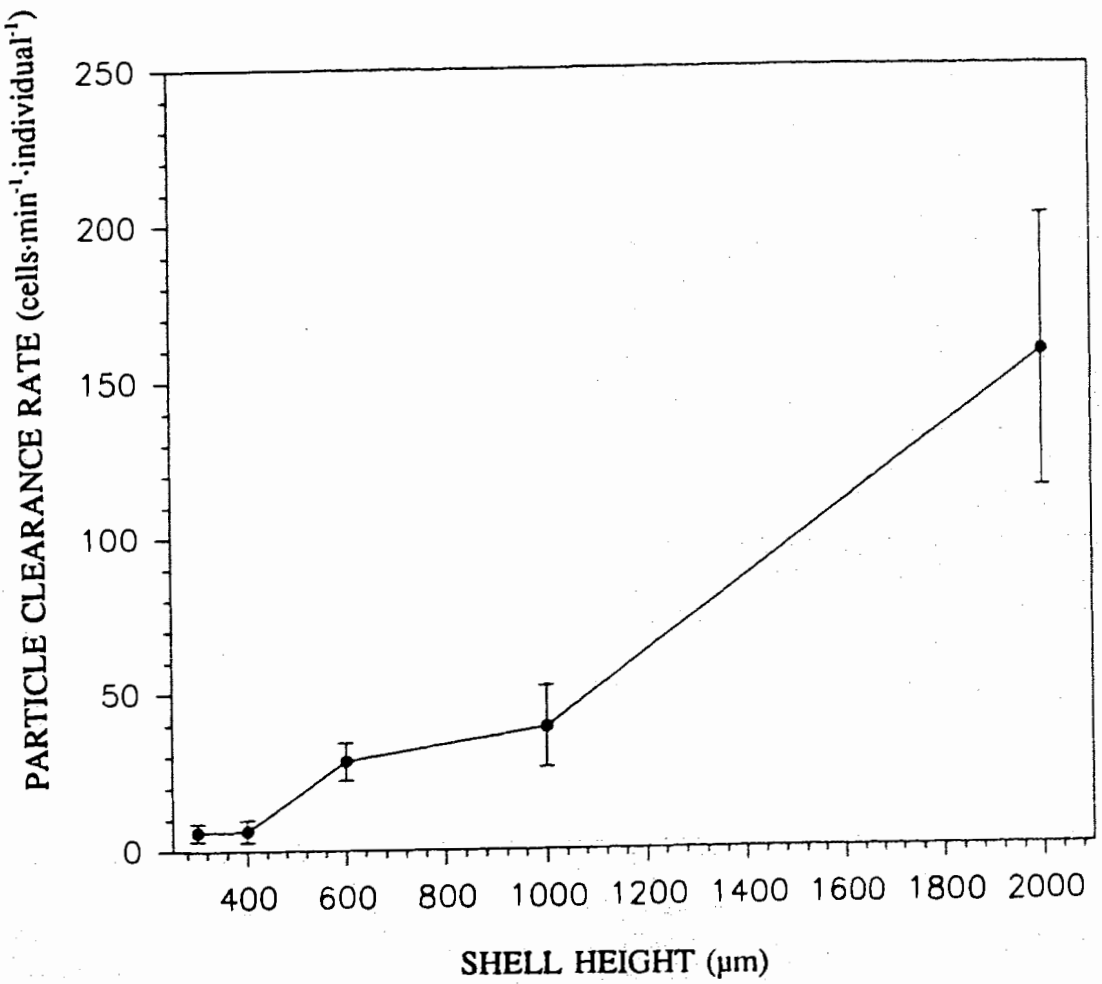

a) NANNO
b) CC
c) 3H
d) CG
e) T-ISO
f) PH
g) 3C
h) RH
0
100
200
300
400
500
10
20
30
40
50
PARTICLE CLEARANCE RATE (cells·min⁻¹·individual⁻¹)
PHYTOPLANKTON CONCENTRATION (1000 cells·mL⁻¹)

Figure 7. Particle clearance rates for juvenile *P. yessoensis* at phytoplankton concentration of 30,000 cells·mL-1 pooled for eight phytoplankton species versus shell height. Error bars = 95% confidence intervals.


PARTICLE CLEARANCE RATE (cells·min-1·individual-1)
250
200
150
100
50
0
400
600
800
1000
1200
1400
1600
1800
2000
SHELL HEIGHT (µm)

Clearance ability increased slightly to 1000 µm and then increased logarithmically between shell heights of 1000 and 2000 µm.

Ingestion experiments

The two size classes of juveniles used in the ingestion experiments were chosen to represent juvenile developmental stages prior to and after efficient filter feeding ability (approx. 300 µm and 1000 µm). Results of these experiments are shown in Table 3. Bead sizes of 2, 6 and 9 µm diameter were ingested by both size classes of juveniles in roughly equal proportions. The two large bead sizes (25 and 55 µm) were not ingested by either size class of juveniles.

Table 3. Number of fluorescent beads ingested by juvenile *P. yessoensis*, of 300 and 1000 µm shell height.

Bead Size (µm)	Exposure (min)	Number of beads in stomach n=30	
		300 µm	**1000 µm**
2.2	5	15.43 (s ± 11.98)	TNC* est >100
6.49	5	15.13 (s ± 13.39)	57.70 (s ± 41.66)
9.33	5	5.39 (s ± 8.19)	70.87 (s ± 42.80)
23.4	10	0 (n>100)	0 (n>100)
54.9	10	0 (n>100)	0 (n>100)

* TNC: Too numerous to count; clumps estimated > 100 beads

DISCUSSION

Particle clearance in juvenile *P. yessoensis* at very low levels was able to be detected by use of the experimental chambers used in this investigation. All species of phytoplankton tested were cleared from suspension by all size classes of juveniles with the exception of *N. oculata.* The calculated clearance rates were sensitive to small differences in particle concentration at the outflow of the chambers. The experimental design simulated normal rearing conditions since juveniles were continually exposed to suspended food materials. Clearance rates therefore reflect the normal ability of the gill to remove particles from suspension. Feeding behaviour may not necessarily remain constant between individuals within the chambers, which would increase the variability in calculated clearance rates. Other factors such as position of individuals relative to the flow within the chambers may have also added to the observed variation. As clearance rates increased with increasing size of the juvenile scallops, variability within the observations increased accordingly. High variability within the observations did not permit comparisons of clearance rates between species of phytoplankton. Data from these experiments do however provide a general indicator of the ability of juvenile *P. yessoensis* to remove phytoplankton from suspension.

Clearance rates for *N. oculata* were very low or non existant for all size classes of juveniles. This was the smallest species of phytoplankton tested, suggesting that this species was too small for efficient capture by juvenile scallops. This contradicted observations with fluorescently labelled beads which indicated that 2 μm particles

were ingested by juveniles. In all experiments it was noted that there was a slight increase in the number of particles in the 1 - 2 μm range (debris) in the outflows of the chambers containing juveniles. Bracketing measured particles to the the phytoplankton ranges for the analysis normally prevented these small particles from interfering with phytoplankton counts. In the case of *N. oculata*, the size peak associated with the algae was so close to the background debris peak that there may have been an overlap that was not resolvable on the basis of particle size alone. Therefore it is believed that use of the Coulter Counter in calculating particle concentrations may have led to an underestimation of clearance of *N. oculata.*

A general trend of clearance rate in response to cell concentration in the surrounding medium occurred. Initially clearance rate was observed to increase with concentration to a maximum level after which clearance rate decreased again. This is similar to the general feeding response reported for other species of bivalves (Winter, 1978). In this model; low clearance rates are observed at low algal concentrations when clearance efficiency is limited by the amount of water passing across the filtering surface. Clearance rate then increases in direct proportion to the number of cells available at increased concentrations and then plateaus as the filtering structures (ctenidia) operate at maximum efficiency. Clearance rate may either remain the same or decrease as clearance is reduced at very high concentrations. Data for *P. yessoensis* juveniles indicate that maximal clearance rate is generally achieved at algal densities between 30 - 40,000 cells·mL^{-1}. This was not observed for *C. calcitrans* the second

smallest species for which clearance rates continued to increase at 50,000 cells·1 μL^{-1} at 600, 1000 and 2000 µm shell heights (Figs. 4b, 5b, 6b).

An important observation from the particle clearance experiments was the sudden onset of ability to clear phytoplankton when scallop juveniles measured approximately 600 µm shell height. Prior to this size, only low levels of particle clearance were noted in post-metamorphic juveniles of 300 µm and 400 µm shell height. After 600 µm shell height, particle clearance ability increased with increased body size of the juveniles. This indicates that until 600 µm shell height (approximately 3 - 4 weeks post settlement), juvenile *P. yessoensis* are inefficient filter feeders and may not be able to filter phytoplankton adequately from suspension. This increase in filtration ability did not affect size specific ingestion ability that was noted for juvenile scallops prior to or after this critical point. Juvenile *P. yessoensis* were able to clear particles as small as 2 µm from suspension. Particles 25 µm and larger were not removed from suspension indicating that the upper limit for ingestion lies between 9 and 25 µm for post-metamorphic scallops. The ability of juvenile scallops to clear smaller particles from suspension, including 2 µm beads and cells of *Chaetoceros calcitrans*, challenges the belief that pectinids are inefficient at filtering particles less than 7 µm. The present data indicates that filtration efficiencies of early juvenile scallops are different to those reported for adult scallops.

CHAPTER 3: ONTOGENY OF FEEDING STRUCTURES IN JUVENILE *PATINOPECTEN YESSOENSIS*

INTRODUCTION

The functional anatomy of feeding structures in adult scallops has been the subject of numerous studies. Much of the basis of present knowledge began with early work by Drew (1906) and extensive studies by Atkins (1936, 1937a, b, c, 1938a, b, c) on the structure of the gills (ctenidia) and ciliation in adult bivalves. Recently there has been renewed interest in feeding structures of the Pectinidae (Owen and McCrae, 1976; Reed-Miller and Greenberg, 1982; Beninger *et al.*, 1988, 1990a, b, 1992; Le Pennec *et al.*, 1988; Beninger, 1991; Beninger and Le Pennec, 1991; Motavkin, 1990). The functional anatomy of adult scallops has been reviewed by Beninger and Le Pennec (1991).

The anatomy of feeding structures has been investigated for various species of larval pectinids (Sastry, 1965; Beaumont and Budd, 1982; Hodgson and Burke, 1988; Cragg and Crisp, 1991 for review). However few studies have been directed toward the study of the structure and function of feeding organs in post-metamorphic and early juvenile scallops. Descriptions of the ontogeny of feeding structures of post-larvae within species of pectinids have been limited primarily to descriptions of their morphogenesis at metamorphosis (Sastry, 1965; Hodgson and Burke, 1988; Bower and Meyer, 1990).

Descriptions of the morphology of feeding structures of post-larvae in genera other than the Pectinidae have included; *Mytilus edulis*, *Venus pullastrata*, *Venus striatula*, and *Panope abrupta* (Rice, 1908; Quayle, 1952; Ansell, 1962; Bayne, 1971; King, 1986). Post-metamorphic development within the bivalvia was the subject of an extensive review by King (1986). A thorough description of the ontogeny of feeding structures in post-metamorphic scallops had not been undertaken.

As with other bivalve groups, metamorphosis is a significant and critical transition period in scallop development. The velum, which is used for swimming and feeding during planktonic larval stages is lost and morphogenesis and reorientation of the internal organs results in the eventual formation of adult feeding structures. In mature larvae (pediveligers), the gill rudiments arise from a mass of undifferentiated cells near the anus into a gill plate composed of a ridge of tissue extending from the mantle into the mantle cavity (Hodgson and Burke, 1988; Bower and Meyer, 1990). At metamorphosis the internal organs undergo a 90° counter-clockwise rotation, the foot moves anteriorly and the mouth anterio-dorsally (Sastry, 1965; Hodgson and Burke, 1988). In post-metamorphic stages of *Aequipecten irradians*, new filaments are added as bud-like processes at the distal ends of the ctenidia and elongate as proximal filaments (Sastry, 1965).

The long, muscular, flexible foot in postlarvae contains byssal glands and an elaborate duct system (Gruffydd *et al.*, 1975; Bower and Meyer, 1990). Lateral and dorsal surfaces of the foot are sparsely ciliated. The base of the foot is covered with simple cilia and has a byssal gland extending along the midline of the longitudinal

axis (Hodgson and Burke, 1988). The structure and orientation of the foot in relation to various other organs has been briefly discussed in descriptions of pedal feeding in *P. yessoensis* (Reid *et al.*, 1992).

In adult scallops, the foot has reduced allometric importance and the primary organs of feeding are the ctenidia. The complex gills of adult scallops are euleutherorahbdic plicate; the W-shaped right and left gills each comprise an outer and inner demibranch composed of two different types of filaments (principal and ordinary), suspended from the gill axis in a corrugated or plicate fashion (Beninger and Le Pennec, 1991). The peribuccal organs, consisting of the large, ridged labial palps, and the arborescent lips are highly developed organs in adults linking the gills with the mouth (Beninger *et al.*, 1990a, b). The functional anatomy of feeding structures in adult scallops has been used as a basis to elucidate mechanisms of feeding. Comparisons with adult structures have been hampered in post-larval and juvenile animals in which morphogenesis of the structures may be incomplete (Beninger, 1991).

The objective of this study was to describe the ontogeny of feeding structures of juvenile Japanese scallops *Patinopecten yessoensis* from metamorphosis until a they were approximately two mm shell height The structures described were primarily the ctenidia, the peribuccal organs (mouth, lips and labial palps) and the foot. This study expands upon previous investigations and extends the descriptions to later juvenile stages.

MATERIALS AND METHODS

Juvenile Scallops

P. yessoensis larvae and juveniles were taken from individuals obtained and maintained for other experiments (Chapter 2).

Fixation

Prior to fixation, juvenile scallops were anaesthetized by adding chilled 15% $MgCl_2$/Filtered Seawater (FSW) dropwise to specimens in a petri dish of FSW (Bower and Meyer, 1990). After a period of 1 - 2 min, specimens were checked for relaxation; determined by shell gaping and extension of the mantle. The juveniles were then killed by adding fixative dropwise to the petri dish. Fluid was drawn off and the specimens were immersed in the appropriate fixative. Post-metamorphic scallops required for histological examination were preserved in Davidson's solution with acetic acid (Howard and Smith 1983). Post-metamorphic scallops used for examination by scanning electron microscopy were preserved in 5% glutaraldehyde in FSW.

Histological Sections

Preparation of specimens for histological examination followed the methods of Bower and Meyer (1990). Samples in Davidson's fixative were stored until the shell had been dissolved by the acetic acid. Specimens were then embedded in a

methacrylate plastic (JB-4, Polysciences, Inc. Warrington, PA 18976, USA). Glass knives were used to cut sections of approximately 2 - 3 µm thickness. Sections were stained with Lee's methylene blue basic fuschin stain as recommended for JB-4 by the supplier. Histological sections were photographed using Kodak technical-pan and T-Max black and white, and Kodak Ektachrome T-160 colour transparency 35 mm film using Koehler illumination microscopy.

Scanning Electron Microscopy

Samples fixed in glutaraldehyde were stored in 10 mM sodium azide in FSW. In preparation for critical point drying, the samples were dehydrated through a graded series of ethanol; 30, 50, 70, 95, 95, 100, 100%, for 10 min each. Samples were critical point dried using a Tousimi Research Corp. SAMDRI-790 critical point dryer using 100% anhydrous ethanol and CO_2 as transitional fluids.

After critical point drying, whole specimens were transferred onto adhesive coated aluminum SEM stubbs. The valves were teased apart with a sharpened tungsten wire dissecting probe while viewing the specimens under a dissecting microscope (King, 1986).

Stubbs were then sputter coated with platinum using a Technics Corp Hummer V sputter coater. Coated stubbs were viewed with an ETEC Corp. Autoscan scanning electron microscope at an accelerating voltage of 20 Kv. Stubbs were photographed with Ilford FP4 and Kodak PXP 120 ASA, 220 black and white film.

RESULTS

Development of the Ctenidia

In pediveligers which are competent to undergo metamorphosis of approximately 260-280 μm in shell length, the ctenidia are present as three to four primordial buds arising laterally to the foot in the mantle cavity (Fig. 8a). During metamorphosis, these undergo rapid histogenesis and elongate to become the first ordinary filaments of the inner demibranchs (Fig. 8b, c). They are sparsely ciliated at this point with simple cilia (Fig. 8d). Organization of the cilia occurs in post-larvae almost immediately after metamorphosis coinciding with initial formation of the dissoconch shell. Ciliary junctions composed of elongated cilia extending from the lateral cilia at the distal ends of the filaments were observed in ctenidia when filaments were approximately 25 μm in length (Fig. 8e, f). These cilia mesh together to create a bond joining the ends of the filaments.

As the filaments increase in length, ciliation increases along the lateral and then the frontal surfaces of the filaments (lateral and frontal cilia respectively) (Fig. 9a). The lateral and frontal cilia are unbranched simple cilia approximately 7 μm in length. The lateral cilia arise from a row of columnar cells along either side of each filament. Less numerous frontal cilia are present along the forward edge of the filaments. Eulatero-frontal or abfrontal cilia are not present.

Figure 8. Ctenidial development of *P. yessoensis* at metamorphosis and immediately post-metamorphosis.

a) Light micrograph of swimming pediveliger prior to metamorphosis. Specimen oriented ventral side up, anterior to the left. v, velum; f, foot; dg, digestive gland; gp, gill primordia m, mouth. Scale bar = 100 µm.

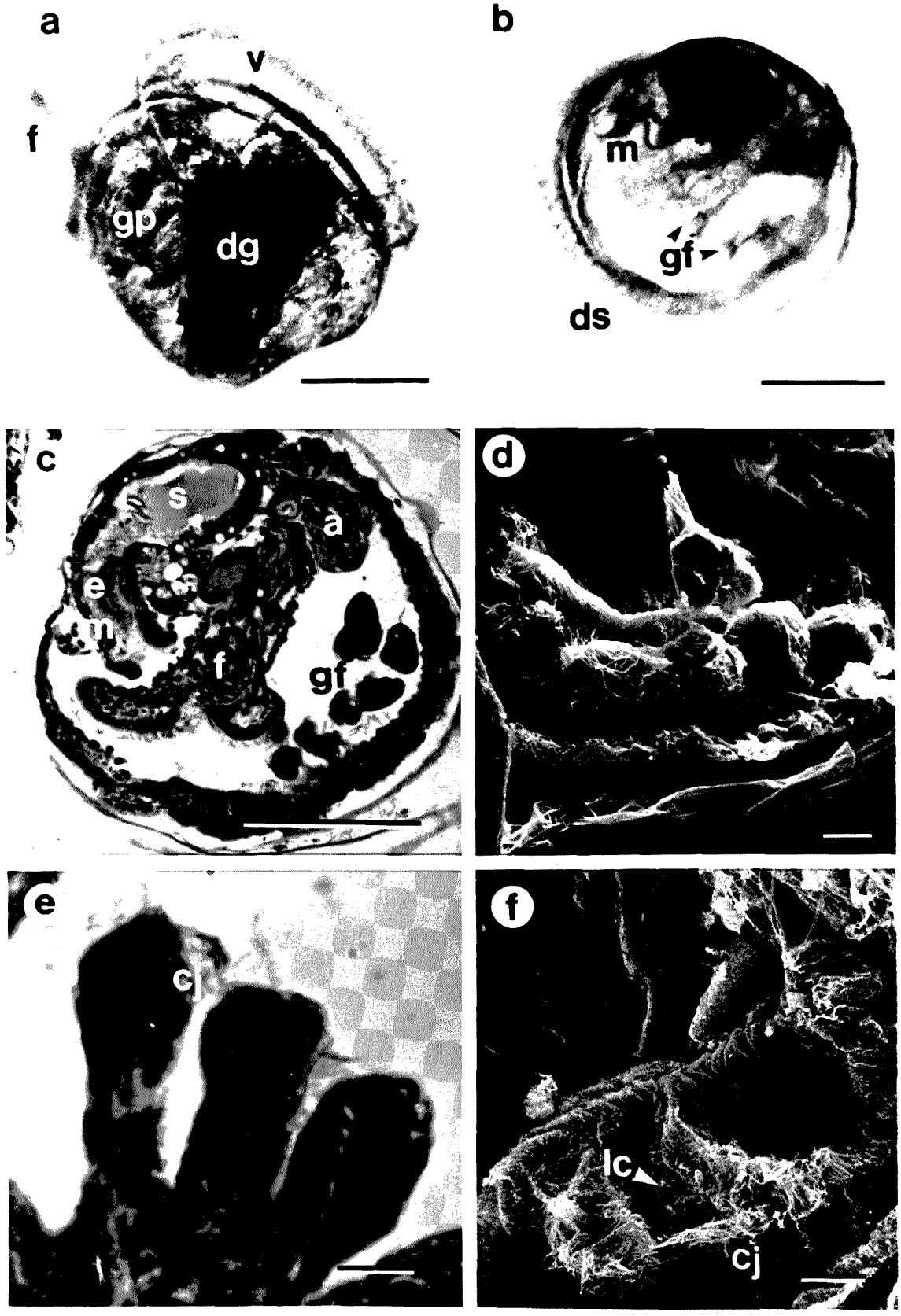
b) Light micrograph of juvenile several days post-metamorphosis. Specimen oriented ventral side down, anterior to the left. m, mouth; gf, gill filament; ds, dissoconch shell. Scale bar = 100 µm.

c) Sagital histological section of juvenile several days post-metamorphosis. Specimen oriented ventral side down anterior to the left. a, adductor muscle; e, eosophagus; s, stomach. Scale bar = 100 µm.

d) SEM of gill filaments in juvenile immediately post-metamorphosis. Scale bar = 10 µm.

e) Longitudinal histological section of gill filaments of juvenile several days post-metamorphosis. cj, ciliary junction. Scale bar = 10 µm.

f) SEM of gill filaments of juvenile several days post-metamorphosis. lc, lateral cilia. Scale bar = 10 µm.


a
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cj
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lc
cj

Figure 9. Structure and ciliation of gill filaments in developing juvenile *P. yessoensis*.

a) Frontal surface of ascending lamellae of ordinary gill filaments from juvenile approximately 600 µm shell height. Filaments are oriented with distal ends at the top. fc, frontal cilia; lc lateral cilia. Scale bar = 10 µm.

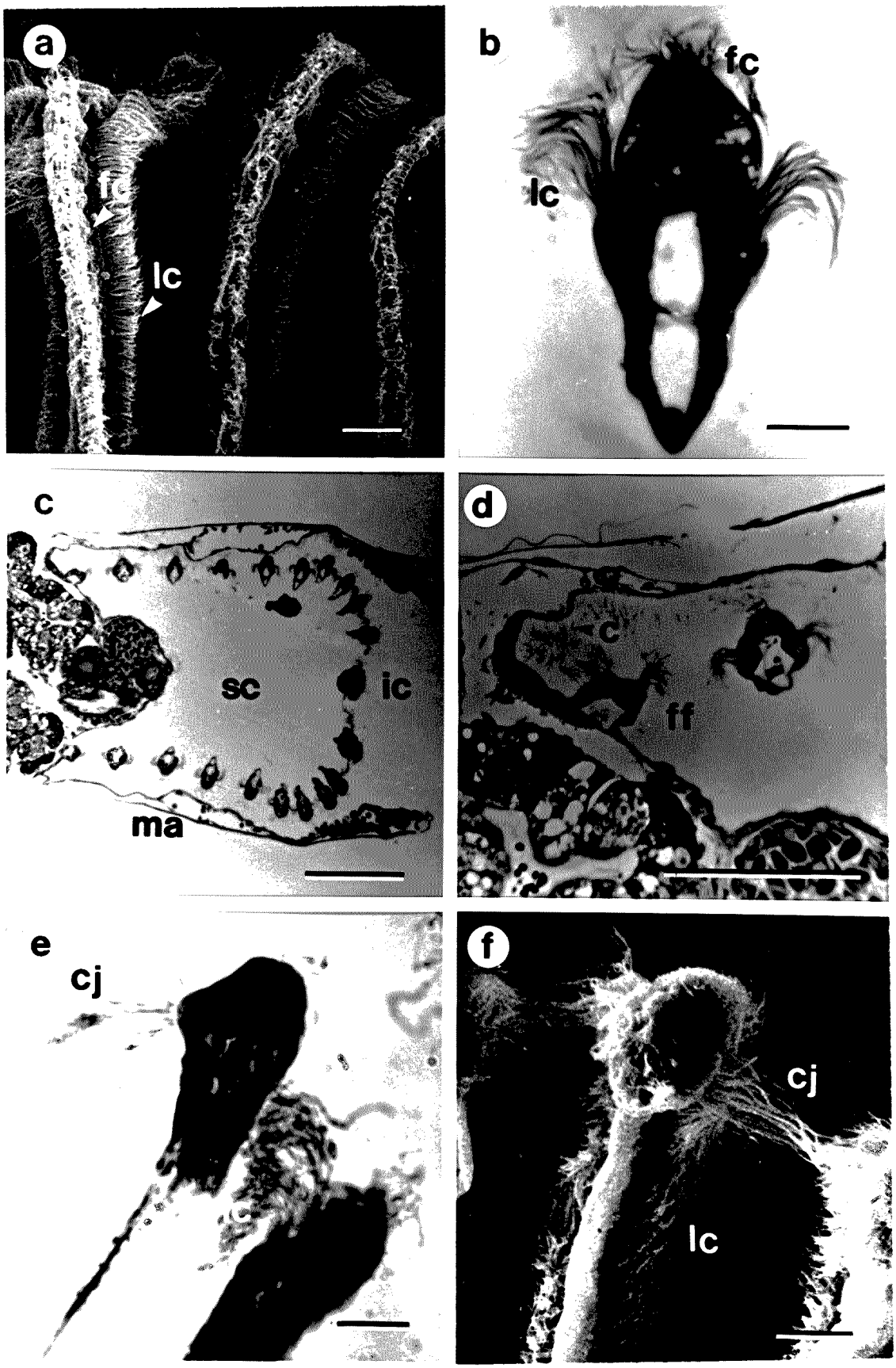
b) Transverse histological section of an ordinary gill filament. Scale bar = 10 µm.

c) Transverse section of juvenile showing arrangement of gill filaments in the mantle cavity. Specimen is oriented with right valve down, dorsal to left. f, foot; ic, infrabranchial cavity; ma, mantle ; sc, suprabranchial cavity. Scale bar = 100 µm.

d) Detail of 9c showing fused filament and first ordinary filament of left inner demibranch. c, cilia (mantle); ff, fused filament. Scale bar = 50 µm.

e) Longitudinal section of ascending lamellae of ordinary filaments showing lateral ciliary junction. cj, ciliary junction. Scale bar = 10 µm.

f) SEM of abfrontal surface of ascending lamellae of ordinary filaments showing lateral ciliary junction. Scale bar = 10 µm.


a
fc
lc
b
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cj
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cj
lc

In cross section the filaments are ovoid and widest at the point of the lateral cilia, approximately 20 μm (Fig. 9b). The central lumen of the filaments is bisected by a thin septum. A nerve ganglion lies in the posterior lumen of the filaments.

At the base of the demibranchs the mantle tissues along either side of the foot evaginate into two ridges to form two filaments which are fused laterally with the mantle along their length. These ridges lie parallel to the rest of the ordinary filaments and they extend along the length of the descending lamellae from the gill axis to a position posterior to the labial palps (Fig. 9c, d). The ventral lateral and frontal surfaces of the ridges are ciliated, with the lateral cilia corresponding to the lateral cilia of the first ordinary filaments. These fused filaments are not connected by ciliary junction to the first ordinary filaments.

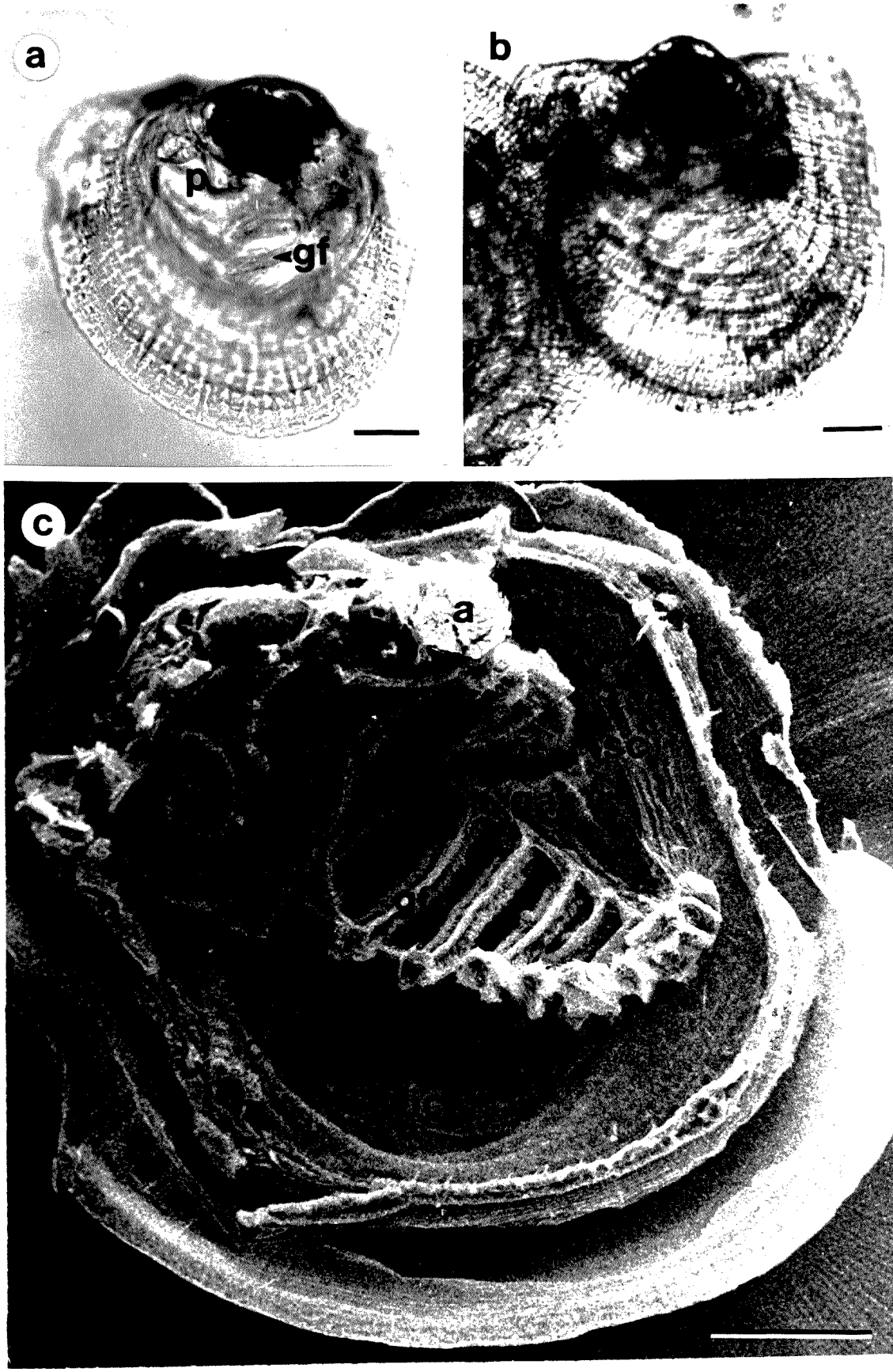
Growth of the inner demibranchs occurs in length and number of ordinary filaments with new filaments arising as buds at the distal ends of the demibranchs. The paired demibranchs extend dorso-ventrally as they grow and the filaments extend anteriorly from the gill axes which are fused with the inner surfaces of the mantle (Fig. 10a, b). The first free filaments at the proximal base of each demibranch are distinct in that they curve ventrally towards the ciliary junction of the next filament at their distal ends (Fig. 10c).

Figure 10. Development of juvenile *P. yessoensis* between 500 and 600 µm shell height. All orientations are anterior to left, dorsal to top.

a) Light micrograph of juvenile approximately 500 µm shell height. p, labial palps; gf, gill filaments. Scale bar = 100 µm.

b) Light micrograph of juvenile approximately 600 µm shell height. Scale bar = 100 µm.

c) SEM of juvenile approximately 600 µm shell height, left valve, mantle and demibranch removed. View is of abfrontal surface of right demibranch. Note curved first ordinary filament. a, adductor muscle; ct, mantle ciliated tract; f, foot; ga, gill axis; ic, infrabranchial cavity; sc, suprabranchial cavity. Scale bar = 100 µm.


a
p
gf
b
c
a

As the filaments extend along each side of the mantle cavity, they begin to curve inwards when the scallops are approximately 500 µm shell height. As the filaments enlarge, the distal ends thicken into small knobs, the capitula (Bayne, 1971). The filaments reach their maximum length prior to reflection when the juveniles are approximately 600 µm shell height (Figs. 10c, 11a).

At this stage each inner demibranch is composed of 8-9 filaments approximately 150 - 175 µm which occupy the majority of the mantle cavity. The frontal surfaces of the capitula become covered with fine junctional cilia approximately 3 µm long. The distal tips of the filaments mesh anteriorly with those of the opposing demibranch. This completes formation of the branchial basket and results in the mantle cavity being divided into infrabranchial and suprabranchial chambers, connected by interfilamentary spaces (Figs. 9c, 12).

The filaments then begin to reflect back upon themselves posteriorly to form the ascending lamellae of the inner demibranchs (Fig. 12). New growth of the filaments extends from the abfrontal surface of the filaments distally to the lateral ciliary junctions. As the filaments reflect, the lateral ciliary junctions remain at the point of reflection and the capitula ascend posteriorly into the mantle cavity. The capitula enlarge and the junctional cilia, which cover the frontal surface of the capitula, increase in number (Fig. 12d). These interdigitate with the cilia on the opposing filaments to maintain the connection of the demibranchs and separation of the infrabranchial and suprabranchial cavities.

Figure 11. Three dimensional diagram of structure and orientation of feeding organs in juvenile *P. yessoensis* prior to reflection of gill filaments (approximately 600 µm shell height). Orientation is anterio-ventral side-view with portions of left valve and mantle cut away. bg, byssal groove; bn, byssal notch; cj, ciliary junction; ct, ciliated tract; ds, dissoconch shell; e, eye; f, foot; fc, frontal cilia; ff, fused filament; ga, gill axis; lc, lateral cilia; lv, left valve; ma, mantle; mc, mantle curtain; p, labial palps; pr, propodium; ps, prodissoconch shell; rv, right valve. Scale bar = 100 µm.

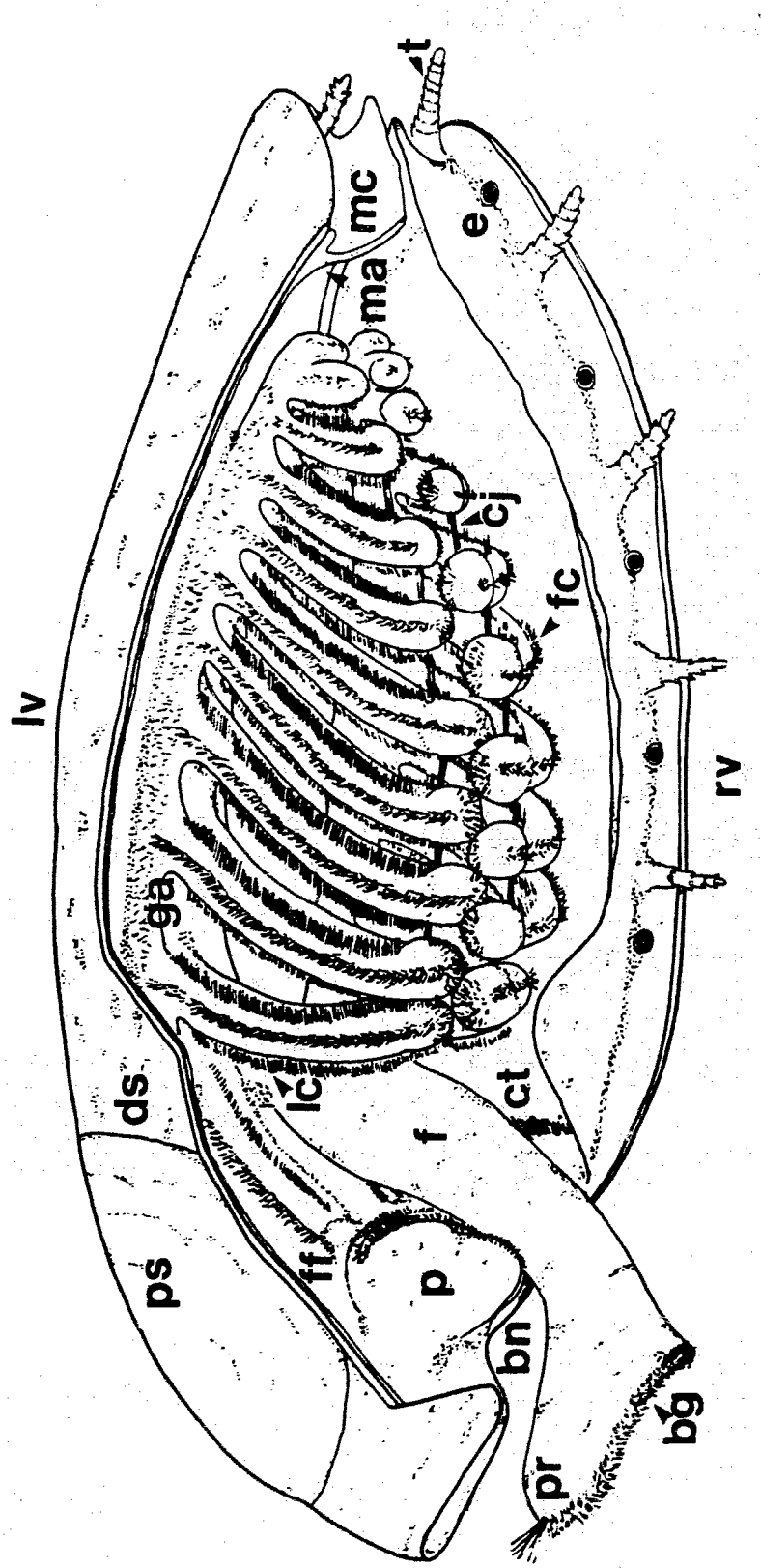

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Figure 12. Reflection of inner demibranch gill filaments in juvenile *P. yessoensis*

a) SEM of abfrontal view of right inner demibranch from juvenile just prior to reflection of filaments. dl, descending lamellae; ga, gill axis; if, interfilamentary space. Scale bar = 50 µm.

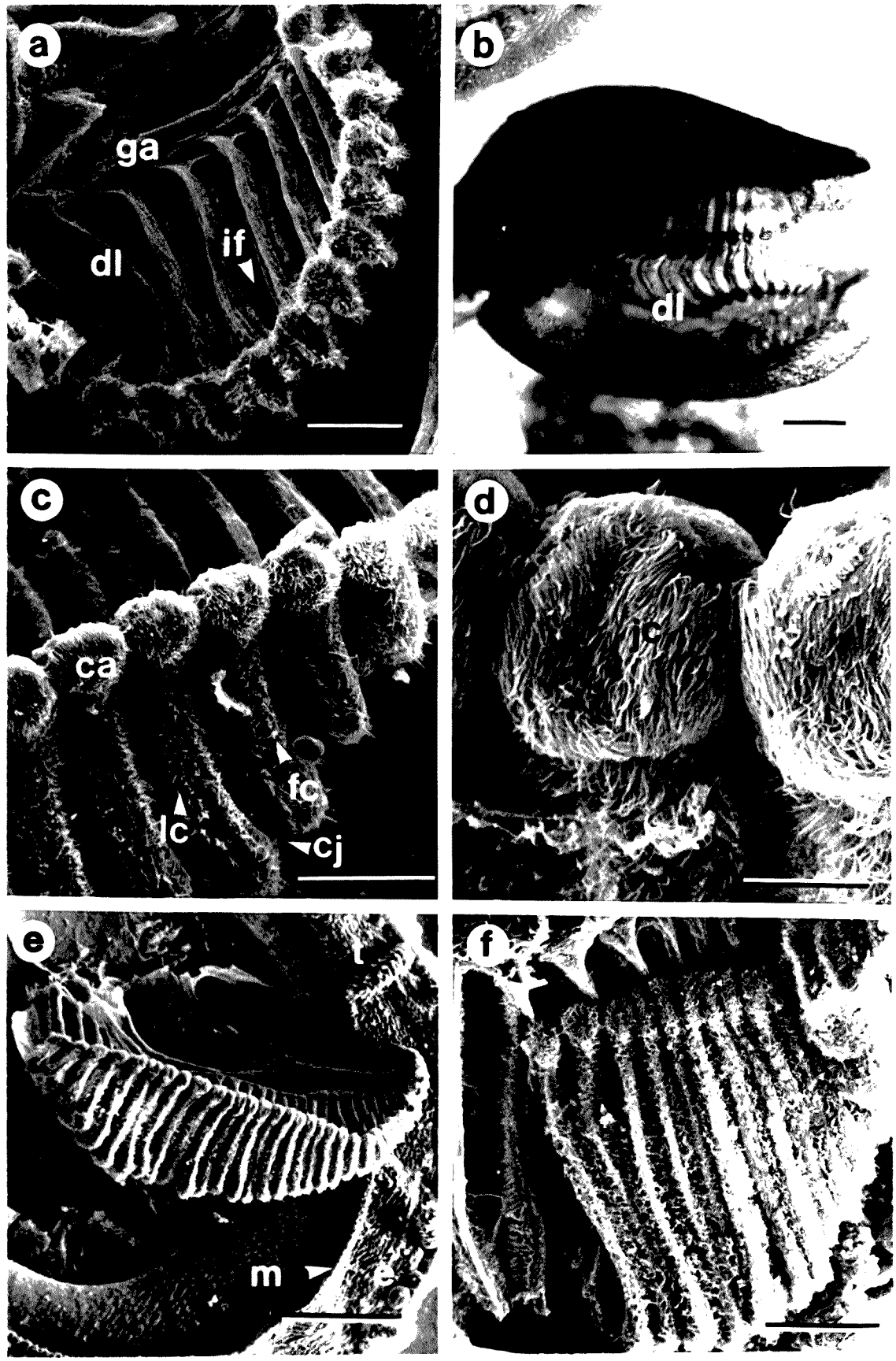
b) Micrograph of anterio-ventral view of live juvenile approximately 800-900 µm shell height showing orientation of demibranchs. al, ascending lamellae; mc, mantle curtain. Scale bar = 100 µm.

c) SEM of frontal surface of ascending lamellae of right inner demibranch in juvenile approximately 1000 µm shell height. ca, capitula; cj, lateral ciliary junction; fc, frontal cilia; lc, lateral cilia. Scale bar = 50 µm.

d) SEM of frontal surface of capitula on ascending lamellae of right inner demibranch in juvenile approximately 1200 µm shell height. jc, junctional cilia. Scale bar = 10 µm.

e) SEM of right inner demibranch from juvenile approximately 1250 µm shell height. Distal (ventral) end of demibranch overlies mantle curtain due to mantle retraction during fixation. e, eye; mc, mantle curtain; t, tentacle. Scale bar = 100 µm.

f) SEM of fully reflected filaments from right inner demibranch of juvenile approximately 1500 µm. Scale bar = 50 µm.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Further ctenidial development involves increases in the reflection, number and overall size of the ordinary filaments of each inner demibranch (Fig. 12e). Moving ventrally along the demibranch, the degree of reflection and length of the filaments increases for the first 6 - 8 filaments reaching maximum reflection and length at the widest portion of the demibranch. As the demibranchs curve posterio-ventrally, relative filament length and reflection decreases towards the distal tip of the demibranchs where new filaments are arising. The new filaments of the opposing demibranchs do not meet (Fig. 12b). In live specimens, the distal tips of the demibranchs were observed to be capable of a large degree of flexion, which presumably enables alteration of the size of this opening. As the overall size of the juvenile increases, the demibranchs extend ventrally and new filaments arise at the distal ends of the demibranchs. The fully elongated primary filaments at the origin of the demibranchs increase in both total length and length of the reflected portion of the lamellae until they are fully reflected back along the descending lamellae (Fig. 12f).

When juvenile shell height range from 1000-1200 µm, there are approximately 20 - 25 ordinary filaments on each inner demibranch and the ascending lamellae are reflected approximately half-way back along the descending lamellae. (Fig. 13a). The primordia of the filaments of the outer demibranchs are first observed during this stage (Fig. 13b). Formation of the outer demibranchs is distinct from the pattern of morphogenesis of the inner demibranchs. At the distal tip of the inner demibranchs where new filaments are being budded off, the gill axis begins to divide and to bud off filaments in both directions simultaneously (Fig. 13d). Histogenesis of the outer

demibranchs then occurs both ventrally and dorsally along the gill axes. New filaments arise along the length of the existing gill axis simultaneously with new filaments of the inner demibranchs at the distal tip of the ctenidia.

Growth and ciliation of the ordinary filaments of the outer demibranchs follows that of the filaments of the inner demibranchs. Initial growth of the filaments is in length. Reflection occurs at filament lengths of approximately 100 -150 µm. Junctional cilia at the distal tips of the reflected filaments make ciliary contact with the mantle walls further dividing the mantle cavity into supra- and infrabranchial chambers. Frontal cilia of both filaments extend across the inner surface of the gill arches, although it is not clear if this results in the formation of an orally directed ciliary tract. When juveniles are approximately 2000 µm shell height, the filaments of the inner and outer demibranchs are almost equal in size and degree of reflection with each demibranch having approximately 35 - 40 filaments (Fig. 13e). In cross section the filaments remain approximately 20 µm wide and the interfilamentary spaces decrease slightly (Fig. 13f). The ascending and descending lamellae of each filament are flattened along each other. Folding (plication) of the demibranchs and filaments resembling principal filaments or marginal food grooves at the ventral margins of the demibranchs were not observed. Increases in shape, number and length of the ordinary filaments with increasing shell height are summarized in Fig. 14.

Figure 13. Outer demibranch development in juvenile *P. yessoensis*.

a) Light micrograph of live juvenile aproximately 1000 µm shell height prior to development of outer demibranch. a, adductor muscle; ct, ctenidia; e, eye; f, foot; t, tentacle. Scale bar = 100 µm.

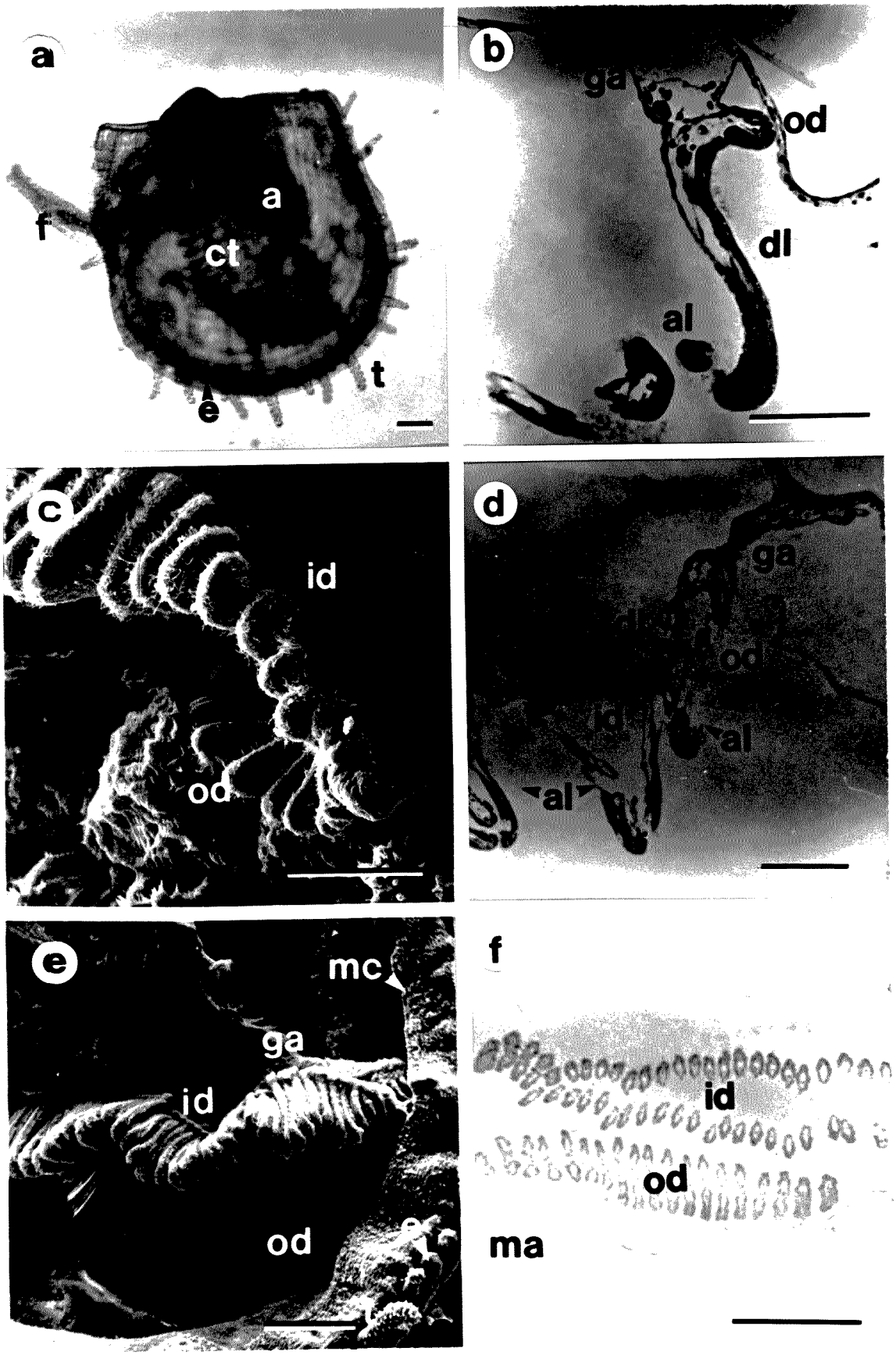
b) Histological longitudinal section through left demibranch filament from juvenile approximately 1200 µm shell height showing initial development of descending lamellae of outer demibranch. Note ascending lamellae of inner deminbranch moves out of plane of section. al, ascending lamellae; dl, descending lamellae; ga, gill axis; od, outer demibranch. Scale bar = 100 µm.

c) SEM of ventral tip of right ctenidia from juvenile approximately 1230 µm shell height. id, inner demibranch. Scale bar = 50 µm.

d) Histological longitudinal section through left demibranch filament from juvenile approximately 2000 µm shell height showing reflected outer demibranch. Scale bar = 100 µm.

e) SEM of right ctenidia from juvenile approximately 2200 µm shell height. Orientation is dorsal to the left. Note mantle cutain is retracted due to fixation. e, eye; mc, mantle curtain. Scale bar = 100 µm.

f) Histological transverse section through ordinary gill filaments of right ctenidia from juvenile approximately 2000 µm shell height. ma, mantle. Scale bar = 100 µm.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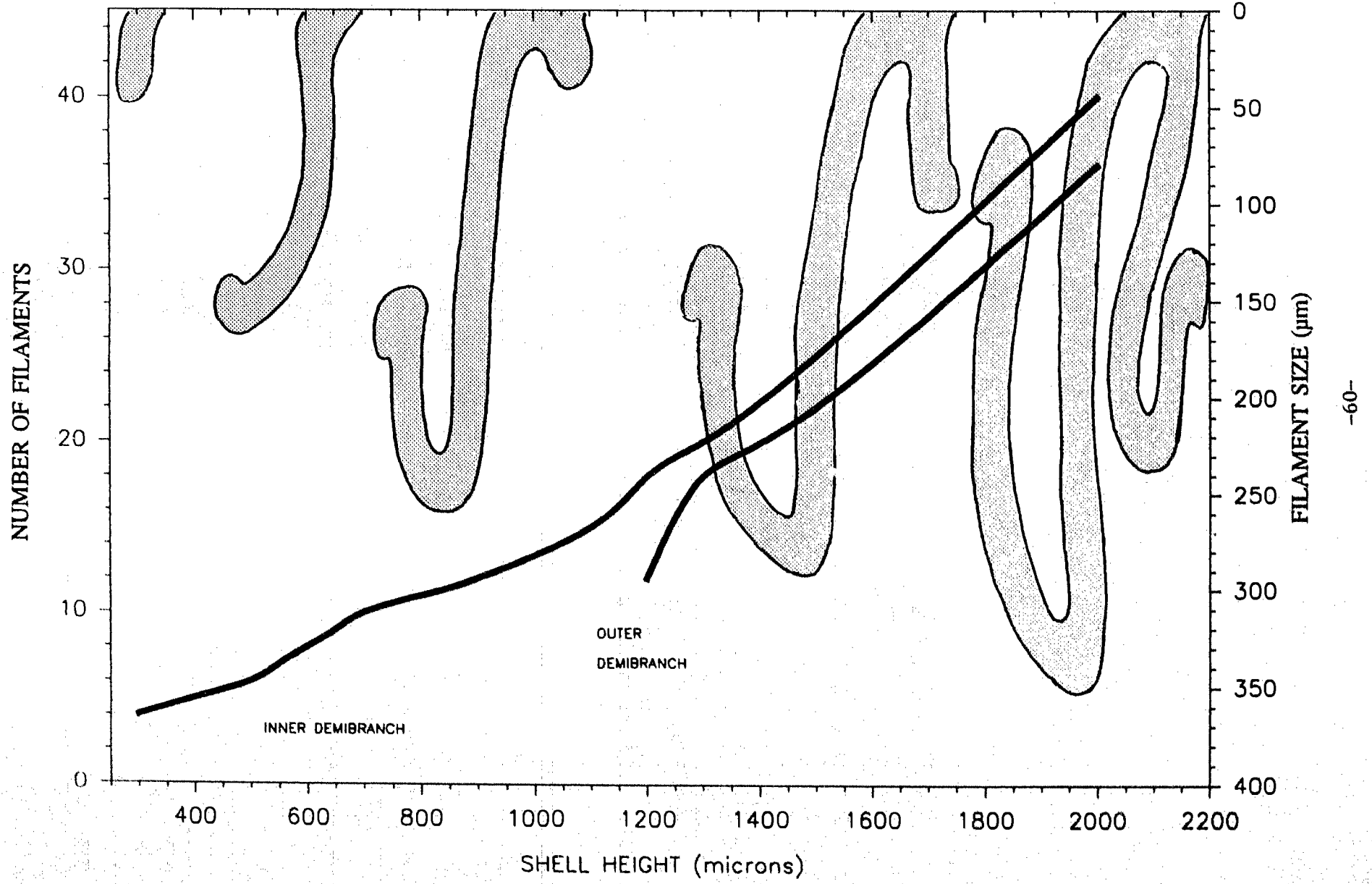
Development of Peribuccal Organs

Immediately after metamorphosis the mouth is located anteriorly in the mantle cavity (Fig. 15a). The lips and labial palps are not well defined and consist of an extension of the oesophageal tract. The inner surface of the palps is uniformly ciliated and the palps form a funnel shaped orifice which narrows into the ciliated oesophagus. In post-metamorphic individuals there is no distinct differentiation between the palps and the lips and the oesophagus leads directly into the stomach. A constriction in the epithelial wall, that would define a boundary between the lips and the oesophagus, does not exist. The inner surface of the labial palps consists of a single layer of columnar epithelial cells supported by a smooth epithelial membrane on the outer surface of the labia by single muscle fibres. The haemocoel between the two epithelia contains numerous haemocytes and single muscle fibres which support the structures. The inner surface of the palps is smooth and uniformly ciliated. The smooth outer surface of the palps is not ciliated.

As development proceeds, the labial palps extend ventrally out into the mantle cavity as a pair of lateral tissue flaps on either side of the mouth (Fig. 15b, c). Each flap is divided slightly to form the upper and lower palps. Using scanning electron microscopy, the palps are observed to form a hood over the mouth. In live specimens, the palps are flexible, enabling alteration of the width of the opening formed by the palps. During development of post-metamorphic juveniles, the palps extend until they are in close association with the first filaments of the inner demibranchs. This occurs simultaneously with the primary filaments achieving their maximum length prior to

Figure 14. Summary of ctenidial growth in juvenile *P. yessoensis*. Left axis; number of developed ordinary filaments on inner and outer demibranchs indicated by solid lines versus shell height (X axis). Right axis; size and shape of ordinary filaments during development indicated by diagrams of longitudinal sections through filaments versus shell height (X axis).


NUMBER OF FILAMENTS
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30
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800
1000
1200
1400
1600
1800
2000
2200
SHELL HEIGHT (microns)
FILAMENT SIZE (µm)
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50
100
150
200
250
300
350
400
OUTER
DEMIBRANCH
INNER DEMIBRANCH

Figure 15. Development of the peribuccal organs in juvenile *P. yessoensis* and structure of the foot.

a) Histological sagittal section through juvenile approximately 400 µm shell height. f, foot; e, eosophagus; g, gastric shield; p, labial palps; pr, propodium of foot; s, stomach. Scale bar = 50 µm.

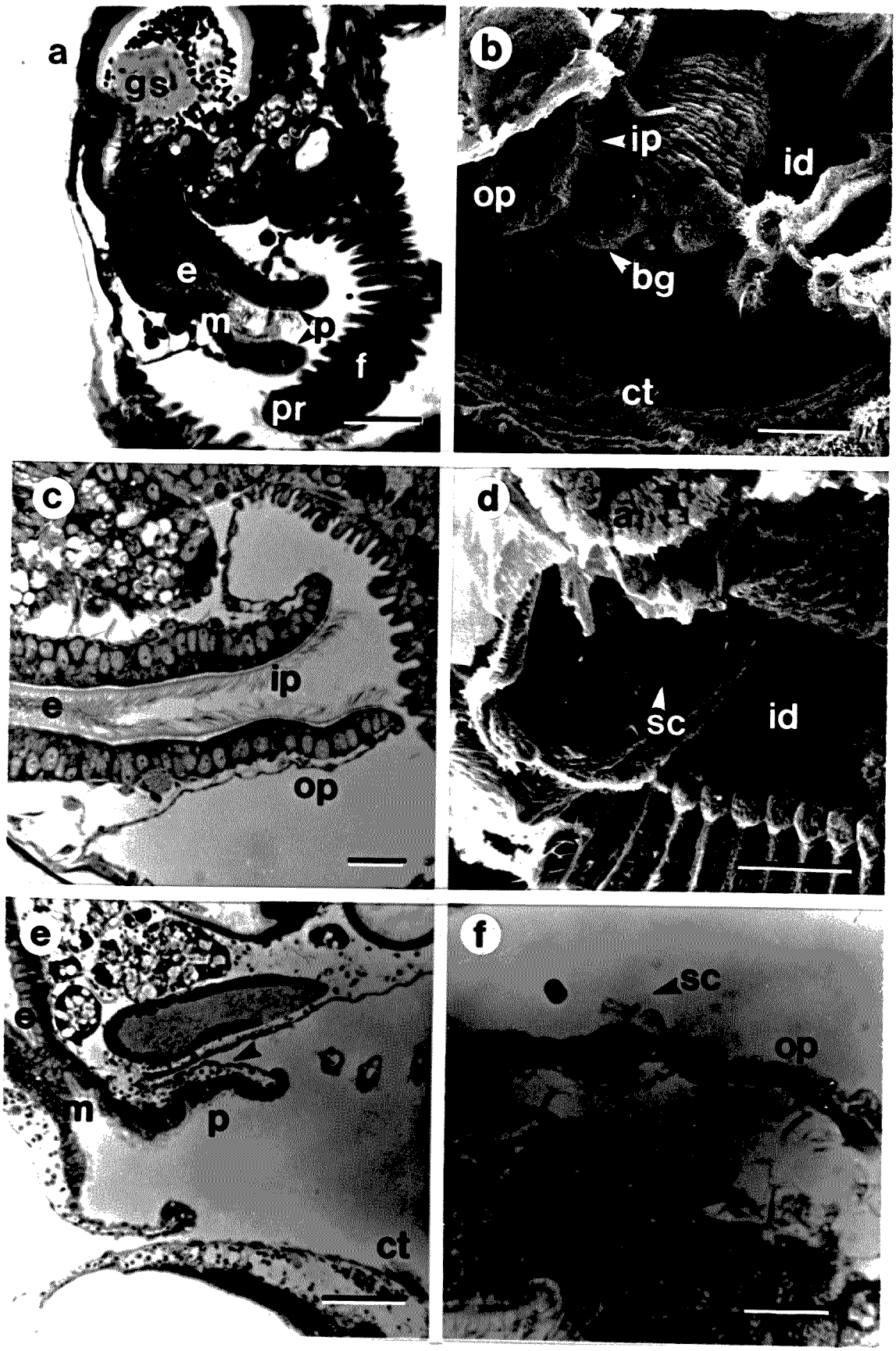
b) SEM of labial palps, foot and first filaments of right inner demibranch of juvenile approximately 600 µm shell height. Propodium of foot is inserted into labial palps. bg, byssal groove; ct, ciliated tract; id, inner demibranch; ip, inner palp surface; op, outer palp surface. Scale bar = 50 µm.

c) Histological sagittal section through labial palps of juvenile approximately 600 µm shell height. Scale bar = 20 µm.

d) SEM of trunk of foot of juvenile approximately 1500 µm shell height showing rows of possible ciliated sensory cells. a, adductor muscle; sc, sensory cell. Scale bar = 100 µm.

e) Histological sagittal section through labial palps of juvenile approximately 2000 µm shell height. Scale bar = 100 µm.

f) Detail of region indicated by arrow in 15e showing possible sensory ciliated cell located in outer palps surface. Scale bar = 10 µm.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reflection when the juveniles are approximately 500-600 µm shell height. In juveniles greater than 1000 µm shell height, the inner and outer surface of the palps begins to thicken (Fig. 15e). The ciliated inner epithelium becomes several cell layers thick and the cells increase in height. The cells of the outer epidermis become thicker and cuboidal, although the outer epidermis remains one cell layer thick. The lower palps extend slightly further into the mantle cavity than the upper, forming two lateral lobes which embrace the foot and the first ordinary filaments.

Further growth of the palps in specimens 1500 - 2000 µm shell height comprises further enlargement with increasing body size. As the palps enlarge they become thicker and the inner surface more convoluted in fixed specimens. Distinct ridging, oral grooves or distinct differentiation of arborescent lip structures, does not appear. At a size of 2000 µm shell height, the palps remain as simple ciliated tissue flaps surrounding the mouth which is a simple opening into the ciliated oesophagus. In specimens greater than 1500 µm occasional ciliated cells (presumed sensory cells) were observed at regular intervals on the smooth outer epidermis of the labial palps.

Associated with the labial palps is a distinct mantle ciliary tract (Figs. 15b, e). This extends from a position between the labial palps and the first ordinary filament to the mantle curtain on the ventral edge of the pedal gape or byssal notch. It is composed of simple ciliated cells and is approximately 20 - 25 µm wide. This tract serves to separate the infrabranchial region of the demibranchs from the peribuccal area and byssal notch.

Development of the foot

The foot is well developed at metamorphosis and reaches its maximum size, relative to the rest of the body tissues, shortly after metamorphosis (Fig. 15a). It originates from the visceral tissues anterio-ventrally of the adductor muscle, centrally between the opposing demibranchs. The bulk of the foot is comprised of muscle tissues and various glandular complexes.

The trunk of the foot extends anteriorly between the first ordinary filaments of each demibranch and the fused filaments attached to the mantle (Fig. 9c). It is highly mobile and typically extends anteriorly through the mantle cavity and ventral to the labial palps and through the byssal notch (Fig. 12a). When the foot is extended the trunk of the foot is aligned with the mantle ciliary tract associated with the labial palp complex. In fixed specimens, the foot often assumes a contracted position in the mantle cavity with the propodium of the foot inserted between the labial palps (Fig. 15b). The base of the foot is covered in dense short cilia and bisected longitudinally by the byssal groove. A tuft of long cilia extends from the tip of the propodium.

In specimens greater than 1000 µm shell height, possible sensory cells were observed on the trunk of the foot (Fig. 15d). These single distinct epithelial cells appeared similar to those observed on the outer surface of the palps. Each cell possessed a tuft of cilia consisting of 10-12 simple cilia 10 µm long, arranged in a straight row. Across the epidermis of the foot these cells are located in irregular rows approximately 10 µm apart.

During post-metamorphic development to 2000 µm shell height, the foot elongates but does not differentiate further. By 2000 µm shell height the foot is decreasing in allometric size.

DISCUSSION

Development of the ctenidia

Formation and early growth of the ctenidial filaments in *P. yessoensis* is similar to descriptions of other pectinid species and agrees with other observations for *P. yessoensis* (Sastry, 1965; Hodgson and Burke, 1988; Bower and Meyer, 1990). The degree of development and ciliation of the filaments at metamorphosis is distinctly different from *Abra alba* (Tellinacea) and *Panope abrupta* (Saxicavea) in which the filaments are well developed and fully ciliated at metamorphosis (Aabel, 1983; King, 1986). Initial morphogenesis of the ctenidia is equal, unlike that of *Ostrea edulis* in which the left ctenidia enlarges initially (Hickman and Gruffydd, 1971). Gross development of the ctenidia is similar to most other bivalves examined (*M. edulis, V. pullastrata, P. abrupta* and *A. alba*) in that new filaments are "budded off" at the distal tips of the demibranchs (Quayle, 1952; Ansell, 1962; Aabel, 1983; King, 1986).

In *Venus striatula* the first filament of the inner demibranchs differs to that found in *P. yessoensis* in that it is composed of a descending limb only and does not

reflect (Ansell; 1962). In *A. alba* the first filament does not reflect and is fused to the visceral mass along its entire length (Aabel, 1983). The fused filaments which arise along the mantle from the base of the gill axes to the palps in juvenile *P. yessoensis* appear similar to those described for *A. alba*.

These fused filaments do not possess a lateral ciliary junction with the ventral tip of the descending lamellae of the first ordinary filament. The curving of the first ordinary filament is believed to result from it being anchored to the rest of the filaments by a lateral ciliary junction on the ventral side only. The close association of the foot, which extends through the demibranchs at this point and past the labial palps, may also influence on the curvature of this filament. In live specimens, the foot was often observed displacing the first ordinary filament ventrally.

The lateral cilia of the fused filaments oppose the corresponding ciliary tract on the first ordinary filament resulting in the interfilamentary space being fully bounded by cilia. This completes the division between the supra- and infrabranchial chambers of the mantle cavity at the base of each demibranch. The fused filaments were not observed to reflect and were associated only with the inner demibranch. It is unclear how the orientation of these fused filaments corresponds to the filaments of the outer demibranchs in the later stages examined.

Reflection of the filaments follows that of other bivalve species except that the junctions of the adjacent filaments are maintained by ciliary connections and do not fuse as reported for *P. abrupta* (King, 1986). King (1986) stated that at this time the six most anterior filaments in *P. abrupta* fuse with the visceral epidermis near the base

of the foot. This was not observed in *P. yessoensis*. Yonge (1926) described the free extremities of the filaments in *O. edulis* spat as being united thin strands of transparent tissue and D'Asoro (1967) described the distal tips of the ordinary filaments in post-metamorphic *Chione cancellata* as being connected by ciliated membranes. Since these observations were both made from whole specimens, it is possible that these observations are of ciliated junctions.

Growth of the ascending lamellae occurred from a fixed point at the interfilamentary ciliary junction of the descending lamellae as described in *A. alba, V. striatula* and *P. abrupata* (Ansell, 1961; Aabel, 1983; King, 1986). Ansell (1962) stated that since this does not involve actual "bending" of the filaments that the term "reflexion" should be avoided.

As discussed by King (1986), the mode of development of the outer demibranchs was unclear in previous work. In *V. striatula* the outer demibranch arises along the supraxial extension and in *A. alba* juveniles the outer demibranch arises from the posterior region of the gill axis (Aabel, 1983; Ansell, 1962). In *P. abrupta* and *Venerupis pullastrata* the filaments of the outer demibranch originate at the dorsal ciliary axes simultaneously (King, 1986; Quayle, 1952). In *P. yessoensis* the distal ends of the ctenidia first begin to give rise to new filaments of both demibranchs from the gill axis just prior to new filaments arising dorsally along the axis. It is not clear from previous work if this is observed in other genera. Further simultaneous development of the outer demibranchs in *P. yessoensis* appears to be similar to previous observations.

In juvenile *P. yessoensis,* development of the outer demibranch occurs when the inner demibranchs are composed of 20 - 25 filaments and the juvenile has a shell height of approximately 1200 µm. This is compared to descriptions for the few other genera that have been examined in Table 4. As in other species of bivalves, development of the outer demibranchs in juvenile *P. yessoensis* does not occur until after reflection of the inner demibranch filaments has taken place. In *P. yessoensis* juveniles, the inner demibranchs possess more filaments at a smaller size prior to development of the outer demibranchs than reported for most other species.

Only certain morphological characteristics of the gill structure of post-larval *P. yessoensis* up to shell heights of approximately 2000 µm resemble those of adult scallops (Beninger *et al.*, 1991; Motavkin, 1990). In cross section, the ordinary filaments achieve the general form and ciliation of the adult structure during the initial elongation of the filaments after metamorphosis. Ciliated spurs are not present on the ordinary filaments during post-metamorphic stages. Distinct tracts of latero-frontal or pro-lateral cilia were not observed as described for *Placopecten magellanicus* or *Chlamys varia* adults (Owen and McCrae, 1976; Le Pennec *et al.*, 1988). It is not known whether this difference in the pattern of ciliation is due to the developmental stage of the juveniles, or to differences between species.

The distal tips of the ordinary filaments in juveniles are not fused laterally and are connected by ciliary junctions arising out of the lateral ciliary tracts. Principal filaments are not present in the ctenidia during the developmental period examined in this study.

Table 4. Shell size and number of filaments on inner demibranchs at point of development of outer demibranchs in juvenile bivalves.

Family	Species	Size	Filaments	Reference
Pectinidae	*Patinopecten yessoensis*	1200	20-25	Present study
Mytilidae	*Modiolaria (Musculus) laevigata*	1900	21	Stasek, 1964
Saxicavea	*Panope abrupta (generosa)*	2000	18-21	King, 1986
Veneridae	*Venus pullastrata*	1000	>12	Quayle, 1952
Scrobiculariinae	*Abra alba*	1500	10	Ansell, 1962
Veneridae	*Venus Striatula*	1000	?	Ansell, 1962

In summary, juvenile ctenidia are filibranchiate (the filaments being connected by cilia), and homorhabdic (all the filaments being composed of one type) and non-plicate. This is in direct contrast to the eulammellibranchiate, heterorhabdic and plicate ctenidia (euleutherorhabdic) of adult scallops. This morphological state reflects more primitive filibranch gill types seen in the bivalvia, exhibiting paedomorphosis as the ctenidia develops towards the eulammellibranchiate form.

Development of the peribuccal organs

Histogenesis of the labial palps from the apical plate after loss of the larval velum in post-metamorphic scallops is similar to that observed in other lamellibranch genera (Yonge, 1926; Cole, 1938; Quayle, 1952; Creek, 1960; Allen, 1961; Ansell, 1962; King, 1986).

The peribuccal organs in adult scallops are highly developed structures (Bernard, 1972; Beninger *et al.*, 1990a, b; Motavkin, 1990). In contrast the peribuccal organs of post-metamorphic *P. yessoensis* to 2000 µm shell height are relatively simple and development mainly comprises of enlargement of the ciliated palps around the mouth. The anatomy of the outer surface of the labial palps, which are not densely ciliated appears similar to the smooth surface of the labial palps in adult scallops (Beninger *et al.*, 1990a).

The ultrastructure of the cells of the upper lip of *P. yessoensis* pediveligers were described by Leask (1991), and were divided into three types; I) numerous cuboidal epithelial cells with 10-30 cilia, II) cells with 100-200 cilia and nerve like

processes at their base, and III) non-ciliated mucocytes. Beninger *et al.* (1990a, b), described three types of epithelial cells in adult *P. magellanicus* and *C. varia*, ciliated (numerous), non-ciliated, and mucocytes. Cells resembling the type II cell described by Leask (1991) were not described in adult *P. magellanicus* or *C. varia*. Observations of developing juveniles using light microscopy suggest that the epithelial cells of the buccal organs persist after metamorphosis and remain similar to the adult structures.

The distinct epithelial cells with tufts of elongated cilia observed on the smooth outer surface of the palps in juvenile *P. yessoensis* appear to be identical to putative sensory cells observed on the principal filaments of adult *P. magellanicus* (Beninger *et al.*, 1988). Beninger (1990a) described the smooth surface of the palps in adult *P. magellanicus* and *C. varia* as displaying scattered clumps of cilia, however extensive anatomical studies of the labial palps, lips and mouth of the scallop *P. magellanicus* failed to identify any sensory cells on the labial palps or lips. This observation was based on ultrastructural examination, scanning electron microscopy and Mann-Dominici staining technique (Beninger *et al.*, 1990a, b).

Morphologically distinct cells in the lower lip of mature larvae and the developing palps of immediately post-metamorphic juveniles of *P. yessoensis* express immunoreactivity to antibodies against the neurotransmitter dopamine beta-hydroxylase (Leask, 1991). These cells bear cilia that appear denser and longer than those of adjacent cells and nerve like bundles of processes were observed at their bases. Leask

(1991) proposed that these cells are chemosensory neurons that monitor incoming water conditions.

Little anatomical or neurophysiological detail of sensory structures is available for the labial palps and the peribuccal organs of bivalves. Dwivedy (1973) investigated electrophysiological properties of chemoreceptors on the labial palps of the American oyster *Crassostrea virginica.* The validity of this study which did not identify the receptor cells, has been questioned (Beninger, 1991). Recordings were taken from the smooth surface of the palps, which were subjected to distilled water rinses. The use of metal electrodes may have promoted artifact recordings. To date this has been the only study of this type performed on the peribuccal organs of any bivalve.

Development of the foot

The structure and anatomy of the foot in post-metamorphic juveniles scallops has been described for *P. yessoensis* and *Pecten maximus* (Gruffydd *et al.*, 1975; Bower and Meyer, 1990). Observations made in this study confirm previous descriptions and the ontogeny of the foot has only been described briefly.

CHAPTER 4.0: ANALYSIS OF FILTER FEEDING BEHAVIOUR IN JUVENILE *PATINOPECTEN YESSOENSIS.*

INTRODUCTION

Mechanisms of feeding in post-larval and early juvenile scallops are poorly understood. Incomplete morphogenesis of the ctenidia and pallial structures make comparisons with adult structures impossible (Beninger, 1991). Direct observations of feeding behaviour are important in understanding the processes by which particle capture occurs and by which feeding and particle selection is regulated.

Paedomorphic development has been reported for many species of bivalves which utilize pedal deposit feeding as a transitional feeding phase in post-larvae (Allen, 1961; Aabel, 1983; Reid *et al.*, 1992; King, 1986 for review). Transitory pedal feeding has been described for *P. yessoensis* post-larvae < 500 µm shell height although the relative importance of this mode of feeding is not clearly understood (Reid *et al.*, 1992).

Anterior inhalant water currents produced by the juvenile foot have been described for many juvenile bivalves examined including *P. yessoensis* (Caddy, 1969; Aabel, 1983; King, 1986). Bayne (1971) described suspension feeding in post-larval mussels *Mytilus edulis* during initial growth of the ctenidial filaments. Food particles were drawn into the mantle cavity by currents produced by the pedal cilia and then directed to the labial palps by ctenidial feeding currents. This has been referred to as

interstitial suspension feeding by Lopez and Holopainen (1987) or interstitial pedal feeding by Reid *et al.* (1992).

Previous authors have suggested that in juvenile bivalves, suspension feeding via ctenidia did not occur until formation of marginal food grooves on the ventral surface of the reflected inner demibranchs (Yonge, 1947; Allen, 1961). Orally directed ciliary currents have been described in juvenile *Venus striatula* and *Modiolaria laevigata* in which reflection of the inner demibranchs had occurred and marginal food grooves were not present (Ansell, 1962; Stasek, 1964). The ability of juvenile *P. yessoensis* to remove particles from suspension increases dramatically when they attain a shell height of approximately 600 µm, suggesting the onset of suspension feeding (Chapter 2). At this point, reflection of the 8-10 ordinary ctenidial filaments has not occurred and marginal food grooves are not present although ciliation of the filaments is well developed. The labial palps and the foot are well developed (Chapter 3).

The objectives of this study were to examine the manner in which suspended particle capture occurred in post-metamorphic *P. yessoensis* at the onset of increased filter feeding ability and to determine mechanisms regulating filtration.

Various hypotheses, based largely on indirect evidence, have attempted to explain the physical mechanisms by which lamellibranch molluscs filter particulate matter. These are generally based on the morphology of feeding structures or observed particle clearance abilities. Two models (or paradigms) describing the function of bivalve feeding structures, particle capture on the gills and transport to the

oral regions, have been described in the recent literature. These are the *mucociliary* and the *hydrodynamic* models (Jørgensen, 1990 for review). The main difference between these two models is the role of mucus in feeding. In the *mucociliary model*, particle capture relies on the secretion of mucus for the capture and transport of particles (Fankboner, 1971; Bernard, 1974; Owen and McCrae, 1976; Ansell, 1981; Le Pennec *et al.*, 1988 for examples). Lateral cilia maintain a flow of water through the mantle cavity. This water is filtered at the entrance to interfilamentary spaces by the latero-frontal cilia which strain particles from the water and sweep them onto the frontal surface of the filaments. The particles adhere to mucus secreted by gland cells on the filaments. Mucus embedded particles are then carried by the frontal ciliary tracts to the ventral or dorsal margins of the gills and then toward the peribuccal organs.

The *hydrodynamic model*, as reviewed by Jørgensen (1990), proposes that particles are retained at the interfilamentary spaces and are concentrated and carried to the mouth in suspension by water currents produced along the food grooves. Mucus is secreted by the filaments under stress or conditions of high particle concentration to cleanse the gill surface. Mucus is also secreted at the labial palps in the formation of pseudofeces (particles rejected prior to ingestion).

Regardless of the mode of feeding utilized by lamellibranchs, bivalves are able to regulate the quantity of food which is ingested and ingest or remove particles from suspension selectively (Vahl, 1972; Foster-Smith, 1975a; Wilson, 1980; Kiørboe and Møhlenberg, 1981; Newell and Jordan, 1983; Cucci *et al.*, 1985; Shumway *et al.*,

1985, 1990; Shumway and Cucci, 1987; Gallager, 1988; Ward and Targett, 1989; Bricelj and Shumway, 1991; Lesser *et al.*, 1991; Iglesias *et al.*, 1992; Stenton-Dozey and Brown, 1992; Ward *et al.*, 1992). Recent investigations by Shumway *et al.* (in prep, pers. comm.) indicate that juvenile scallops selectively remove some species of phytoplankton when presented with mixed assemblages. Direct evidence does not exist to indicate a controlling mechanism at any one organ, but indirect evidence suggests that one may be present at several locations in the feeding pathway.

The mantle edge may be capable of some particle screening. Guard tentacles on the innermost fold of the mantle margin in oysters and scallops may perform a screening function in medium to high particle concentrations (Nelson, 1938; Palmer and Williams, 1980). The mantle may also regulate feeding by altering the size and position of inhalant and exhalant openings (Beninger and Le Pennec, 1991).

Previously, the bivalve gill was believed to be the primary site of particle selection which was based on the mechanical sieving properties of the lateral and latero-frontal cilia (Dral, 1967). The lack of eurolateral and latero-frontal cilia in pectinids is believed to be responsible for a decreased ability to sieve particles < 5 - 7 µm (Vahl, 1972; Møhlenberg and Riisgård, 1978; Palmer and Williams, 1980; Cranford and Grant, 1990; Lesser *et al.*, 1991). Regulation of feeding could possibly occur from changes in the speed of the ciliary beat; the cilia may beat at a steady rate, faster, slower or stop suddenly (Aiello, 1990). Changes in the plicate structure of the gill filaments have been noted at high particle concentrations and this may affect particle selection (Owen and McCrae, 1976; Beninger, 1991; Beninger *et al.*, 1992).

Classically, the labial palps have been considered to be the structures responsible for particle sorting prior to ingestion (Nelson, 1938; Kiørboe and Møhlenberg, 1981; Newell and Jordan, 1983; Shumway *et al.*, 1985). This hypothesis has been debated because of the inability of the palps to separate particles embedded in mucus (*mucociliary model*) and because in the *hydrodynamic model* particles are transferred directly to the mouth in suspension (Beninger *et al.*, 1990a; Jørgensen, 1990; Beninger and Le Pennec, 1991). Beninger (1991) concluded that the palps are capable of accepting or rejecting only groups of mucous-bound particles and not selecting individual particles for ingestion or rejection. During rejection, the mucous string is diverted from the mouth and the mucous bound particles are expelled (pseudofeces). The lips are generally believed to be responsible for retaining strings of mucus directed towards the mouth (Beninger *et al.*, 1990).

Little attention has been given to the role of the mouth or oesophagus in regulating feeding. Visual observations of tethered hard clam larvae (*Mercenaria mercenaria*) using high speed video microscopy have shown that particle rejection may occur in the oesophagus by a characteristic sudden flexion of the oesophagus which expels particles from the mouth (Gallagher, 1988; C. Langdon, pers comm.). It is not known if a similar behaviour occurs in adult bivalves.

Previous investigations of particle capture and transport have been made on isolated gill filaments, gill fragments and intact gills of specimens with severed adductor muscles (Jørgensen, 1982). Attempts to observe feeding in undisturbed bivalves have included the use of windows in the valves, removal of the anterior

portion of the upper valve and more recently the use of video endoscopy (Bernard, 1974; Foster-Smith, 1975b; Beninger *et al.*, 1992). Difficulties in observing feeding may be partially overcome in early juvenile bivalves in which the prodissoconch (larval) and early dissoconch (juvenile) shells are semi-transparent allowing observations of normal functioning of the pallial organs (Dral, 1967; Bayne, 1971).

Analysis of normal feeding behaviour, including particle interception and ciliary function of tethered bivalve larvae, have been conducted using video microscopy (Gallager, 1988). In the present study this technique was applied to tethered *P. yessoensis* juveniles to analyze the activity of the pallial organs and particle movement within the mantle cavity through the dissoconch shell.

MATERIALS AND METHODS

Tethering and maintenance of juvenile scallops

All experiments were conducted at the Oregon State University, Hatfield Marine Science Center (HMSC), Newport Oregon. Juvenile scallops were obtained from Island Scallops Ltd., Qualicum B.C. and shipped to Newport in chilled seawater. At HMSC juvenile scallops were maintained on Nitex screens in flowing, sand-filtered seawater at 15°C.

Post-metamorphic scallops were tethered to coverglasses by pipetting several at a time onto a very thin film of fast setting epoxy glue (DEVCON 5-Minute Epoxy,

Devcon Corp. Wood Dale Il. 60191 USA) and then drawing off the seawater with an absorbent paper. When the epoxy had set (approximately 4-5 min), the coverglass was immersed in the flow-through chamber, and the specimens given time to recover from any toxic shock of the epoxy. After adhesion to the coverslips, a small percentage of juveniles were oriented such that the mantle activity could be readily observed. Normal behaviour was considered to be present when opening of the valves, ciliary activity of the gills, and no retraction of the mantle tissues was observed. Post-metamorphic scallops to approximately 700 µm shell height were used. After this size, thickening of the dissoconch shell decreased the transparency of the shell.

Observations of feeding behaviour

Feeding behaviour was observed by tethering specimens in glass flow-through chambers which could be mounted on a microscope stage. Design of this chamber was similar to the type b Perspex cuvette used by Dral (1967). A peristaltic pump with two pump heads working in opposing directions pumped seawater into one end of the chamber and equally out at the other end (Fig. 16). Sleeves were built for the microscope objective lenses from laboratory tubing with round cover glasses over the optics. This enabled immersion of the objective lenses in the flow-through chamber. Filtered seawater containing phytoplankton or artificial particles was pumped through the chambers at 2 - 4 $ml \cdot min^{-1}$ from reservoirs maintained at a constant temperature of 15°C. Further temperature control was achieved by conducting experiments in an air-conditioned room maintained at 15 - 17°C.

A black and white video camera attached to the microscope recorded observations of feeding behaviour. An electronic time code generator was used to inset a time code display (hrs:min:sec:30^{-1}sec) into each frame to provide a time reference. Individual particle pathways within the mantle cavity were traced by analyzing video footage in 30^{-1} second intervals.

Materials used in feeding observations

Seawater solutions containing both natural (phytoplankton) and artificial particles were used for observations. The following species of phytoplankton were used: Caribbean *Isochrysis sp.* (CCMP463), *Thalassiosira pseudonana* (CCMP1015), *Thalassiosira rotula* Meunier (CCMP1018), and *Prorocentrum micans* Ehr (CCMP691). Artificial particles used included tripalmatin lipid beads, ethylcellulose beads (C. Langdon in prep). Polystyrene spheres (Analychem Corp. Ltd. 721 Victoria Park Ave. Unit 16, Markham Ontario L3R 2Z8) of various sizes and colloidal graphite (Aquadag) were also used.

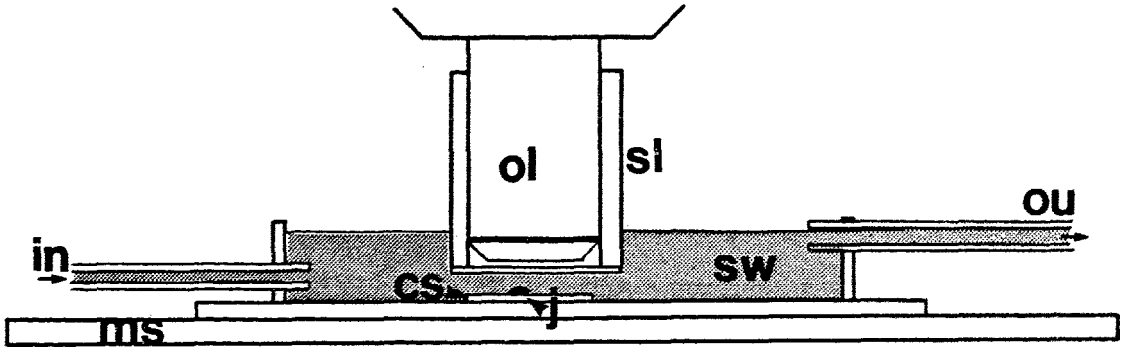


Figure 16. Schematic diagram of glass flow-through chamber used to observe feeding behaviour in juvenile *P. yessoensis*. cs, coverslip; in, inlet; j, juvenile scallop; ms, microscope stage; ol, microscope objective lens; ou, outlet; sl, objective lens sleeve; sw, seawater.

RESULTS

Focusing through the semi-transparent dissoconch shell allowed for observation of ciliary beating, movements of the filaments and labial palps, and the speed and paths of particles within the mantle cavity. Tethered juvenile scallops resumed normal feeding behaviours in the flowing seawater chambers, including extension of the mantle, ctenidia and foot and normal gaping of the shell. Scallops fixed to the coverslips by the right or left shell exhibited similar behaviour. Quantification of the number of particles accepted or rejected at each point of the feeding pathway over the entire mantle cavity was not possible because of limitations of depth of field with the microscope optics and resolution through various parts of the dissoconch shell. It was possible to trace individual particles through the mantle cavity to determine function and fluid flow patterns within the mantle cavity.

Tethered juveniles exhibited regular periodic rapid shell closure (clapping), preceded by ciliary arrest (simultaneous cessation of the cilia) which resumed as the valves opened. External disturbances, eg vibration of apparatus, also produced valve clapping.

The mantle curtains on each valve of juveniles meet along the posterior ventral edge and openings are present along the anterio-ventral and posterio-dorsal edges. These are the inhalant and exhalant openings respectively. The mantle curtains also separate at the byssal notch. Primary currents within the mantle cavity are created by the lateral and frontal cilia of the ordinary filaments. The lateral cilia beat in distinct metachronal waves moving from the base of the filaments to the distal end along the

dorsal (oral) side of the filament and from the tip to the base along the ventral (aboral) side of the filaments. Distinct metachronal waves could not be ascertained in the bands of frontal cilia.

Particles are drawn into the mantle cavity along the anterior ventral margin of the mantle into the infrabranchial chamber. Frontal cilia draw water along the length of the filaments towards the gill axis. The lateral cilia create strong currents which draw water through the interfilamentary space into the infrabranchial cavity. The net result is a flow of water and suspended particulates into the mantle cavity posterio-dorsally across the gill filaments which are then drawn through the interfilamentary spaces (Figs. 17, 18a).

Particles in suspension only come into direct contact with cilia as they move across the frontal and interfilamentary surfaces of the filaments. Once within the suprabranchial cavity, particles shift direction and flow dorsally in free suspension. Particles which enter the suprabranchial cavity near the tips of the filaments move posterio-dorsally towards the base of the gill axis. Particles which enter the suprabranchial cavity nearer the base of the filaments immediately change direction 90° and flow parallel to the gill axis dorsally.

At the base of the ctenidia, particles were observed to flow around the base of the foot and then move either anteriorly towards the labial palps, or flow dorsally between the gill axes to be rapidly rejected from the mantle cavity. Particles which were directed anteriorly moved in more random paths, the result of coming in contact with ciliated mantle surfaces. Generally particles moved between the fused filaments

Figure 17. Typical particle movement through inner demibranch filaments of juvenile *P. yessoensis* prior to being directed orally. Series of video frames following of a clump of three *Thalassiosira pseudonana* cells. View is of right inner demibranch filament viewed through right (bottom) valve. Orientation is anterior to left ventral to top. ac, algal cells; ga, gill axis; gf, gill filaments. Time codes are seconds:30^{-1}s, scale bar = 100 µm

a) Algal cells enter infrabranchial cavity and move across frontal surface of filaments posterio-dorsally.

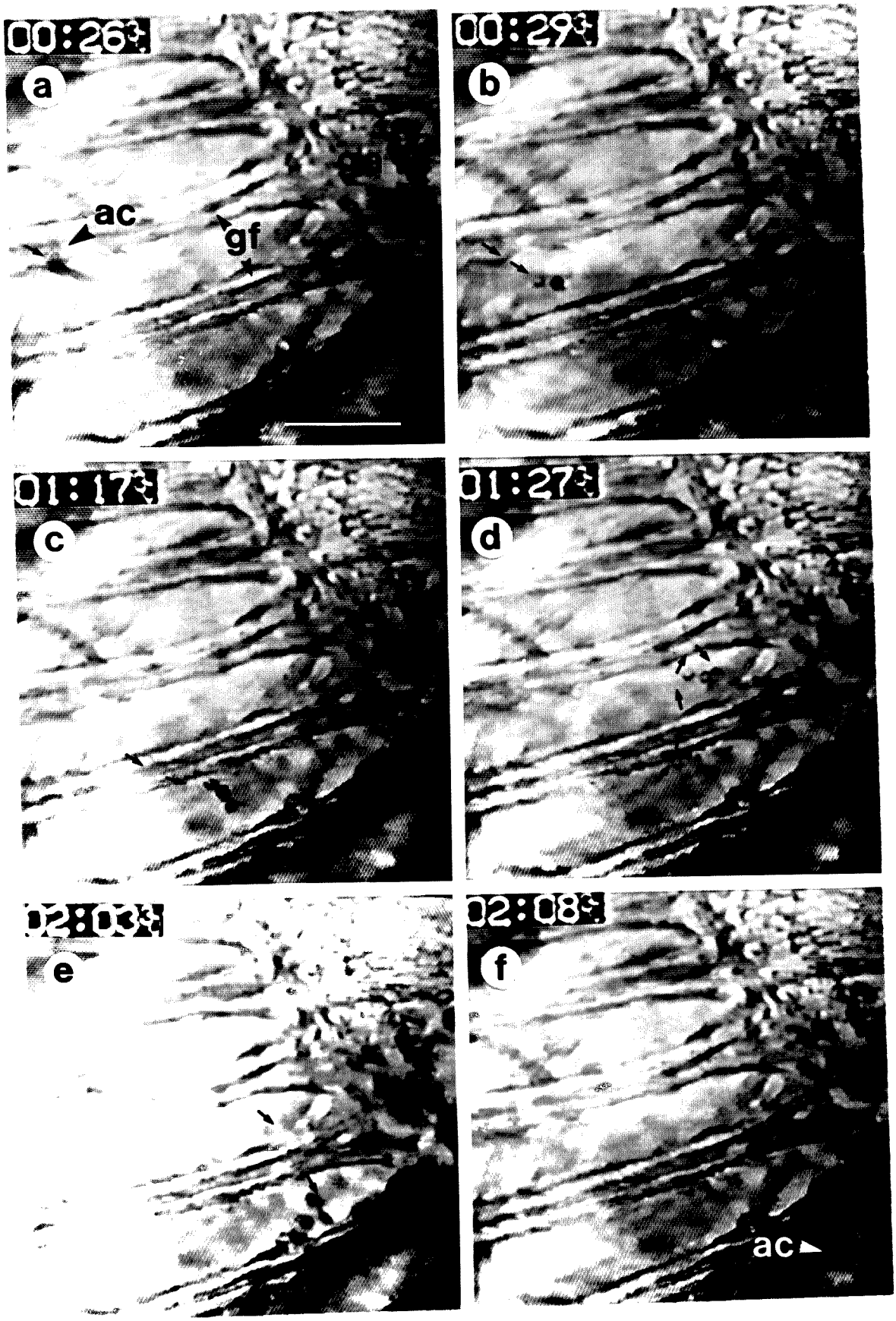
b) Cells move across to frontal surface of next filament. Time elapsed 0.1 s. Small arrows indicate path from previous frame

c) Algal cells caught in interfilamentary space by lateral cilia. Time elapsed 0.7 s.

d) Algal cells deflected by frontal cilia and move posteriorly back across filament and through interfilamentary space. Time elapsed 1.03 s

e) Algal cells in suprabranchial cavity move dorsally in suspension. Time elapsed 1.23 s.

f) Algal cells moving dorsally towards base of foot in suprabranchial cavity. Time elapsed 1.4 s.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ac
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c
01:27
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e
02:08
f
ac

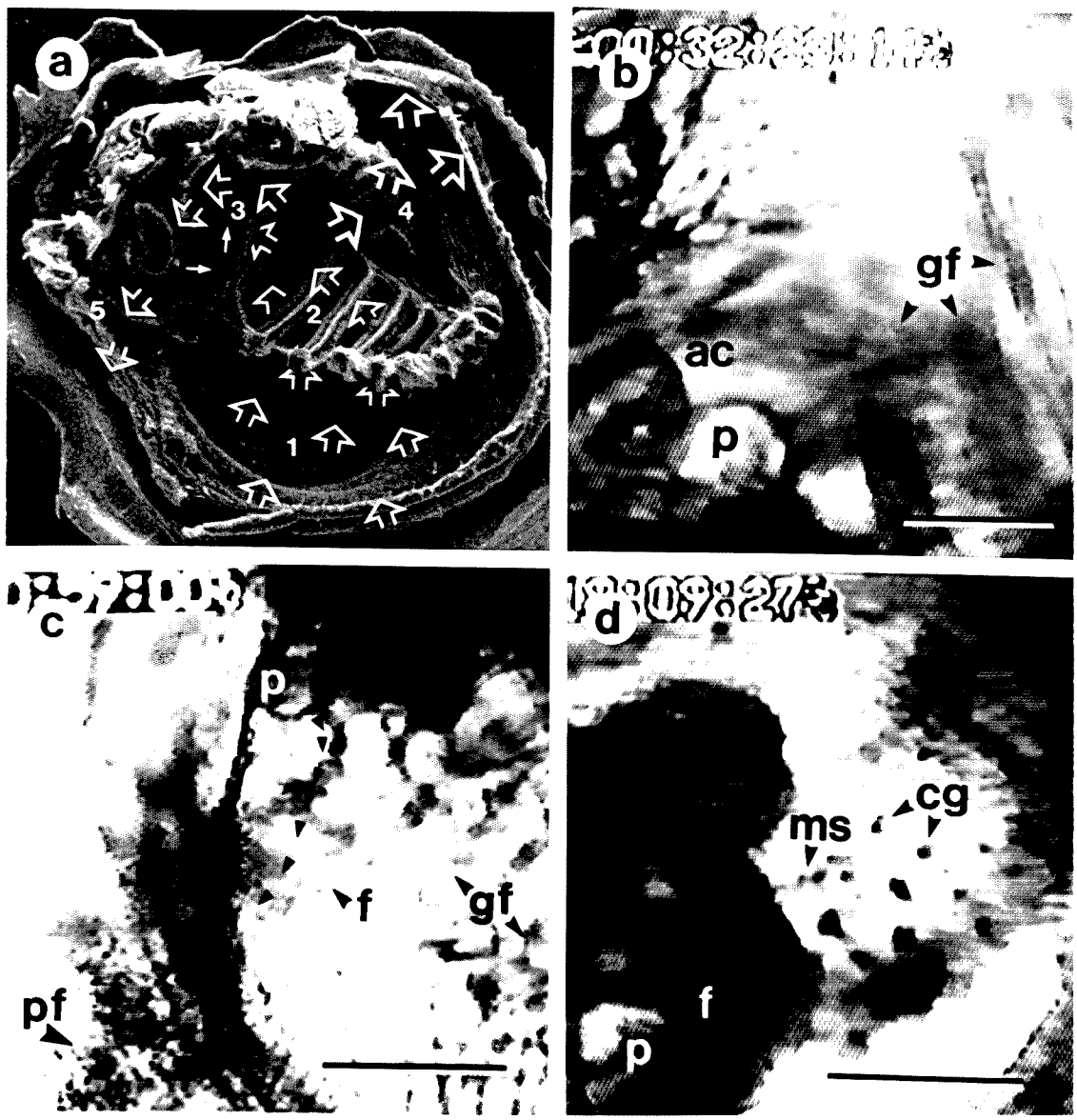
Figure 18. Patterns of feeding in juvenile *P yessoensis*.

a) SEM of juvenile approximately 600 µm shell height, left valve, mantle and demibranch removed (Fig. 10c). Arrows indicate directions of fluid and particle flow through mantle cavity. 1) Inflow into mantle cavity across anterio-ventral margin, and posterio- dorsal flow across frontal surface of filaments. 2) Dorsally directed flow within demibranchs. 3) Flow around base of foot, directed orally by fused filaments to labial palps. Recirculation in peribuccal region. 4) Suprabranchial posterio-dorsal rejection stream from mantle cavity. 5) Weak anteriorly directed stream and pseudofeces path through byssal notch along mantle ciliary tract.

b) Video frame of peribuccal region of tethered juvenile showing algal cell (C-ISO) prior to being directly ingested. View is through right (bottom) valve, orientation is anterior to top, ventral to right. ac, algal cell; gf, gill filaments; p, labial palps. Scale bar = 50 µm.

c) Video frame of peribuccal region of tethered juvenile actively producing pseudofeces (C-ISO) . View is through left (upper) valve; orientation is anterior to left, dorsal to top. Small arrows indicate path taken by algal cells from labial palps along foot and through byssal notch. f, foot; pf, pseudofeces. Scale bar = 100 µm.

d) Video frame of colloidal graphite particles on gill filaments of tethered juvenile and mucus strings being produced on the frontal surface of the demibranch. View is through left (upper) valve, orientation is anterior to bottom, dorsal to left. cg, colloidal graphite; ms, mucus string. Scale bar = 100 µm.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and were directed to or past the inner surfaces of the labial palps. The palps were observed to be highly mobile and flexed continually.

As particles came into contact with the rapidly beating cilia of the inner surfaces of the palps, they were observed to:

1) be swept into the oesophagus by cilia of the inner palp surface and ingested directly (Fig. 18b);

2) be immediately deflected back into the mantle cavity by the cilia;

3) enter the labial palps and be ejected from the palps by flexion of the palps (Fig. 19).

Particles which were returned to the mantle cavity drifted around in the region bounded by the foot, the gill axis and the visceral epidermis between the fused filaments. Random and immediate changes in particle direction indicated occasional ciliary contact, although particles were observed to be still free in suspension and not embedded in mucus. Particles in suspension in this region generally were directed in a counter clockwise rotation until they contacted the palps again or exited the mantle cavity through the strong posterio-dorsal current or from a weaker anteriorly directed stream (Fig. 17a). Particles that were free in suspension and which contacted the labial palps were also observed to be rejected in the form of pseudofeces in some specimens (Fig. 17c). Pseudofeces emerged from the palp region as clumps of phytoplankton bound in mucus which moved anterio-ventrally down the length of the foot and ciliary tracts of the mantle surface to be deposited outside the byssal notch (Fig. 17c). The speed which pseudofeces clumps were moved was much slower than

Figure 19. Particle rejection by labial palps in juvenile *P. yessoensis*. Series of video frames of peribuccal region of tethered juvenile showing ethylcellulose bead within demibranchs being directed orally to labial palps and rejected from palps by palp flexion. View is through left (upper) valve, orientation is dorsal to left, anterior to bottom. b, ethylcellulose bead; gf, gill filaments; p labial palps. Scale bar = 50 μm.

a) Bead in suspension moving dorsally about to pass behind first ordinary gill filament (curved filament).

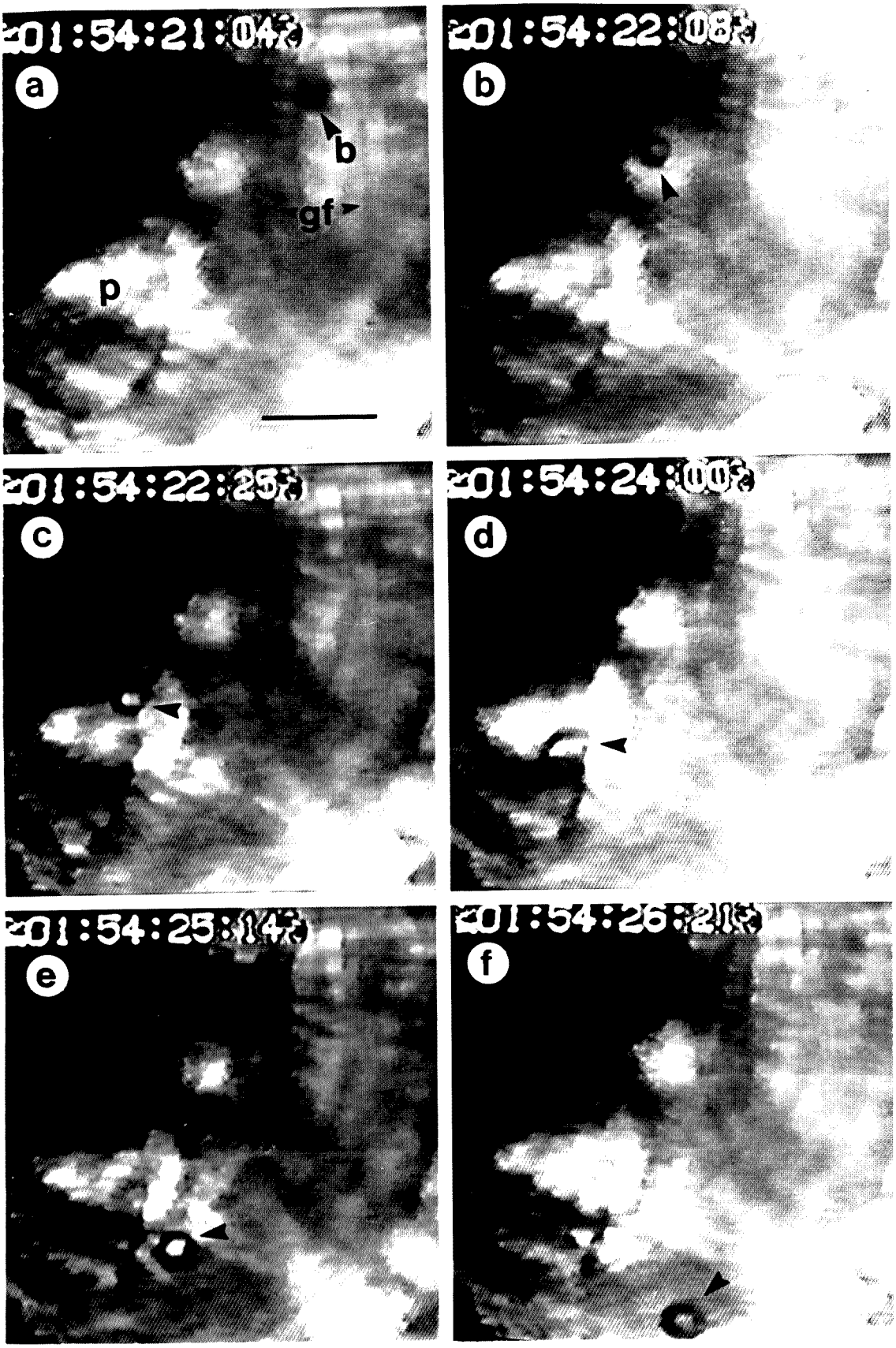
b) Bead at mantle wall between fused filaments being deflected anteriorly towards labial palps. Time elapsed 1.13 s.

c) Bead entering labial palps. Time elapsed 1.7 s.

d) Bead within palps in contact with cilia of inner palp surface. Time elapsed 2.87 s.

e) Bead being ejected from palps by flexing of palps. Time elapsed 4.33 s.

f) Ejected bead moving away from palps. Time elapsed 5.57 s.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b
gf
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b
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suspended particles and followed a specific route suggesting that they were moved directly by the cilia and not in loose suspension. During active pseudofeces production, suspended particles within the oral region were also observed to exit the mantle cavity via the posterio-dorsal exhalant stream.

Particles with diameters greater than the interfilamentary width which were drawn into the mantle cavity did not pass into the suprabranchial chamber. These included the diatom *Thalassiosira rotula,* (~25 x 20 µm, chains), the large dinoflagellate *Prorocentrum micans* (~40 x 20 µm) and artificial particles (ethyl cellulose or tripalmitan beads). In each case, these particles were moved across the frontal cilia of the filaments, but were unable to be drawn through the interfilamentary spaces. These particles were kept moving by the frontal cilia and eventually arrived at the base of the ctenidia where they circulated in the infrabranchial chamber until they were rejected. Rejection usually occurred by means of periodic valve clapping of the juvenile scallop which expelled all free contents of the mantle cavity.

The upper size limit of objects that passed through the ordinary filaments was approximately 20 µm. Particles between 15 - 20 µm passed through the filaments with difficulty and were observed to be caught in interfilamentary spaces at various points across the gill before passing into the suprabranchial cavity (Fig. 20). Short chains of diatoms longer than the interfilamentary distances moved across the frontal surfaces of the filaments randomly until they were rejected or were oriented parallel to the filaments and were able to be drawn through the filaments.

Figure 20. Particle movement of objects too large for passage through interfilamentary spaces of *P. yessoensis* juveniles. Series of video frames showing ethylcellulose bead 15 µm in diameter moving acoss frontal surface of gill filaments. View is through right (bottom) valve of tethered juvenile. Orientation is anterior to top, dorsal to left.

a) Bead drawn into mantle cavity caught in interfilamentary space in contact with lateral cilia. a, adductor muscle; b, ethylcellulose bead; f, foot; gf, gill filaments; p, labial palps. Scale bar = 100 µm.

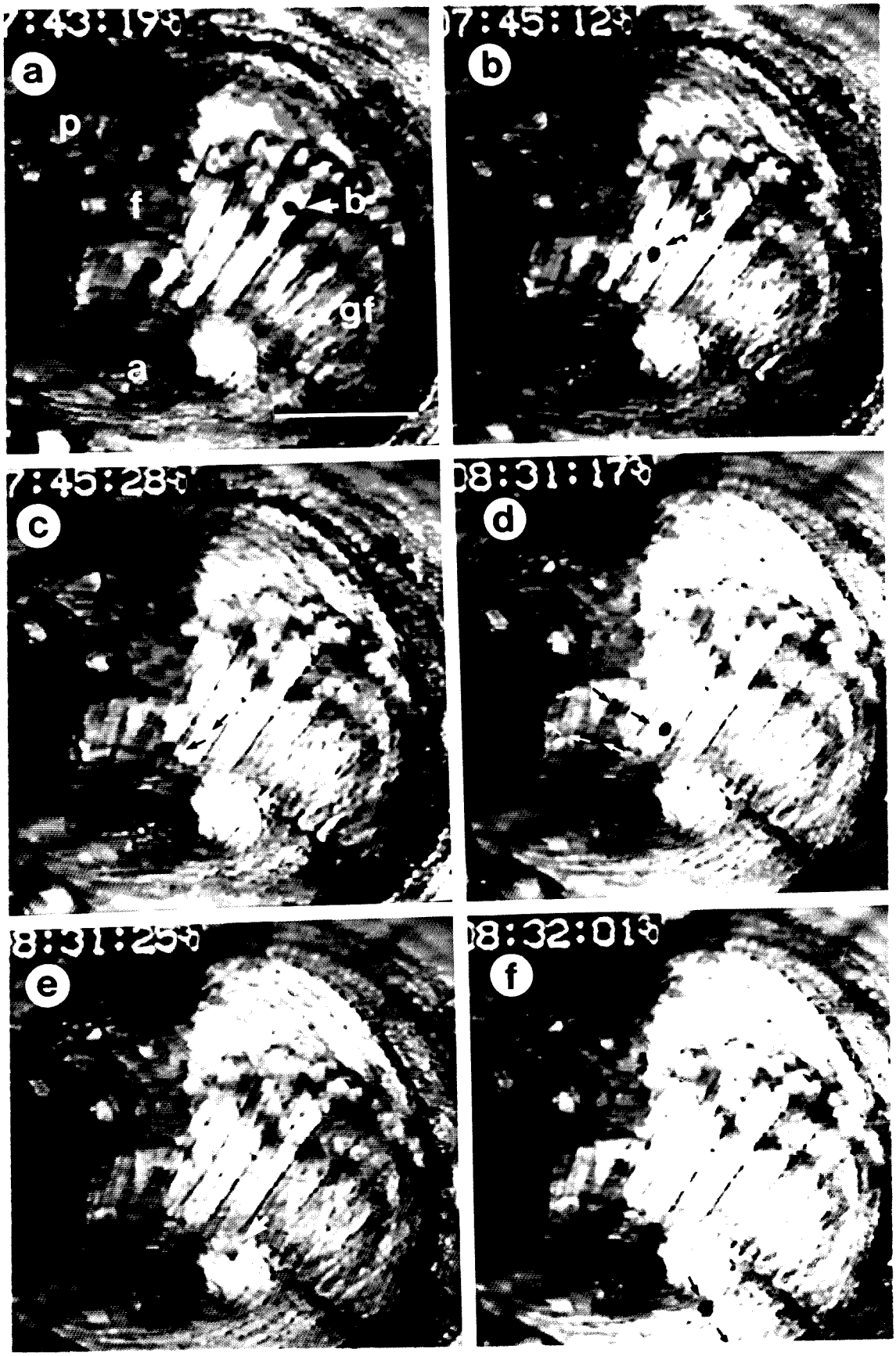
b) Bead breaks away and is moved posterio-dorsally across frontal surface of demibranchs. Small arrows indicate particle path from previous frame. Time elapsed 1.77 s.

c) Bead at base of gill filaments moves dorsally across frontal surface of gill axis. Time elapsed 2.09 s.

d) Bead at dorsal base of demibranch and gill axis in contact with cilia circulates randomly prior to moving ventrally across frontal surface of filaments and becomes caught in interfilamentary space. Time elapsed 47.93 s.

e) Bead moves through interfilamentary space into suprabranchial cavity and into posterior directed exhalant stream ventral to the adductor muscle. Arrow indicates position of bead below gill axis of right demibranch. Time elapsed 48.2 s.

f) Bead in exhalant stream prior to exiting mantle cavity. Time elapsed 48.4 s.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The response of the juvenile scallop filtering apparatus to colloidal graphite in solution was distinctly different to that of other particle types. Colloidal graphite particles which contacted the frontal cilia after being drawn into the mantle cavity became ensnared on the frontal surfaces of the filaments and mucus production on the filaments was observed immediately (Fig. 17d). As more colloidal graphite was drawn into the mantle cavity, the frontal surfaces of the filaments became increasingly fouled with mucus and colloidal graphite. Colloidal graphite which did not contact the frontal cilia passed directly in suspension into the infrabranchial cavity and moved orally. Particles which became bound in mucus were not moved in a distinct acceptance or rejection tract. Some mucus clumps were observed to break off and were ejected from the mantle via valve clapping or were randomly moved dorsally across the frontal surface of the filaments.

DISCUSSION

Juvenile *P. yessoensis* can remove particles from suspension prior to the development of the outer demibranchs, the reflection of ordinary filaments or the presence of marginal food grooves. The lateral cilia of juvenile scallops beat in distinct metachronal waves. This results in constant water currents through the interfilamentary spaces produced by the oscillatory movements of the enveloping surface of the ciliary band (Jørgensen, 1982). Juvenile scallops showed extreme sensitivity in ciliary control and were capable of undergoing immediate ciliary arrest in response to external disturbances as was observed in the latero-frontal cilia of juvenile

Mytilus edulis (Dral, 1967). Natural and artificial particles entering the mantle cavity moved through the interfilamentary spaces into the suprabranchial cavity via the through currents and were not retained on the ordinary filaments. Particles arrived at the labial palps free in suspension where they were ingested or rejected. Particles were not observed to be passed dorsally in a dorsal ciliated tract as described by Reid *et al.* (1992). This mode of feeding is similar to that described by Bayne (1971) for post-metamorphic *Mytilus edulis* except that the primary feeding currents were created by the lateral cilia of the ordinary filaments and not by the cilia of the foot. Orally directed ciliary currents produced by the foot may occur during pedal feeding in post-metamorphic *P. yessoensis*. In tethered individuals, the foot was also able to aid in rejection of pseudofeces carried in ciliated tracts.

Mucus did not play an active role during normal particle capture. Observations with colloidal graphite indicated that the ordinary filaments were capable of producing mucus in response to particles that were caught on the frontal surfaces of the filaments. Since this was only observed with the graphite particles, it is believed that this was an unnatural response to a foreign substance. Particles rejected from the palp region embedded in mucus as pseudofeces suggested that mucus is produced normally by the labial palps and oesophagus. This may occur only during active production of pseudofeces. Phytoplankton and artificial particles were rejected from the surface of the palps (by cilia of the inner palp surface) and from the mouth-oesophagus (by palp flexion) as free suspended particles.

Ordinary filaments in early juvenile *P. yessoensis* differ from juvenile mussels in that they do not possess latero-frontal cilia, yet they are capable of clearing and ingesting particles less than 7 µm diameter (Chapter 2). Seven micrometers is believed to be the critical size limit for particle retention in adult pectinids lacking laterofrontal cilia (Møhlenberg and Riisgård, 1978). Based on hydrodynamic principles, it has been proposed that the function of the laterofrontal cilia in bivalve gills is not particle retention (seiving), but they act in conjunction with the frontal cilia to produce water currents along the surface of the gill filaments (Jørgensen, 1981). In this theory, particle retention occurs via velocity gradients and larger forms of laterofrontal cilia will result in greater efficiency of particles being retained by the frontal cilia. Post-metamorphic scallop gill structures were not observed to retain particles on the frontal surface of the gills. This suggests that critical minimum size limits for particle retention are independent of the presence or absence of the laterofrontal cilia on the gills in early juvenile scallops. The absence of shear forces created by laterofrontal cilia result in particles being passed through the interfilimentary spaces without being deflected towards frontal tracts. In isolated gill filaments of *Mytilus edulis*, in which the laterofrontal cilia had been arrested with serotonin, particles were observed to disappear between the filaments to be carried away with the interfilamentary currents (Jørgensen, 1982). The tendency to pass through the interfilamentary spaces without being affected by lateral cilia increased with decreasing particle size. In his study, Jørgensen (1982) noted that when the

laterofrontal cilia were inactive, currents produced by the recovery phase of the lateral metachronal wave extended as much as 50 μm above the frontal surface of the gill.

The potential for regulating feeding and size selective clearance during this mode of feeding appears to exist at several levels within the juveniles. This is accomplished either by directing fluid flow within the mantle cavity or acting directly on individual particles. As observed in adult bivalves, the mantle currents may be affected by altering the size of the exhalant or exhalant openings. When the valves are held further apart, separation of the gill axes is increased thereby enlarging the posteriorly directed exhalant (rejection) stream and causing more particles to exit the mantle cavity before being directed towards the palps. Conversely valve closure would direct more particles orally.

The foot is the largest and most mobile organ in the early juvenile scallop and may also play an important role in altering flow patterns within the mantle cavity. It arises directly between the inner demibranchs and extends across the labial palps. The propodium was often observed being inserted into the labial palps effectively inhibiting ingestion. By being retracted into the mantle cavity anteriorly of the palps, the foot may cause more particles to be recirculated back towards the palps. When the foot is extended anteriorly from the mantle cavity, inhalant ciliary currents direct seawater into the mantle cavity (Reid *et al.*, 1992). In addition to this function, reversal of the ciliary current by the foot assists in rejection of particles and pseudofeces from the mantle cavity. Bower and Meyer (1990) postulated that the foot may aid in feeding by transferring food particles caught on the gills to the mouth.

This does not appear to be a normal feeding behaviour. The foot was occasionally observed to sweep across or probe the demibranchs and it may serve to perform a cleaning function.

The width of the interfilamentary space limits the size of particles which enter the orally directed infrabranchial stream. The interfilamentary widths remained constant between 20 - 25 µm. Variations in the width in response to different particle concentrations were not observed as described for juvenile *Mytilus edulis* (Dral, 1967). In *M. edulis* it is believed that the oscillating currents of the lateral cilia block the interfilimentary passageway to about the level of the ciliary tips during the effective stroke, leaving 10 - 15 µm for the through current (Jørgensen, 1982). In *P. yessoensis,* 15 - 20 µm particles appeared to be at the upper limit for through particle passage. This is approximately equal to the diameter of the oesophagus. Therefore the filaments reduce passage of particles to those which are capable of being ingested.

The labial palps are the final and most active of the feeding organs which may exert control over ingestion. Particles may be ingested directly, deflected from the inner surface of the palps or rejected from the mouth/oesophagus via palp flexion. This suggests that the palps may perform a direct or indirect sorting function controlling particle ingestion. Interception of particles arriving free in suspension at the palp cilia may be affected by size, shape, specific gravity or electrostatic surface charge of the particle (Rubenstein and Koehl, 1977; Jørgensen, 1983a; Gallager, 1988; Gallager *et al.*, 1988; Solow and Gallager, 1990). The presence of spines may also

affect particle capture as copepods have been observed to treat spined diatoms as if they were larger particles (Gifford *et al.*, 1981).

M. mercenaria veligers increase rejection of phytoplankton cells from the oesophagus when gut satiation is achieved (Gallager, 1988). Post-metamorphic scallops retain many of the characteristics of the larval alimentary system and this is a plausible explanation for direct rejection. Rejection or acceptance based on biochemical properties of the captured particle, as discussed by Gallagher (1988), requires the presence of biochemical receptors within the peribuccal organs. These have not been found by histological methods in adult or large juvenile pectinids (Beninger *et al.*, 1990a, b, 1991; Beninger, 1991). Leask (1991), using immunochemical staining techniques, detected possible biochemical sensory cells in the lip of mature *P. yessoensis* larvae. The role of these cells in feeding is not known or if these persist in the oesophagus after metamorphosis. Further investigation is required to quantify particle selection or rejection at the palps and to determine whether selection is actively controlled by the juvenile or if it is the result of indirect forces.

The *hydrodynamic* and *mucociliary* suspension feeding models both describe captured particles being moved in frontal tracts of the gill filaments. As this was not observed to occur in post-metamorphic scallops, the observed mode of feeding behaviour represents a unique hydrodynamic mode of suspension feeding which serves to bridge pedal feeding immediately after metamorphosis with development of more efficient adult feeding structures. Recent observations of near-natural suspension

feeding in adult *Placopecten magellanicus* indicate particles are retained at the gill and moved dorsally along the frontal surfaces of the principal filaments. Particles in a mucus slurry are then transported hydrodynamically in dorsal ciliated tracts to the palps (Ward *et al.*, 1991; Beninger *et al.*, 1992). Visual observations through the dissoconch of early juvenile *P. yessoensis* are limited in specimens greater than 700 µm shell height and it is unknown at what point during development particle retention and transport on the frontal surfaces of the filaments occurs. Presumably this may not be until development of the outer demibranchs is completed and/or the morphogenesis of principal filaments and the plicate structure of the adult gill is achieved.

CHAPTER 5. CONCLUSIONS

Post-metamorphic *Patinopecten yessoensis* exhibited low particle clearance rates for all species of phytoplankton tested through shell heights to approximately 400 µm. At shell heights of approximately 600 µm, particle clearance rates increased dramatically indicating initiation of efficient filter feeding ability. The ability of juvenile scallops to capture small particle sizes differed from adult scallops which are inefficient at capturing particles less than 7 µm in diameter. Juvenile scallops were capable of ingesting artificial particles between 2 µm and 9 µm in diameter, and cleared the small diatom *Chaetoceros calcitrans* from suspension. Particles 23 µm in diameter and larger were not ingested by juveniles. Particle clearance rates increased slightly in juveniles 600 - 1000 µm shell height and logarithmically from a size of 1000 to 2000 µm shell height. Rates of clearance typically increased from densities of 10,000 phytoplankton cells·mL^{-1} to maxima between 30 and 40,000 cells·mL^{-1} for all juvenile sizes examined.

Post-metamorphic growth of *P. yessoensis* juveniles is marked by the overall growth of the dissoconch shell and the morphogenesis of simple gill structures from larval primordia. Initial ctenidial growth is in number and length of the descending lamellae of ordinary filaments. Ciliation patterns of the filaments are achieved during initial development, with the filaments possessing only lateral and frontal tracts of cilia. Lateral ciliary junctions connect the distal ends of the descending lamellae.

During this period the labial palps enlarge and extend into the mantle cavity as extensions of the larval mouth apparatus. The labial palps form a ciliated hood around the mouth embracing the foot and in close association with the first gill filaments. It is during this period that the highly mobile foot reaches its greatest allometric size.

The ordinary filaments achieve their maximum length prior to reflection when juveniles are approximately 600 µm shell height when there are 8-9 filaments on each inner demibranch. Simultaneously the opposing filaments of each inner demibranch mesh via junctional cilia on the frontal surface of the distal ends to separate the branchial cavities. Observations of fluid flow and particle capture within the mantle cavity indicate that at this stage the metachronal beating of the lateral cilia produce a coordinated flow of water within the mantle cavity. Suspended particles drawn into the mantle cavity pass through the interfilamentary spaces and are directed orally where they are ingested by interception with the mobile labial palps or rejected from the mantle cavity. Behavioural and morphological observations indicate that the width of the interfilamentary spaces restricts juveniles from capturing particles less than 15 - 20 µm in diameter.

Continued growth and development of the gill structures involves increases in the size and complexity of the demibranchs and the labial palps. The reflection of the ascending lamellae of the inner demibranchs effectively increases the surface area of the inner demibranchs. Formation of the outer demibranchs occurs when juveniles are approximately 1000 - 1200 µm shell height when there are 20 - 25 filaments on each inner demibranch. This results in further increases in the surface area of the

demibranchs, increasing particle clearance abilities. By 2000 µm shell height, *P. yessoensis* juveniles possess more than 35 - 40 fully reflected ordinary filaments on each inner and outer demibranch. Although the ordinary filaments resemble the ordinary filaments of adult scallops, principal filaments are not present and the juvenile ctenidia remain distinctly different from adult structures. The juvenile gill is filibranchiate (the filaments being connected by cilia), and homorhabdic (all the filaments being composed of one type) and non-plicate.

When juveniles are approximately 600 µm shell height stage (8 - 9 pairs of inner demibranch filaments) which is approximately 3 - 4 weeks post-settlement it is a critical point in post-metamorphic growth, and a period of high mortality in nursery rearing (Ó Foighil *et al.* 1990). This point of initial coordination of the filaments and functioning of the feeding organs was associated with the sudden increase or initiation of particle clearance ability. Ó Foighil *et al.*, (1990) noted that growth rates increased for *P. yessoensis* juveniles after 3 - 4 weeks in nursery culture. Similar results were obtained for the rock scallop *Crassadoma gigantea* in nursery culture (Whyte *et al.*, 1992).

Post-metamorphic rock scallops showed a 58.3% decline in total energy during the first 25 days of post-metamorphic development (Whyte *et al.*, 1992). Similar results have been noted for *P. yessoensis* (Whyte, unpublished data, pers comm 1993). Whyte *et al.* (1992) concluded that; "energy reduction from unchecked shell formation during post-metamorphic growth of an early juvenile with insufficient energy remaining after metamorphosis would lead quickly to total energy exhaustion and

death". Observations made during this investigation concur with the energy decline noted by Whyte *et al.* (1992), suggesting that increased mortality during early post-metamorphic growth is the result of juvenile scallops being unable to acquire sufficient energy to overcome the metabolic demands of early growth.

Survival through this critical phase requires that energy reserves sequestered during larval growth are sufficient to support post-metamorphic growth until functional feeding structures develop. Increased lipid levels have been suggested as an index of bivalve larval viability (Gallager *et al.*, 1986). Lipid levels have been noted to increase dramatically in the third and fourth weeks of pelagic larval life of *Placopecten magellanicus, C. gigantea* and *P. yessoensis* (Manning, 1986; Whyte *et al.* 1987, 1992). Further research into methods to increase energy levels prior to metamorphosis is required to enable survival through the first month of post-metamorphic growth during ctenidial development.

The decline in post-metamorphic energy content is shorter in oysters in which development of feeding apparatus is faster than scallops. *Ostrea edulis* juveniles show a decrease in energy during the first four days after metamorphosis (Holland and Spencer, 1973). It is believed that oyster gill structures become effective at a much earlier stage than scallops (Yonge, 1926; Hickman and Gruffydd, 1971). Presumably this is necessary in the absence of a well developed juvenile foot which may engage in pedal feeding.

Trophic routes of energy acquisition other than the capture of suspended particles may also be important during the early post-metamorphic development of

scallops; these are pedal feeding and the uptake of dissolved organic matter (DOM). Pedal feeding is important in juvenile clams (King, 1986; Reid *et al.*, 1992). The juvenile scallop foot reaches its greatest allometric size immediately post-metamorphosis and pedal feeding behaviour has been observed in *P. yessoensis* juveniles by Reid *et al.* (1992). Juvenile scallops show a preference for substrates with epifaunal growth (Ó Foighil *et al.*, 1990). At present the importance of pedal feeding in energy acquisition is poorly understood. Qualification of optimal substrates, or quantification of feeding on these substrates has not been investigated.

Bivalve tissues are capable of the uptake of DOM and these may be nutritionally significant (Wright, 1982; Jørgensen, 1983b). Uptake of free dissolved amino acids by the lecitrophic larvae of the abalone *Haliotis rufescens* have been estimated to account for 39 - 70% of the metabolic energy demand during development (Jaeckle and Manahan, 1989). Autoradiographic studies have detected the uptake of [^{3}H]glycine in the developing gill filaments of post-metamorphic oysters *Crassostrea gigas* and the scallop *Pecten maximus* (Manahan and Crisp, 1983).

The contribution of pedal feeding and DOM during post-metamorphic growth remains unknown although during this critical period, all possible contributions to the total energy requirements of the juvenile may be important in determining its success. Increasing survival of post-metamorphic scallops in commercial nursery culture will require that energy reserves accumulated during larval growth are maximized to carry post-metamorphic development through initial ctenidial development. Phytoplankton diets of high nutritional content must be made available to early juveniles in order that

maximum benefit may be achieved from the low numbers of cells captured by the developing ctenidia. Further research may determine means to supplement energy uptake through pedal feeding or dissolved organic materials.

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