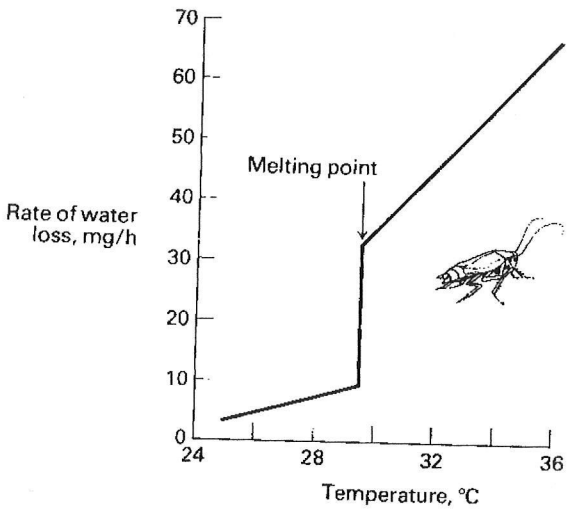
UNIVERSITE DE NANTES **FACULTE DES SCIENCES**

M1 TPE BE

ECOPHYSIOLOGIE ANIMALE ET ETHOLOGIE

Illustrations cours écophysiologie

Prof. P. G. Beninger



ÉCOPHYSIOLOGIE ANIMALE ET ÉTHOLOGIE

Contenu des CM

Écophysiologie

- Le champ de l'écophysiologie ; adaptation et écophysiologie
- Réponses comportementales vs. Réponses physiologiques
- Le raisonnement écophysiologique par des exemples :

♦ le problème de l'osmolarité

- la solution des animaux marins et terrestres
- le problème de l'eau douce
- le cas des Chondricthéens et des Osteichthéens
- les anadromes-catadromes

♦ la tolérance au gel – les réponses graduées d'une adaptation générale : mécanismes biochimiques, relation salinité – osmolarité - gel

♦ écophysiologie de l'alimentation

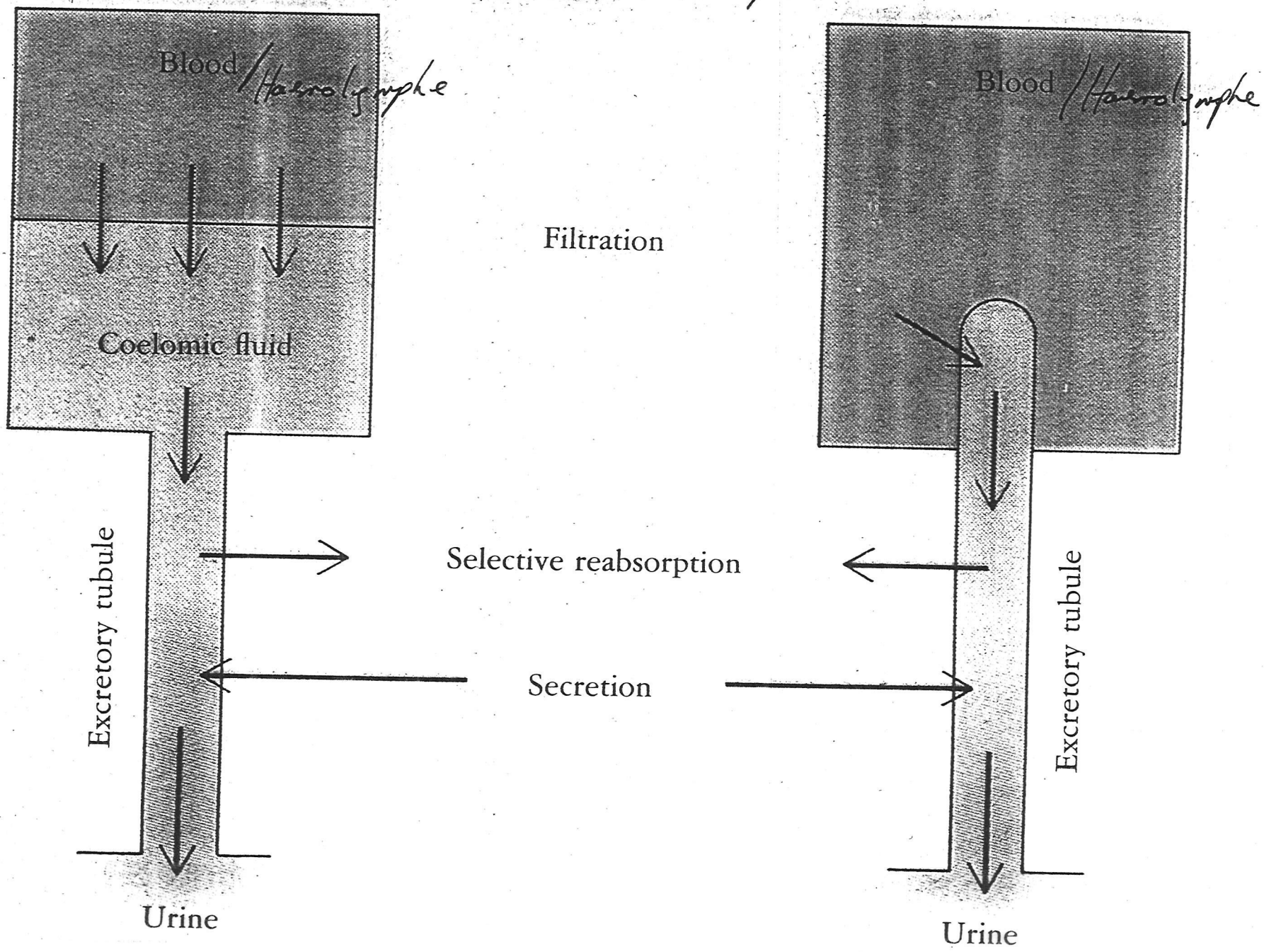
- la prédation – refuge de taille, conséquenceS sur l'Évolution des métazoaires
- l'exploitation optimale des proies

- les suspensivores – les approches 'boîte noire', indices de production ; les approches 'mécanismiques' : de la configuration des structures alimentaires aux mécanismes de régulation
- écophysiologie de la compétition alimentaire
- écophysiologie des espèces invasives

Références :

Beninger, P.G. Ecophysiologie animale – Illustrations du cours et références primaires y citées.

Figure 16.3 Model of two principal types of excretory organ[illegible] - Metanephridies



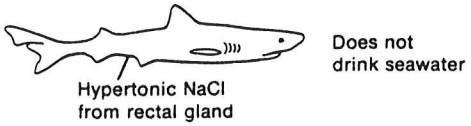
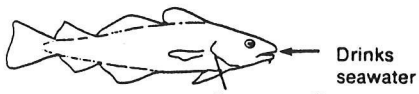
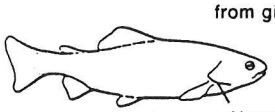
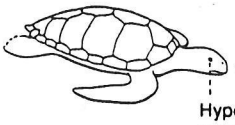
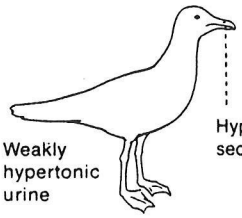
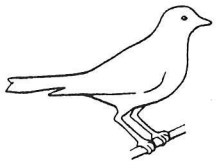
Composition of extracellular fluids of representative animals (concentrations in millimoles per liter of H_2O).

	Habitat†	Milliosmoles	$[Na^+]$	$[K^+]$	$[Ca^{2+}]$	$[Mg^{2+}]$	$[Cl^-]$	$[SO_4^{2-}]$	$[HPO_4^{2-}]$	Urea
Seawater‡		1000	460	10	10	53	540	27		
Coelenterata										
Aurelia (jellyfish)	SW		454	10.2	9.7	51.0	554	14.6		
Echinodermata										
Asterias (starfish)	SW		428	9.5	11.7	49.2	487	26.7		
Annelida										
Arenicola (lugworm)	SW		459	10.1	10.0	52.4	537	24.4		
Lumbricus (earthworm)	Ter.		76	4.0	2.9	—	43	—		
Mollusca										
Aplysia (sea slug)	SW		492	9.7	13.3	49	543	28.2		
Loligo (squid)	SW		419	20.6	11.3	51.6	522	6.9		
Anodonta (clam)	FW		15.6	0.49	8.4	0.19	11.7	0.73		
Crustacea										
Cambarus (crayfish)	FW		146	3.9	8.1	4.3	139	—		
Homarus (lobster)	SW		472	10.0	15.6	6.7	470			
Insecta										
Locusta	Ter.		60	12	17	25				
Periplaneta (cockroach)	Ter.		161	7.9	4.0	5.6	144			
Cyclostomata										
Eptatretus (hagfish)	SW	1002	554	6.8	8.8	23.4	532	1.7	2.1	3
Lampetra (lamprey)	FW	248	120	3.2	1.9	2.1	96	2.7	—	0.4
Chondrichthyes										
Dogfish shark	SW	1075	269	4.3	3.2	1.1	258	1	1.1	376
Carcharhinus (freshwater shark)	FW	—	200	8	3	2	180	0.5	4.0	132
Coelacantha										
Latimeria	SW	—	181	51.3	6.9	28.7	199	—	—	355
Teleostei										
Paralichthys (flounder)	SW	337	180	4	3	1	160	0.2	—	—
Carassius (goldfish)	FW	293	142	2	6	3	107	—	—	
Amphibia										
Rana esculenta (frog)	FW	210	92	3	2.3	1.6	70	—	—	2
Rana cancrivora	FW	290	125	9	—	—	98	—	—	40
	80% SW	830	252	14	—	—	227	—	—	350
Reptilia										
Alligator	FW	278	140	3.6	5.1	3.0	111	—	—	—
Aves										
Anas (duck)	FW	294	138	3.1	2.4		103	—	1.6	—
Mammalia										
Homo sapiens	Ter.		142	4.0	5.0	2.0	104	1		
Lab rat	Ter.		145	6.2	3.1	1.6	116	—		

†SW = seawater; FW = fresh water; Ter. = terrestrial.

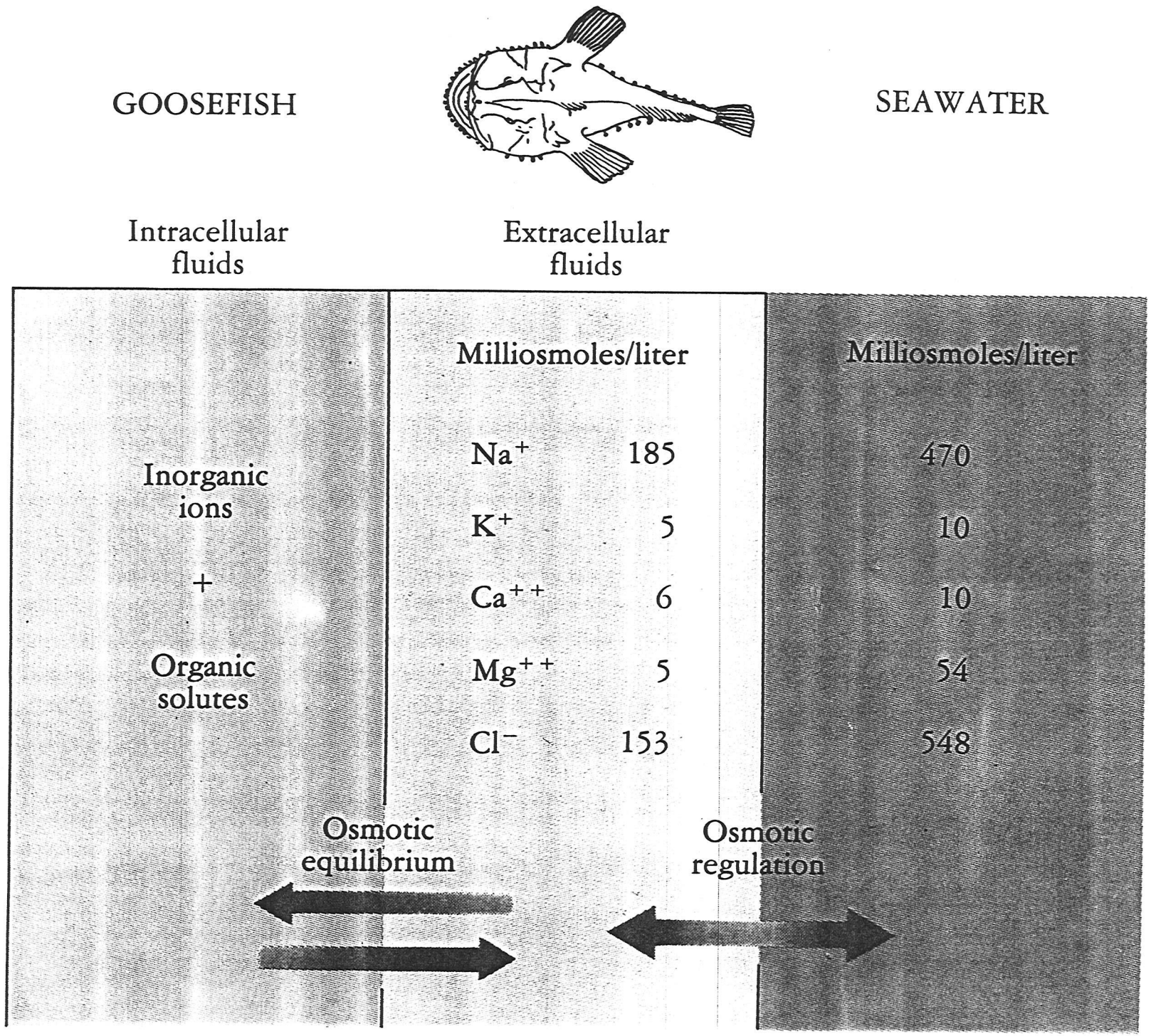
‡The osmolarity and composition of seawater vary, and the values given here are not intended to be absolute. The composition of body fluids of osmoconformers will also vary, depending on the composition of the seawater they are tested in.

Sources: Schmidt-Nielsen and Mackay, 1972; Prosser, 1973.

	Blood concentration relative to environment	Urine concentration relative to blood	
Marine elasmobranch	Isotonic	Isotonic	 Does not drink seawater Hypertonic NaCl from rectal gland
Marine teleost	Hypotonic	Isotonic	 Drinks seawater Secretes salt from gills
Freshwater teleost	Hypertonic	Strongly hypotonic	 Drinks no water Absorbs salt with gills
Amphibian	Hypertonic	Strongly hypotonic	 Absorbs salts through skin
Marine reptile	Hypotonic	Isotonic	 Drinks seawater Hypertonic salt-gland secretion
Desert mammal	—	Strongly hypertonic	 Drinks, no water Depends on metabolic water
Marine mammal	Hypotonic	Strongly hypertonic	 Does not drink seawater
Marine bird	—	Weakly hypertonic	 Drinks seawater Hypertonic salt-gland secretion Weakly hypertonic urine
Terrestrial bird	—	Weakly hypertonic	 Drinks fresh water

Exchange of water and salt in some vertebrates. Only active exchange is indicated; passive loss of water through the skin, lungs, and alimentary tract is not indicated.

Figure 16.1 Ionic content of a goose fish compared to sea water



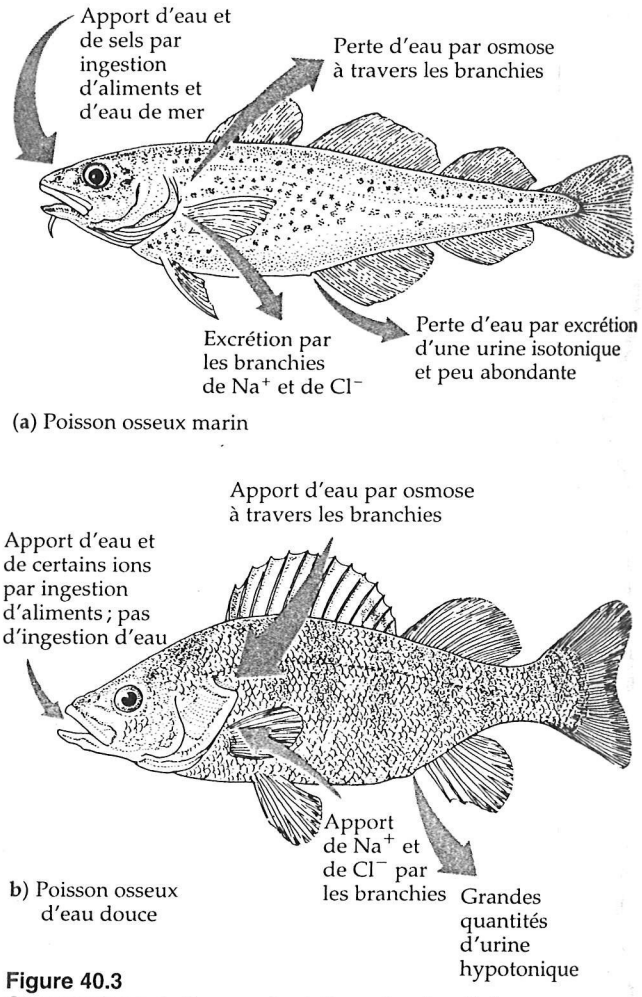
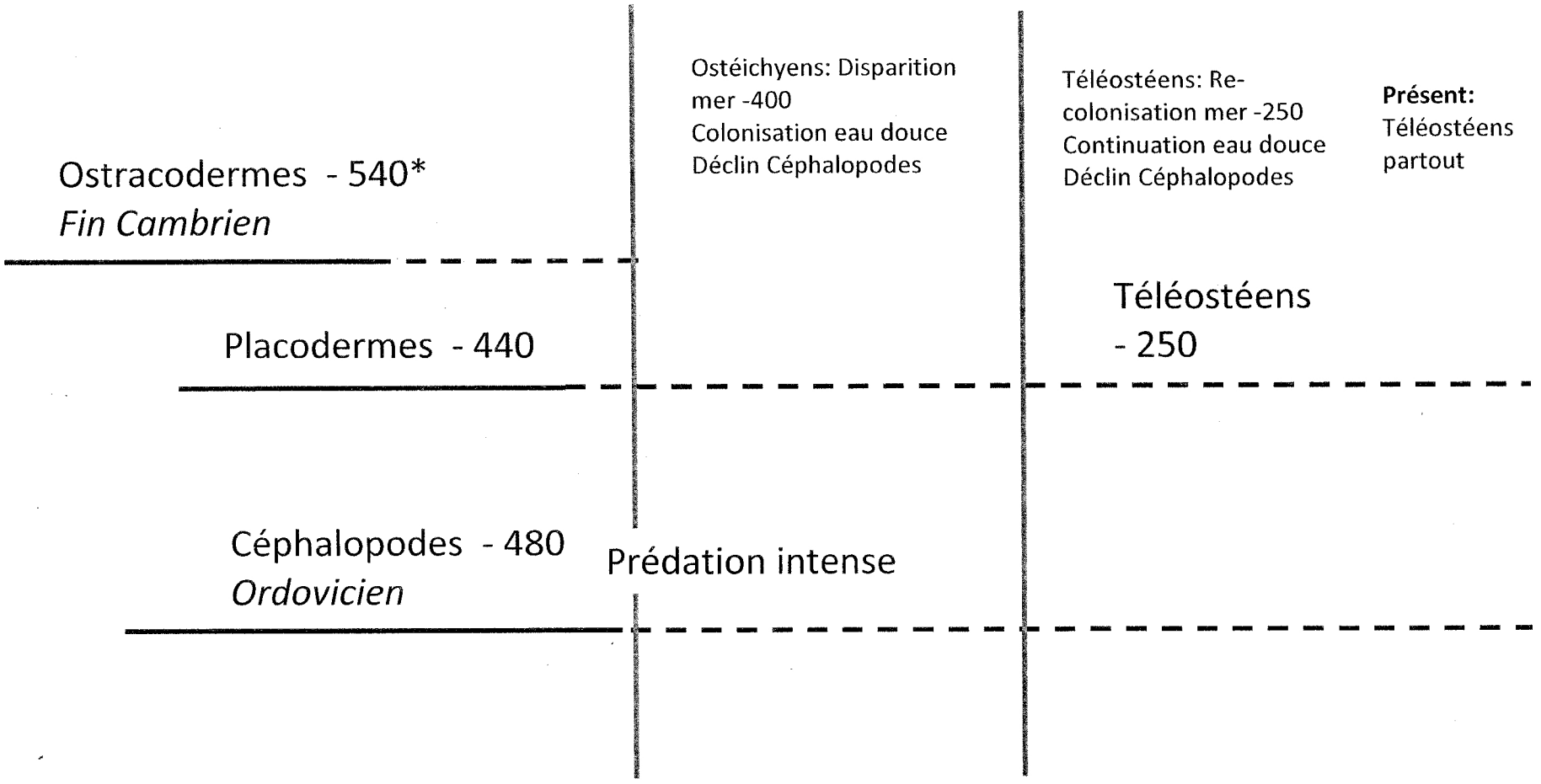
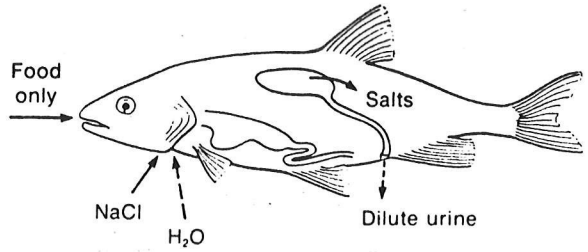


Figure 40.3
Comparaison de l'osmorégulation chez les Poissons osseux marins et d'eau douce. (**a**) Les Poissons marins, comme cette Morue, sont hypotoniques par rapport à l'eau de mer environnante et perdent donc constamment de l'eau par osmose, principalement à travers l'épithélium des branchies. Ces Animaux compensent la perte de liquide en buvant de grandes quantités d'eau de mer et en éliminant ensuite le surplus d'électrolytes de leur organisme à travers l'épithélium des branchies. L'urine est peu abondante, et isotonique par rapport à leurs liquides biologiques. (**b**) Les Poissons d'eau douce, comme cette Perche, font face au problème inverse : ils accumulent continuellement, par osmose, l'eau de leur environnement hypotonique. Ils équilibrent l'apport d'eau par une excrétion abondante d'urine, qui est hypotonique par rapport à leurs liquides biologiques. Bien que l'urine soit diluée, ces Animaux perdent des électrolytes importants au cours de l'excrétion et compensent par l'apport actif d'ions à travers l'épithélium des branchies.

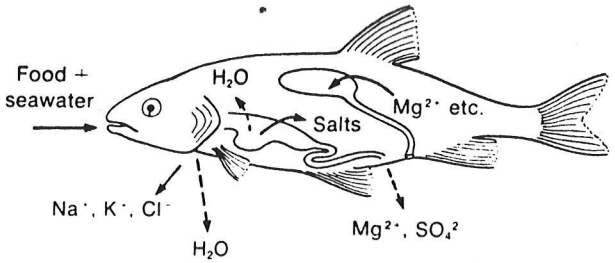
Hypothèse 'refuge' pour expliquer osmolarité des Téléostéens



* = x 10^{6} ans

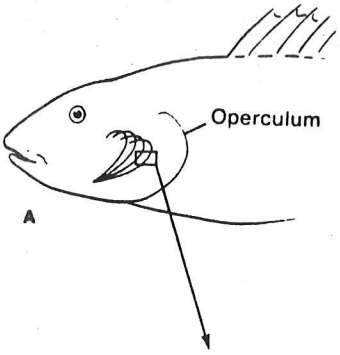


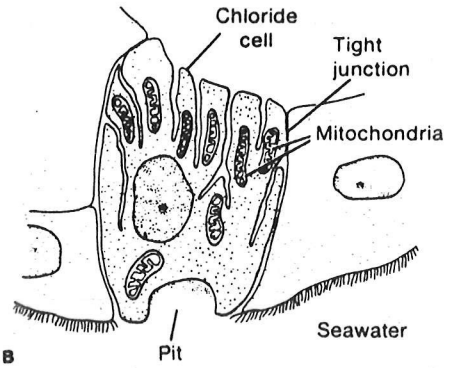
A Freshwater teleost

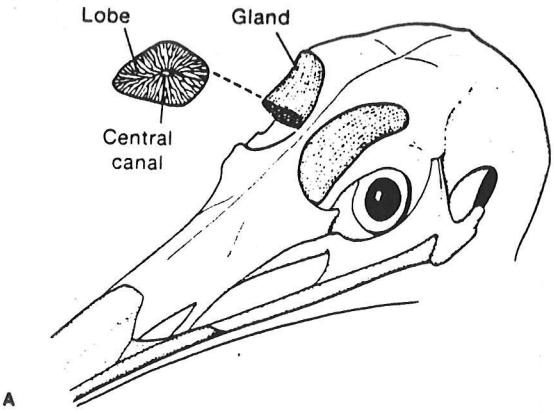


B Marine teleost

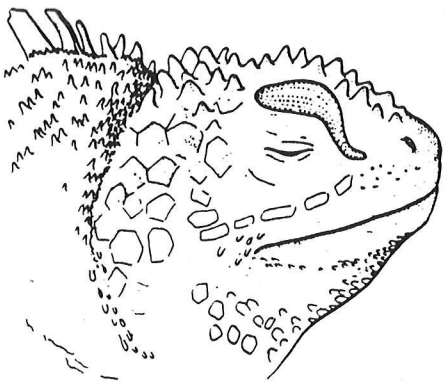
12-37 Salt and water exchange in (**A**) freshwater and (**B**) marine teleosts. Solid arrows indicate active processes; broken arrows, passive processes. Note the active role of the gills in salt transport in both groups. [Prosser, 1973.]







(**A**) Avian salt glands are located above the orbit and drain via ducts into the nasal region. The gland consists of a longitudinal arrangement of many lobes. Each lobe consists of tubules and capillaries (see Figure 12-29) arranged radially around a central canal. The secretion collects in a duct that empties into the nasal region. [Schmidt-Nielsen, 1960.] (**B**) Reptilian salt glands are found associated with eyes, nostrils, cheeks, and tongues in various groups. A marine iguana is shown here with the subdermal salt gland in color. [Dunson, 1969.]



ELSEVIER

Aquaculture 177 (1999) 277–283

Aquaculture

Gill lipid metabolism and unidirectional Na^+ flux in the European eel (*Anguilla anguilla*) after transfer to dilute media: the formation of wax alcohols as a primary response

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Accepted 1 October 1998

Abstract

The present study investigated the mechanism involved when fish tighten their gill membrane after transfer to dilute media. Ten individually assayed eels adapted to fresh water (FW) and labelled with radioactive ^{22}Na in the plasma showed mean exchange rates in equilibrium with ambient radioactive ^{24}Na in FW at 70 μmol kg^{-1} h^{-1}. Just after transfer to demineralized water (DW), the mean Na^+ release rate went up to 120 μmol kg^{-1} h^{-1}; after 24 h in DW it came down again and levelled off at 40 μmol kg^{-1} h^{-1} during a period of up to 2 weeks. During the same period the mean Na^+ uptake rate was 12 μmol kg^{-1} h^{-1}, i.e., the general picture in DW was a net Na^+ loss. In another similar experiment, groups of three FW-adapted eels were incubated in vivo for up to 24 h with (^{14}C) acetate added as lipid precursor to the ambient water. Incubation in FW showed about 20% of the total ^{14}C-activity incorporated into gill lipids as (^{14}C) wax alcohols (WA; octadecanol and eicosanol). This percentage went up to 50% shortly after transfer to DW and came down again to about 20% after 2 weeks in DW. Single eels labelled with ^{22}Na in the plasma showed a statistically significant positive linear correlation of percentage (^{14}C) wax alcohols with log [^{22}Na efflux]. Based on the observed parallel between Na^+ flux and gill lipid metabolism, it is suggested that the eel reacts at first to a loss of Na^+ by synthesizing wax alcohols that can tighten the gill membrane. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Eels; Eicosanol; Gills; Lipid metabolism; Octadecanol; Osmoregulation; Sodium flux

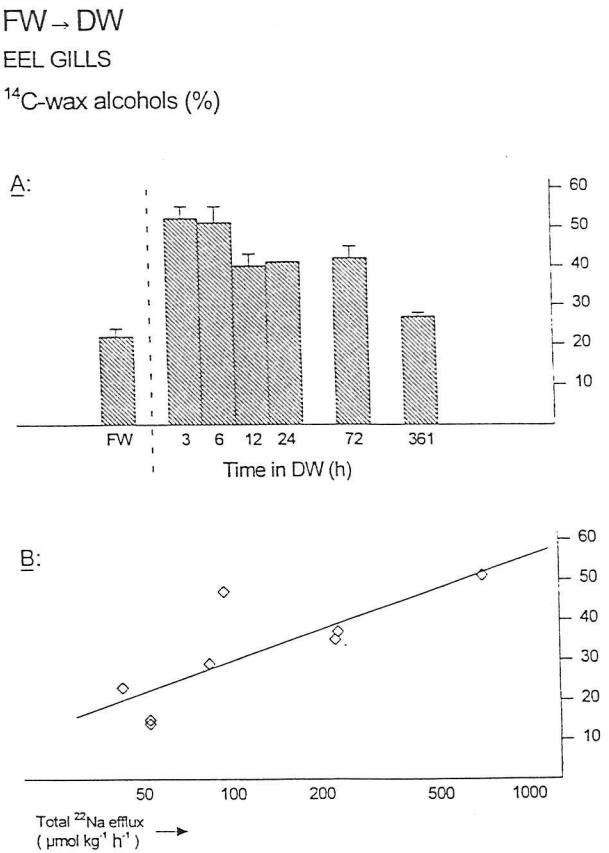
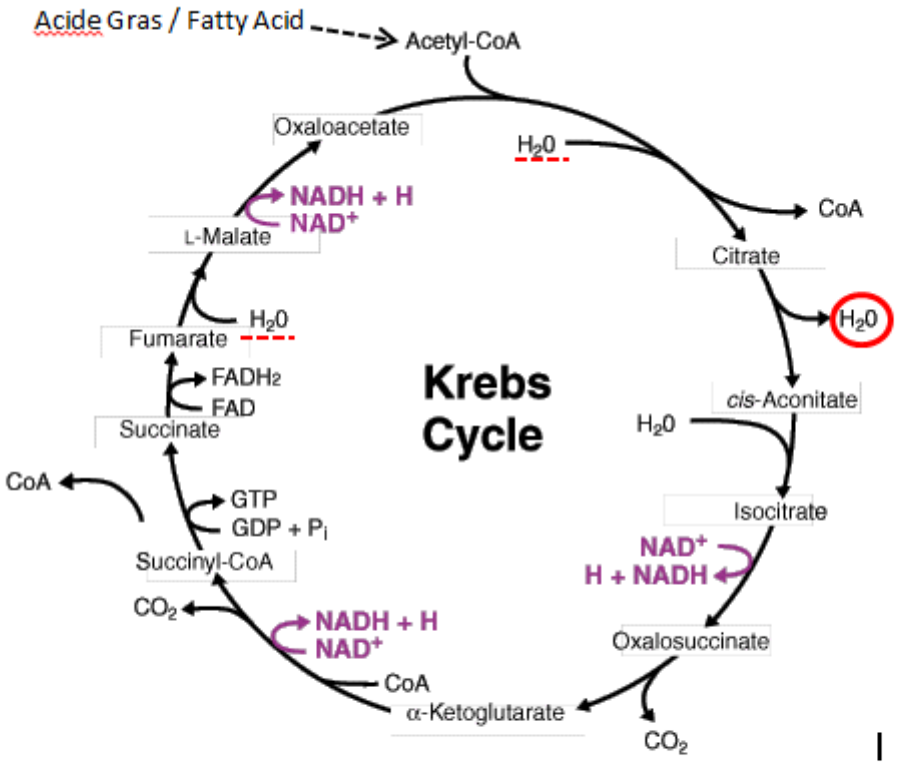


Fig. 2. The incorporation in vivo into gill wax alcohols of (1-^{14}C) acetate, added to the water in the incubation tank, expressed as percentage of total (^{14}C) lipids. (A) Presented as a function of time in DW, similar to Fig. 1; means ± SEM of groups of three individually assayed eels. (B) Presented as a function of Na^+ efflux; single eels.

Water from Lipid /De l'eau à partir des lipides



C 16: 0 + 23 O_2 + 129 P_i + 129 ADP → 16 CO_2 +145 H_2O + 129 ATP

Nutrient	Metabolic water produced by oxidation	
	mL/100g	*mL/100 kcal*
Lipids	107	11.8
Carbohydrates	55	15
Proteins	41	10

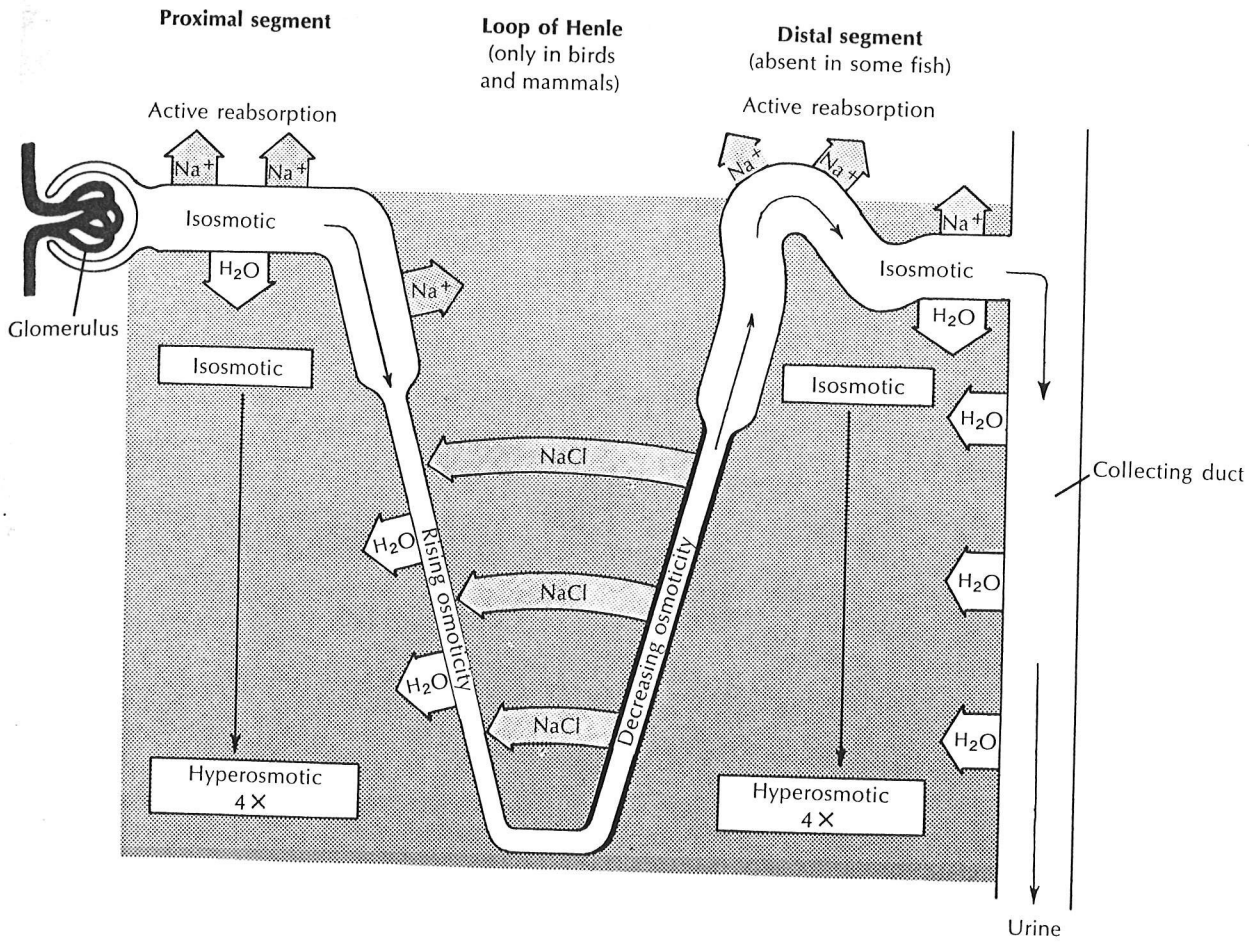


Figure 30-10

Mechanism of urine concentration in humans. Sodium chloride is actively pumped from the ascending limb of loop of Henle and passively reenters the descending limb, building up the osmotic concentration to approximately four times that of blood. This creates an osmotic gradient for the controlled reabsorption of water from the collecting duct. Many rodent and desert mammals have relatively longer loops of Henle and can produce much more concentrated urine than humans.

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Adaptations with Respect to Salinity*

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Synopsis

Amino acids contribute up to about 50% of the intracellular osmotic pressure of aquatic invertebrates. Since their concentration varies according to the salinity of the medium (high in sea water, low in fresh water) euryhaline invertebrates are good models for studying the mechanisms involved in the control of amino acid metabolism. During hyperosmotic stress CO_2 production and O_2 consumption decrease whereas the reverse is true when the animal is submitted to a hypo-osmotic stress. Nitrogen excretion (as NH_3) increases in media of low salinity and the concentration of cyclic AMP increases during hyperosmotic stress. Moreover, blood proteins and haemocyanin are more concentrated in individuals adapted to media of low salinity. To explain the situation, three main mechanisms can be considered: (*a*) hydrolysis and synthesis of blood proteins; (*b*) transport of amino acids across the cell membrane; (*c*) control of the turnover rate of some amino acids. Results obtained on whole animals as well as on isolated tissues indicate that some amino acids are released from the cells and carried via the haemolymph to the posterior pairs of gills where they are oxidized (mechanism *b*) or to an organ (hepatopancreas ?) where they are used for blood protein synthesis (mechanism *a*). The use of labelled substrates demonstrates that the turnover rate of amino acids is controlled by the salinity of the environment (mechanism *c*). It is suggested that inorganic ions trigger the metabolic response by directing reducing equivalents toward oxygen or 2-oxo acids through control of the catalytic activity of dehydrogenases.

Reference: *Biol. Bull.* **163**: 405–419. (December 1982)

INVERTEBRATE CELL VOLUME CONTROL MECHANISMS: A COORDINATED USE OF INTRACELLULAR AMINO ACIDS AND INORGANIC IONS AS OSMOTIC SOLUTE

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ABSTRACT

All cells have some capacity for cell volume regulation when confronted with a hypoosmotic stress. The basis of this physiological response is an extrusion of intracellular osmotic solute. The cells of euryhaline osmoconforming invertebrates are capable of regulating volume over a wide range of external osmotic concentrations. Most of the existing data indicate that these cells utilize free amino acids from a substantial intracellular pool as the solute source. However, recent studies indicate that these invertebrate cells utilize inorganic ions as osmotic solute as well. The relative contribution of each solute type varies from species to species and, perhaps, from cell type to cell type. The two solute types are regulated by different mechanisms and often with different time courses, but both solute control systems function in a coordinated manner to regulate cell volume. In addition, evidence is appearing demonstrating a role for organic solutes in the volume regulatory processes of vertebrate cells. At present, it seems that the volume regulatory mechanisms utilized by all cells may be more similar than currently thought, differing in relative contributions of the two solute types rather than kind of solute utilized.

Relative Contributions of the Most Abundant Free Amino Acids in the Total Tissue Pools of Different Marine Bivalves[a,b]

Amino acid	Rangia cuneata[c]		Mytilus edulis[d]	Venus gallina[e]	Scapharca cornea[e]
Taurine	0	0	30–40	40	65
Alanine	45	70	2–7	15	3
Glycine	10	7	10–40	22	7
Aspartate	2	1	3–6	8	7
Glutamate	20	8	5–10	7	8
Proline	0.3	6	1–2	—	1
Total pool size[f]	11	80	100–220	80	45

[a] From Zurburg and DeZwaan (1981).

[b] Expressed as percentage of total amino acid pool.

[c] Data from Henry et al. (1980) for the adductor muscle. Salinity, 2 and 20‰ for the first and second value, respectively.

[d] Data from Kluytmans et al. (1980) for the total animal. The range of values during an annual cycle is given. Salinity 25–31‰.

[e] Data from Zurburg and Carpene for the total animal (Zurburg and DeZwaan, 1981). Salinity 37‰.

[f] Total free amino acid concentration in units of μmol/g fresh weight (or per milliliter in the case of hemolymph).

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PURIFICATION AND CHARACTERIZATION OF PYRUVATE KINASE FROM THE FOOT MUSCLE OF THE COCKLE, *CARDIUM TUBERCULATUM**

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(*Received* 24 *January* 1985)

Abstract—1. Pyruvate kinase (PK) of the foot muscle from the bivalve mollusc, *Cardium tuberculatum*, was purified 650-fold to near homogeneity by a heat step (50°C, 15 min), ammonium sulfate precipitation (30–50%), ion exchange chromatography (DEAE-Sephacel), affinity chromatography (Blue-Sepharose CL-6B) and gel filtration (Ultrogel AcA 22) to a final specific activity of 181 U/mg protein.

2. The native molecular mass was 282 Kda and that of the subunits 70 Kda, indicating that the PK is tetrameric.

3. The PK exhibited a slight positive cooperativity for the substrate phosphoenolpyruvate (PEP) with K_m values of 0.07 mM at pH 7.2 and 0.09 mM at pH 6.6, both determined at 2 mM ADP.

4. Fructose-1,6-bisphosphate (FBP) activated the enzyme. The PK was inhibited by L-alanine, but the degree of inhibition was dependent on the concentrations of PEP and Mg^{2+}. Furthermore, FBP relieved the inhibition caused by alanine.

5. ATP inhibited the enzyme only at low Mg^{2+} concentrations. D-Lactate and citrate also inhibited enzyme activity.

6. The enzyme showed normal Michaelis–Menten kinetics for adenosine-diphosphate (ADP): K_m 0.33 mM at 0.5 mM PEP. This PK displayed partial activity when guanosine-diphosphate or inosine-diphosphate were substituted for ADP in the basic assay.

7. Monovalent and divalent cations are necessary for activity. K^+ is besides NH_4^+ the most effective monovalent cation. Mg^{2+} is the primary divalent cation activator for PK, as Mg^{2+} can only be partly substituted for by Mn^{2+}. Ca^{2+} is a strong inhibitor of the enzyme when Mg^{2+} is the used cation, but less inhibition was obtained when Mn^{2+} was used.

Marine Biology (2006) 149: 1387–1396
DOI 10.1007/s00227-006-0303-7

RESEARCH ARTICLE

Sandra Kube · Anke Gerber · Jeroen M. Jansen
Doris Schiedek

Patterns of organic osmolytes in two marine bivalves, *Macoma balthica*, and *Mytilus* spp., along their European distribution

Table 3 Slopes, determination coefficients and levels of significance (*$P<0.05$, **$P<0.01$, ***$P<0.001$) for the linear regression of individual amino acids and habitat osmolality

	Macoma		*Mytilus*	
	0–600 (mmol kg^{-1} H_2O)	500–981 (mmol kg^{-1} H_2O)	143–600 (mmol kg^{-1} H_2O)	500–1143 (mmol kg^{-1} H_2O)
	$N = 163$	$N = 86$	$N = 124$	$N = 172$
Alanine				
Slope	0.16	0.15	0.03	−0.002
R^2	0.56***	0.09**	0.26***	<0.001
Glycine				
Slope	0.12	−0.02	0.17	0.02
R^2	0.41***	0.01	0.41***	0.01
Taurine				
Slope	[a]0.18	−0.005	0.38	−0.002
R^2	[a]0.69***	<0.001	0.79***	<0.001
Glutamate				
Slope	0.01	−0.01	0.03	−0.005
R^2	0.06**	0.13***	0.42***	0.03*
Aspartate				
Slope	0.03	0.03	0.03	−0.005
R^2	0.49***	0.11**	0.42***	0.03*
Arginine				
Slope	0.01	0.02	0.02	−0.01
R^2	0.05**	0.11**	0.12***	0.02
Leucine				
Slope	−0.02	0.001	−0.02	−0.003
R^2	0.18***	0.02	0.26***	0.03*
Proline				
Slope	−0.005	0.001	0.005	0.002
R^2	0.19***	<0.001	0.07	0.007
Valine				
Slope	−0.005	0.002	0.003	0.001
R^2	0.11***	0.1*	0.05	0.002

Habitat osmolality is subdivided into two ranges: minimum level to 600 mmol kg^{-1} H_2O and 500 mmol kg^{-1} H_2O to maximum level
[a] Linear regression for taurine is calculated for habitat osmolalities > 100 since taurine was absent at lower osmolality values ($n=108$)

Comp. Biochem. Physiol. Vol. 82A, No. 4, pp. 945–949, 1985
Printed in Great Britain

0300-9629/85 $3.00 + 0.00
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LONG-TERM VARIATIONS IN CATION CONTENT OF TWO POPULATIONS OF ADULT MARINE CLAM (*TAPES DECUSSATUS* L. AND *T. PHILIPPINARUM*) REARED IN A COMMON HABITAT

PETER G. BENINGER*
Laboratoire de Zoologie, Université de Bretagne Occidentale, 29283 Brest Cedex, France

(*Received* 19 *April* 1985)

Abstract—1. The levels of four major cations, Na^+, K^+, Mg^{2+} and Ca^{2+} were measured using flame spectrophotometry over a 16-month period in two populations of adult marine clam reared in a common habitat.

2. The variations in absolute and relative values of Na^+ and Mg^{2+} were virtually identical within each species; the relative values showed a marked increase during the period of negative energy balance previously reported, even when the external salinity decreased.

3. Calcium levels showed very irregular long-term variations, reflecting its multiple roles as a metabolic, osmotic and structural element.

4. A close relationship was observed between the levels of K^+ and organic constituents, especially glycogen.

5. An adaptive benefit of this relationship is postulated.

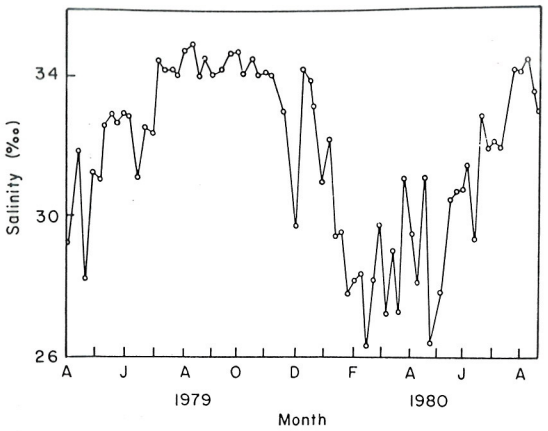


Fig. 1. Weekly variation of salinity in the external medium of the two populations studied.

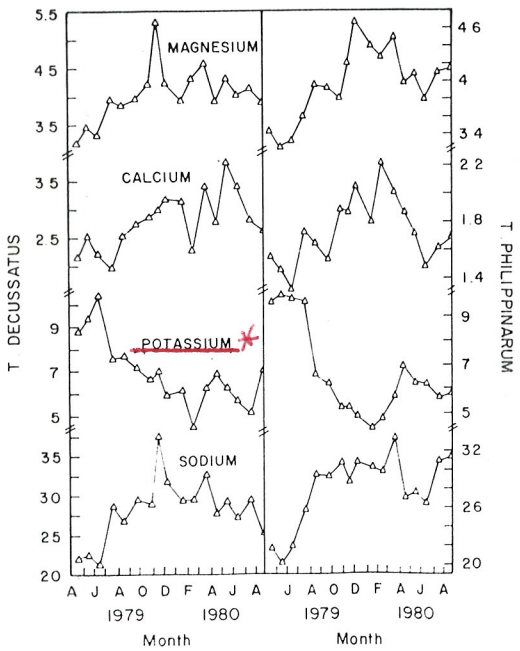
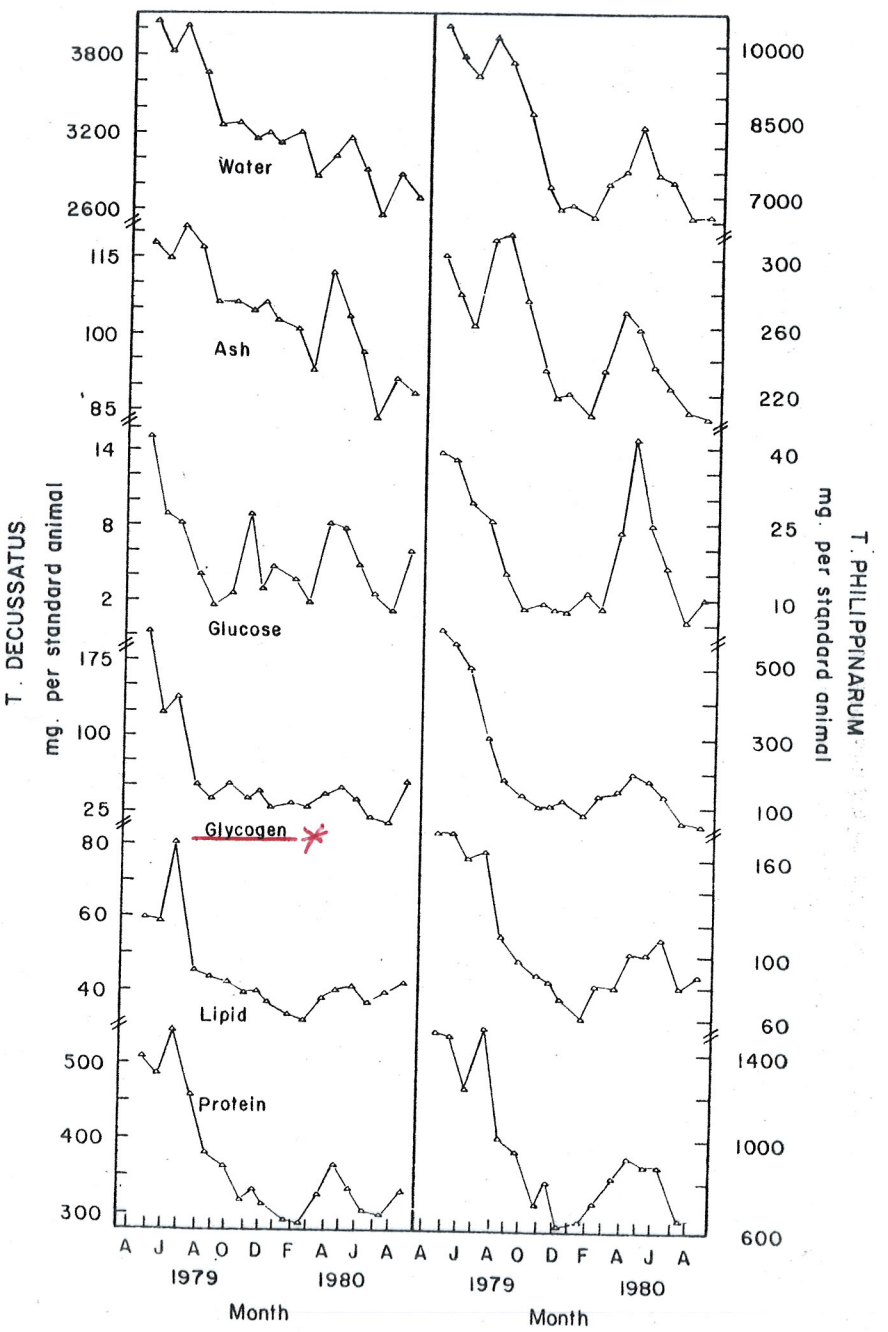


Fig. 4. Tissue levels of the four major cations in the two populations studied, expressed as % wet tissue weight × mean wet tissue weight of *T. decussatus* (relative values: see text for details).





Moules prises dans la glace

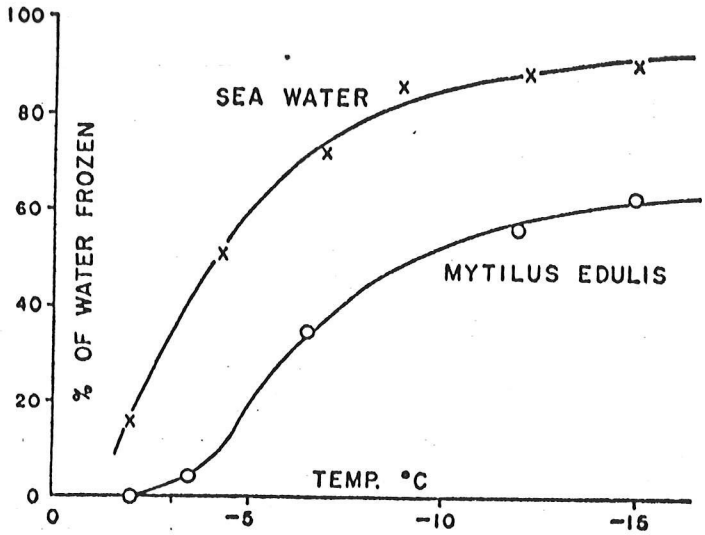


FIGURE 3. Graph of ice determined in the same fashion for a vial of sea water and a mussel. The difference is believed to represent unfreezable or bound water.

Kanwisher JW 1955 Freezing in intertidal animals. Biol. Bull. 109, 56 – 63

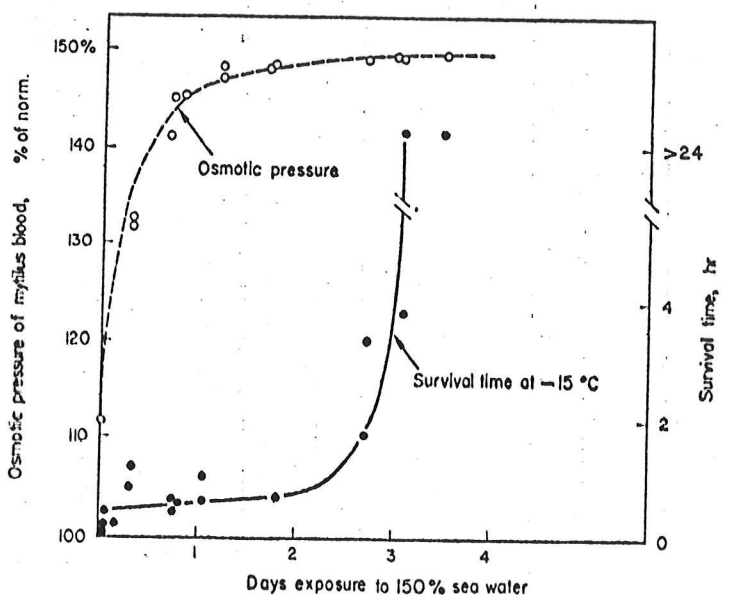


FIG. 3. The changes in osmotic adaptation and tolerance to freezing as a response to changed osmotic environment. Animals adapted to normal sea water were placed in 150% sea water and tested after intervals. The osmotic pressure of the blood, measured as a freezing-point depression in an osmometer, increased until it was again the same as the external environment and appears to be a first-order process. The survival time of excised tissues frozen at −15°C increased only after the second day of exposure, suggesting its relation to cellular osmotic adaptation.

Williams, RJ 1970 Freezing tolerance in *Mytilus edulis*. Comp Biochem Physiol 35, 145 - 161

Comp. Biochem. Physiol., 1970, *Vol.* 35, *pp.* 145 *to* 161. *Pergamon Press. Printed in Great Britain*

FREEZING TOLERANCE IN *MYTILUS EDULIS*

ROBERT J. WILLIAMS*

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(*Received* 15 *December* 1969)

Abstract—1. *Mytilus edulis* tolerates freezing to a tissue temperature of −10°C, while *Venus mercenaria* tolerates only −6°C.

2. In both species, tissues are injured when 64 per cent of cellular water has been removed to form ice. In *Mytilus*, 20 per cent of cell water appears osmotically inactive, requiring lower freezing temperatures to remove 64 per cent of intracellular water.

3. Adapting *Mytilus* to 150% salinity increases freezing tolerance to −15°C, but does not change the amount of osmotically inactive water or the dehydration limit. Lower salinities reduce freezing tolerance.

4. The adaptations permitting tolerance of varying salinities and of freezing appear to be independently evolved.

Oceanography and Marine Biology: an Annual Review 1995, **33**, 337–350
© A. D. Ansell, R. N. Gibson and Margaret Barnes, Editors
UCL Press

FREEZING TOLERANCE OF MARINE INVERTEBRATES

STEPHEN H. LOOMIS
Department of Zoology, Connecticut College, New London, CT 06320, USA

Abstract Intertidal invertebrates living in temperate regions of the world are generally freezing tol erant, and must be able to limit the osmotic and mechanical damage to their tissues that results from freez ing of the extracellular fluids. General mechanisms of protection include production of proteinaceous ic nucleators, antifreeze proteins, cryoprotectants, changes in the composition of membrane phospholipid and metabolic rate reduction. Various intertidal invertebrates, including *Melampus bidentatus*, *Littorin littorea*, *Geukensia demissus*, *Mytilus edulis*, and *Balanus balanoides*, utilize these strategies to greater o lesser extents. All have seasonal changes in the extent of their freezing tolerance that most likely result from seasonal presence of ice nucleators (either proteinaceous or bacterial) and cryoprotectants (whic may be related to exposure to anaerobic conditions). All also have a lower metabolic rate in the winte resulting from exposure to lower temperatures. However, *Mytilus* collected in Norway appears to be th only intertidal invertebrate for which antifreeze proteins may play a role in freezing tolerance, althoug there is some question of the significance of these findings, since no antifreeze proteins were found i *Mytilus* collected from Connecticut or Massachusetts. Changes in the composition of membrane phosphol ipids (especially sphingomyelin) have been correlated with changes in freezing tolerance in *Balanus balc noides*, but little is known of this strategy in other intertidal invertebrates.

Table 1 Adaptions of intertidal invertebrates to freezing tolerance.

	Adaptions					
Species	Ice nucleator	Cryoprotectants	Antifreeze proteins	Anaerobiosis	Membrane lipids	Metabolic rate depression
Melampus bidentatus	protein	unknown	absent	likely	unknown	yes
Littorina littorea	protein (N) none (C)	unknown	absent	unknown	unknown	yes
Geukensia demissa	INA bacteria	alanine proline	absent	yes	unkown	yes
Mytilus edulis	protein (N) none (C)	strombine alanopine octopine taurine succinate acetate alanine	present (N) absent (C)	yes yes	unknown unknown	yes yes
Balanus balanoides	likely	unknown	absent	unknown	sphingomyelin	yes

N – Norway; C – Connecticut.

Cryobiology **41,** 257–279 (2000)
doi:10.1006/cryo.2000.2289, available online at http://www.idealibrary.com on IDEAL®

REVIEW

Ice Nucleation and Antinucleation in Nature

Karl Erik Zachariassen and Erlend Kristiansen

Laboratory of Ecophysiology and Toxicology, Department of Zoology, Norwegian University of Science and Technology, 7491 Trondheim, Norway

Plants and ectothermic animals use a variety of substances and mechanisms to survive exposure to subfreezing temperatures. Proteinaceous ice nucleators trigger freezing at high subzero temperatures, either to provide cold protection from released heat of fusion or to establish a protective extracellular freezing in freeze-tolerant species. Freeze-avoiding species increase their supercooling potential by removing ice nucleators and accumulating polyols. Terrestrial invertebrates and polar marine fish stabilize their supercooled state by means of noncolligatively acting antifreeze proteins. Some organisms also depress their body fluid melting point to ambient temperature by evaporation and/or solute accumulation. © 2000 Academic Press

Key Words: ice nucleators; antifreeze proteins; polyols; supercooling; fish; insects; plants.

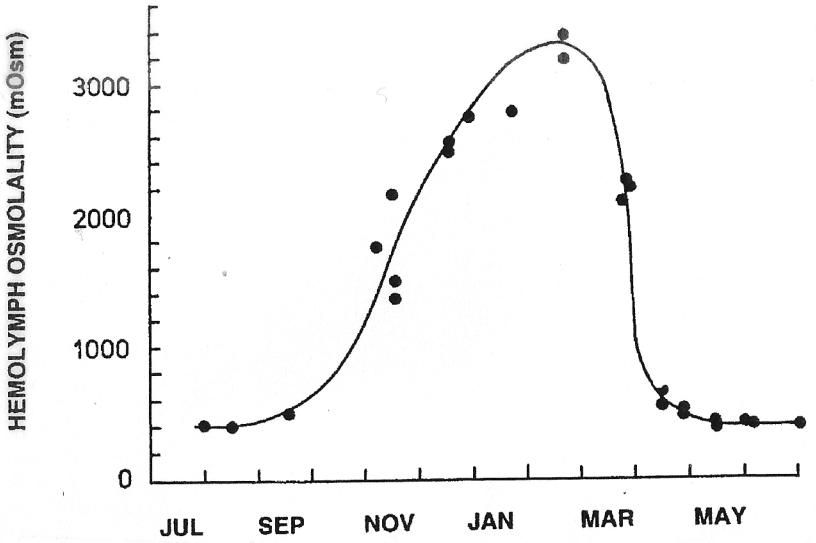


FIG. 2. Hemolymph osmolality in the freeze-avoiding longhorn beetle *Rhagium inquisitor* during the year. From Zachariassen (108).

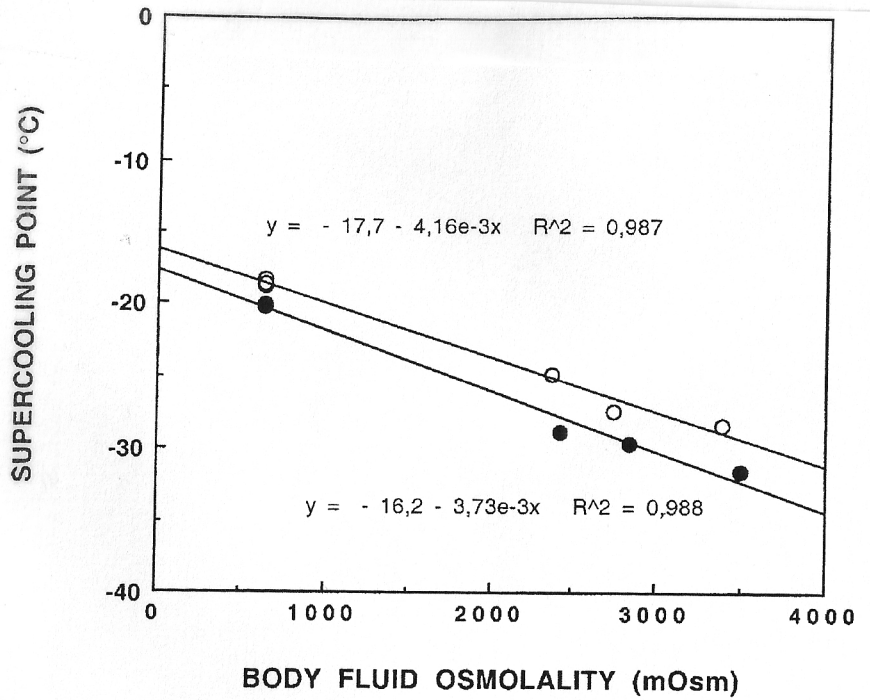


FIG. 5. Supercooling points of freeze-avoiding *Ips acuminatus* bark beetles as a function of body fluid osmolality. ●, Beetles with antifreeze proteins; ○, beetles without antifreeze proteins. Lines are linear regression lines. Data from Gehrken (39).

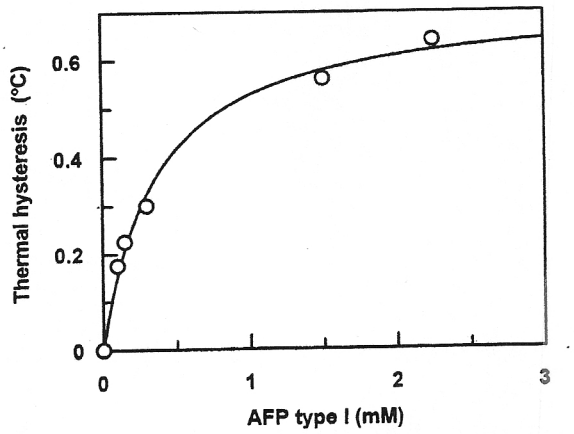


Fig. 1. Thermal hysteresis activity of type 1 fish Antifreeze protein [95].

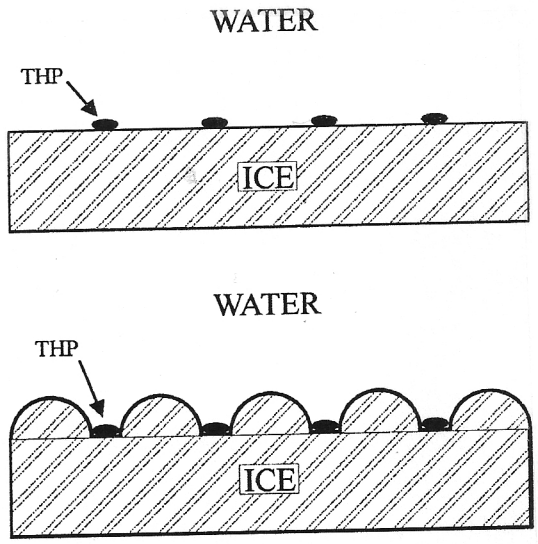
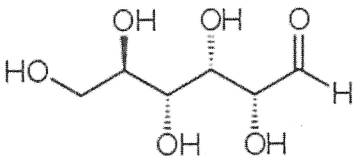
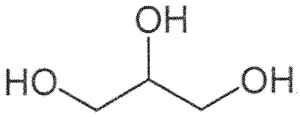


Fig. 2. The 'mattress button' model of thermal hysteresis protein (THP) interaction with ice [15]. Where the THPs bind to the ice surface they restrict further growth at that point and the ice face between them becomes more curved.

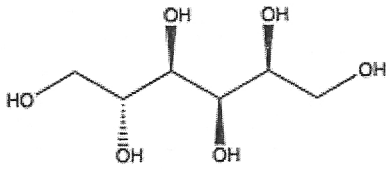
Cryoprotectants



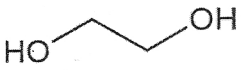
Glucose



Glycérol

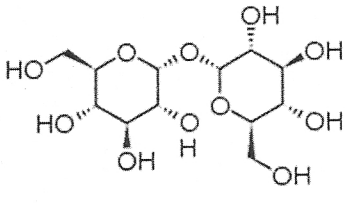


Sorbitol

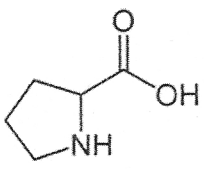


Ethylène glycol

Prévention de formation de glace



Tréhalose



Proline

Stabilisation membrane cellulaire

A scientific forum about organisms that can catalyze the freezing of water and their roles in nature

About

In the absence of catalysts for freezing, water can remain in a metastable liquid state at temperatures well below 0°C. Water in the liquid state at temperatures below 0°C is referred to as supercooled water. Spontaneous freezing of supercooled water occurs below –39°C. Hence, freezing catalysts are clearly essential for many natural freezing processes.

A wide range of substances are able to catalyze ice nucleation. Various inorganic crystalline solids (the most well known of which is silver iodide), amino acid crystals, monolayers of long chain alcohols and organic compounds such as phloroglucinol and metaldehyde can catalyse the freezing of supercooled water. Substances that are abundant in atmospheric aerosols, such as soot, mineral dusts and metallic particles are also ice nucleation active. In the atmosphere near forests, ice nucleation-active combinations of terpenes and of other tree oils with iodine have been detected. But among all the different ice nucleators naturally present in the environment, biological ice nucleators are among the most active. Ice nucleation-active (INA) organisms include plants, fungi, bacteria, vertebrates and invertebrates.

Atmosphere physicists have long sought the ice nucleators responsible for various freezing processes in nature. Several decades ago, when INA micro-organisms were detected in atmospheric aerosols and in clouds, biologists joined in the heightened effort to investigate the role of biological ice nucleators in atmospheric processes that require freezing – such as rain formation. Recently there has been renewed interest in the unanswered questions about this role. Assessments of an atmospheric role for biological ice nucleators are gathering momentum as techniques of detection improve, as understanding of biological sources is growing, and as observations and modelling of clouds elucidate the role of early ice nucleation in the formation of precipitation and in determining other cloud characteristics. Progress in understanding the role of biological ice nucleators in these atmospheric processes that define our climate requires close collaboration of scientists from the physical and the biological sciences. This discussion forum is dedicated to serving this need for interdisciplinary communication.

This current forum on WordPress is a newly formatted version of the former site created with different blogging tool (**http://bio-ice.forumotion.com/ (http://bio-ice.forumotion.com/)**). I started moving the site on 4 Sept 2011 and hopefully it will proceed quickly. Most of the pertinent information will be transfered in addtion to the updates made for this new site.

Institute for Evidence-Based Cryonics



Vitrification agents in cryonics: M22

08. July 2008 · Comments Off · Categories: Cryonics · Tags: 21st Century Medicine, Brain, Brian Wowk, Cryonics, Cryopreservation, Gregory Fahy, Vitrification
Written by: Aschwin de Wolf on July 8, 2008.

Recommander Une personne recommande ça.

M22 represents the culmination of decades of work in applied cryobiology by researchers Gregory Fahy , Brian Wowk, and others to develop a vitrification agent that can recover complex organs (such as the kidney) from cryogenic temperatures without ice formation and minimal toxicity. In 2005, M22 was licensed by the patent holder 21st Century Medicine (21CM) to the Alcor Life Extension Foundation to replace their previous vitrification agent B2C. As a result, the least toxic vitrification agent for complex organs that has been documented in peer review journals is currently being used for cryonics patients at Alcor.

M22 incorporates a number of important discoveries in cryobiology:

1. High concentrations of a cryoprotective agent (or a mixture of different cryoprotective agents) can prevent ice formation during cooldown and warming.

2. The toxicity of some cryoprotectants can be neutralized by combining them with other cryoprotective agents.

3. The *general* toxicity of a vitrification agent can be predicted by using a measure called qv*, allowing for the rational formulation of less toxic vitrification agents.

4. Within limits, non-penetrating agents can reduce the exposure of cells to toxic amounts of cryoprotectants without reducing vitrification ability.

5. Synthetic "ice blockers" can be included in a vitrification mixture to reduce the concentration of toxic cryoprotective agents necessary to achieve vitrification.

6. Substituting methoxyl (-OCH3) for hydroxyl groups (-OH) in conventional cryoprotective agents can decrease viscosity, increase permeability, and reduce the critical cooling rate necessary to avoid ice formation.

7 Chilling injury can be eliminated by introducing the vitrification agent with a hypertonic concentration of non-penetrating solutes.

8. In cryonics, with a minor proprietary modification, M22 can be used for whole body perfusion without causing severe edema that has been a problem for some other solutions.

Vitrification is the solidification of a liquid without crystallization. When a solution is cooled down to the glass transition point (-123.3°C for M22) the extreme elevation in viscosity will produce a glass in which all translational molecular motions are arrested. Although water vitrifies at cooling rates exceeding a million of degrees Celsius per second, such cooling rates are relaxed when other solutes are substituted for water. In cryobiology solutions with high concentrations of cryoprotective agents can be used to vitrify complex organs such as the kidney or the brain.

Vitrification has a number of clear advantages over conventional cryopreservation. The most important advantage is the elimination of ice formation. Although the adverse effects of ice formation can be mitigated by the use of cryoprotective agents (glycerol, DMSO) and optimization of cooling rates, massive ice formation does not permit recovery of complex organs with full viability. Another advantage is that vitrification eliminates the need to strike a balance between the risk of intracellular freezing induced by fast cooling on the one hand, and cell dehydration and solution concentration induced by slow cooling on the other hand.

The challenge in formulating successful cryoprotective agents is to design vitrification solutions that are non-toxic but allow for vitrification at realistic cooling and warming rates. For more than a decade the least toxic vitrification agent was Greg Fahy's VS41A, which is an 55% weight/volume equimolar mixture of DMSO and formamide plus propylene glycol. The "1A" in VS41A reflects the solution's ability to vitrify at normal atmosphere pressure (as opposed to an older, more dilute solution, VS4, which requires 1000 atmospheres of pressures to vitrify). The equimolar concentrations of DMSO and formamide reflect Baxter and Lathe's research who concluded that amides can neutralize the toxicity of DMSO, a finding that Greg Fahy later revised in favor of the theory that it is actually DMSO that neutralizes the toxicity of formamide. The ability of DMSO to neutralize the toxicity of formamide (up to certain concentrations) allows for the formulation of vitrification agents with reduced toxicity. This finding has been so fundamental that an equimolar concentration of DMSO and formamide remains the core of M22.

UNIT 5: SOLID FROGS - THE ART OF CRYOPRESERVATION

When it gets cold, all we have got to do is just put on our coats or turn up the heating. But, what about animals? What can they do when the thermometer drops below zero? Well, some just change places - they migrate south away from the cold climate. Others hibernate deep down in the soil or, for fish, at the bottom of rivers or lakes. However, certain animals have developed alternative physiological strategies. For instance, they may go through complex biochemical changes and develop antifreezes, such as glycerol which allow their bodies to go below zero without ice crystals forming. But there is a fourth category of animal which freezes solid and, strange as it may seem, still survives. When summer comes they just melt and start living again. Harriet Green is here to explain just what happens.

The first thing to remember of course is that normally, freezing is a very dangerous thing for organisms. There are quite a few reasons for this; in the first place when the ice crystals form they burst through the cell walls, thus damaging the membranes. Not only is the membrane destroyed, but as a result, the metabolic processes in individual cells are contaminated by processes in the next cell. This means that it is absolutely essential that any ice that forms should only be in extracellular fluid; for example in blood plasma, in urine, abdominal fluids and between the muscles. But apart from that, there is another problem. As freezing halts breathing and blood circulation, organs have no access to oxygen and nutrients normally conveyed by the blood.

Yes, but what happens, what can animals do about this? How can they prevent it happening? How is it possible for animals to survive?

There are several species of animals that survive freezing, but let's take the example of the frog. When freezing begins, that is to say, when ice crystals begin to form on the surface of the frog's skin, this triggers off a complex reaction which results in glucose being pumped throughout the animal's system. You see, enzymes are produced in the animal's liver, thereby converting glycogen into glucose in massive quantities. Average levels of 4500 milligrams per millilitre may be reached.

Is that very high?

Well just remember that human blood has a normal glucose content of about 50 - 100 per millilitre - maybe three or four times higher in the case of diabetes.

Really. But what exactly happens, could you tell me in more detail ?

Well, the brain reacts to the formation of ice by liberating enzymes. This is done by releasing hormones into the blood supply. As we have said, this activates the production of glycogen which is subsequently transformed into glucose. The heart then proceeds to pump this through the major organs, hence protecting them against freezing. So then, once the vital organs are protected by the high glucose content, the rest of the body cavities and extra-cellular space can be filled up with ice without causing injury to the organism. This is just what happens to the abdomen, the urine and the spaces between the muscles. As much as 65% of the total body water can become ice. The animal is then frozen solid.

So, are there no body functions left at all ?

Well, clearly there is no respiration, heart beat or blood circulation, and as far as neurological activity is concerned it is hardly perceptible.

But what about the muscle and body tissues? How can they survive without access to the blood supply ?

In fact, freeze tolerant animals can survive without oxygen for a considerable time. Some animals can even survive a whole winter without breathing. We think that the glucose may provide a fuel to supply energy to cells that have no access to oxygen from the blood. In addition, there is some evidence to suggest that high glucose levels may fulfil another role, namely, helping to reduce the rate of metabolism in the organs. Reducing the metabolic rate means that smaller amounts of body fuels are required thereby enhancing the chances of survival.

Harriet Green. Thank you very much.

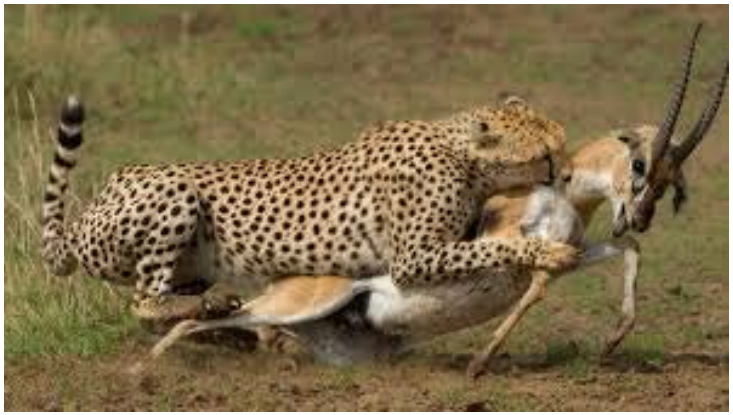
Ecophysiologie: Alimentation: Optimal Foraging Theory
La théorie de la recherche optimale de nourriture











OPTIMAL FORAGING THEORY BASIC RULE:

GREATEST ENERGY GAIN WITH THE MINIMAL ENERGY COST

Optimal Foraging Model

- **Optimal foraging model** views foraging behavior as a compromise between benefits of nutrition and costs of obtaining food
- The costs of obtaining food include energy expenditure and the risk of being eaten while foraging
- Natural selection should favor foraging behavior that minimizes the costs and maximizes the benefits

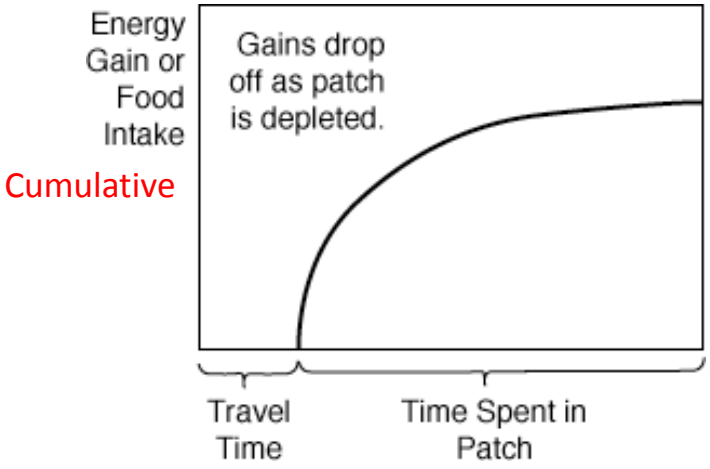
<u>Gain</u>:

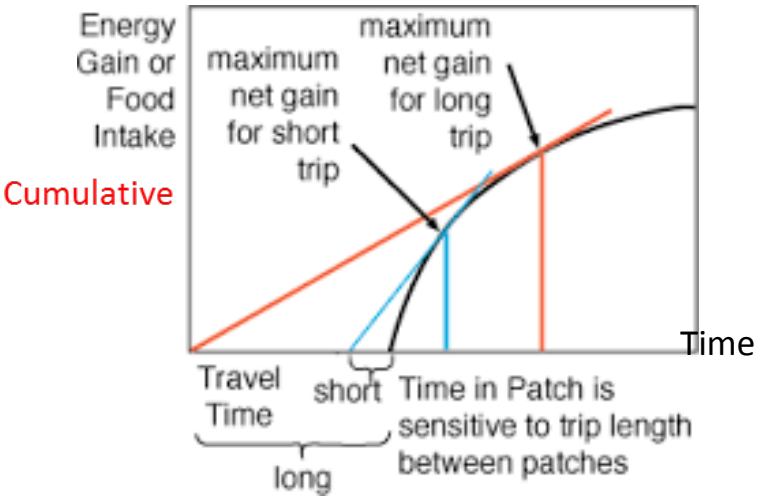
- Energie
- Nutriments

<u>Coûts</u>:

- voyage
- préparation proie
- risque de prédation
- forfait d'autres activités à haute valeur sélective (ex. reproduction)

Optimal foraging – effect of travel time to patch
Recherche optimale de nourriture – effet du temps de trajet





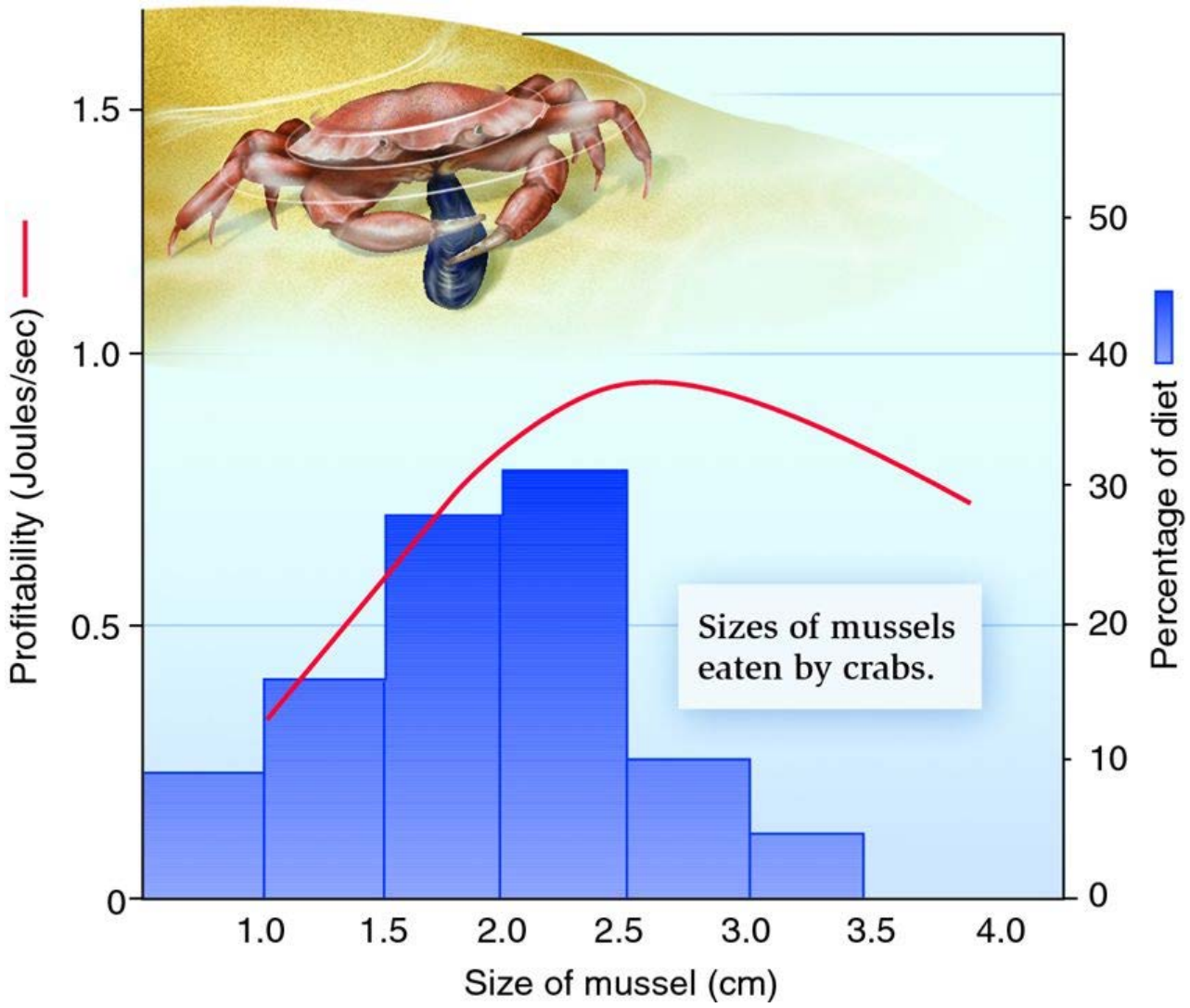
Maximum net gain = highest rate of gain for a given travel time, before gains diminish significantly



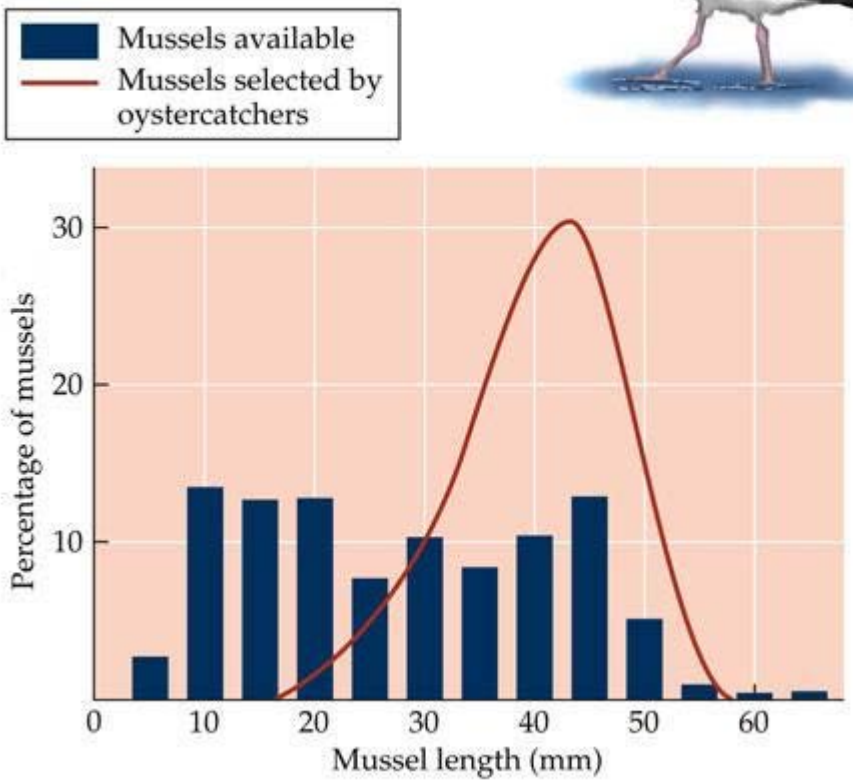
"I think we finally mastered foraging theory."

Optimal foraging theory – type of prey

Théorie optimal de recherche de nourriture – type de proie

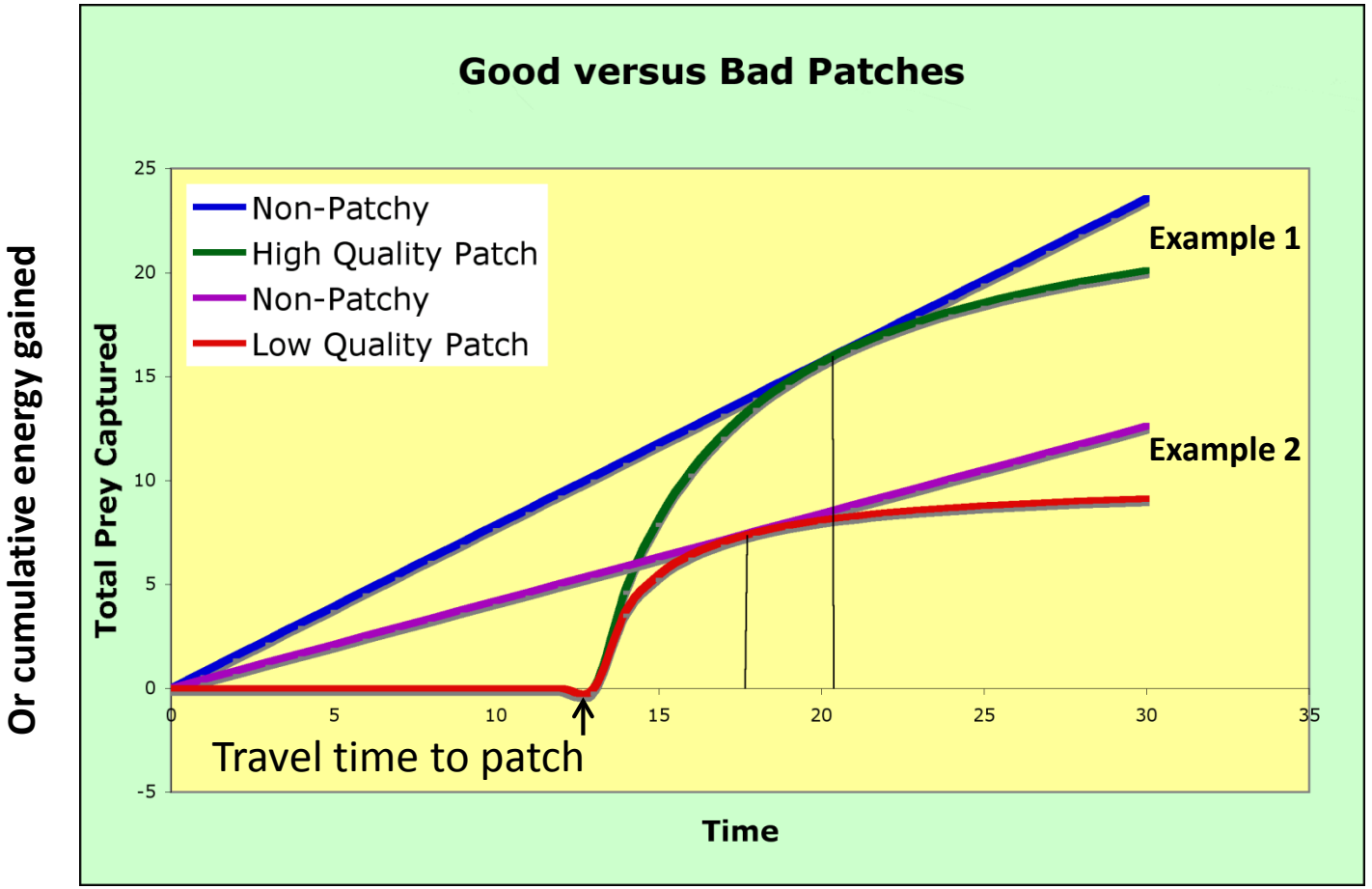


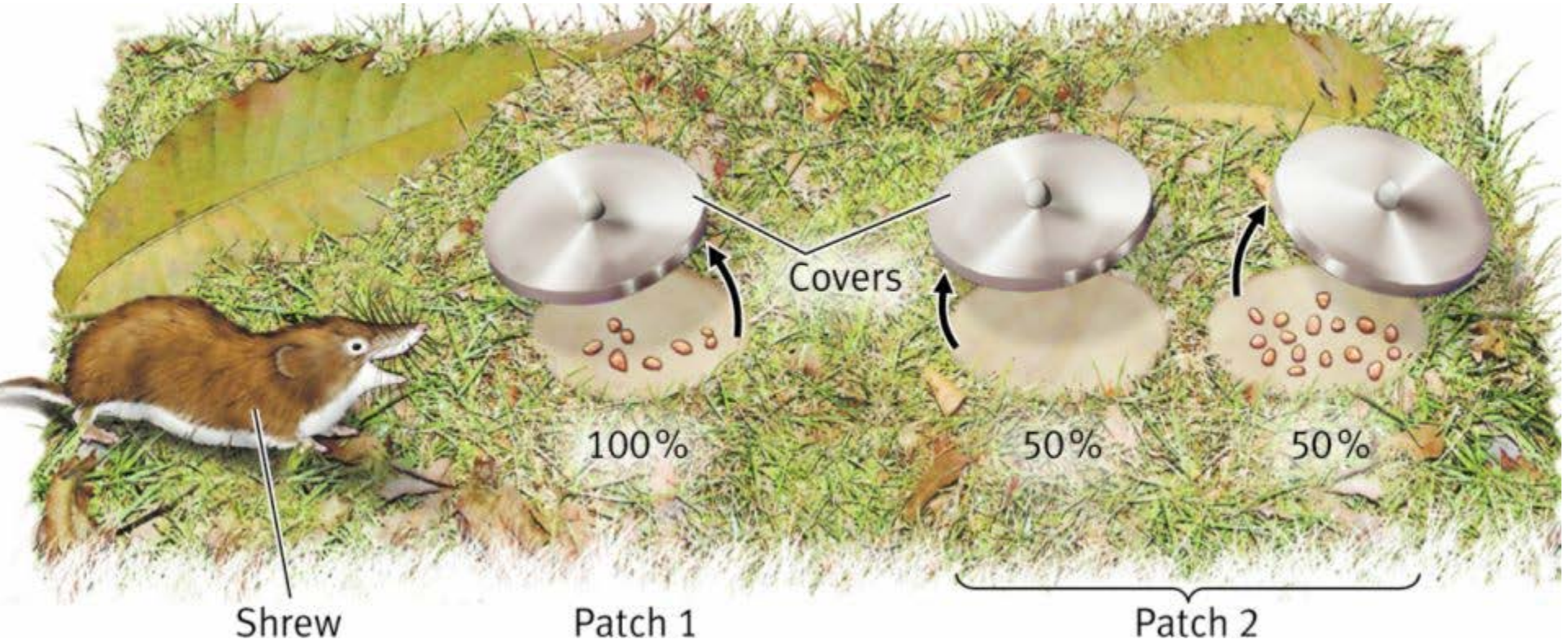




Contrainte: ne peut pas ouvrir les + gros (refuge de taille)

Optimal foraging – effect of patch quality (density, energy or nutritive value)
La recherche optimale de nourriture – effet de la qualité (densité, energie, valeur nutritive) du patch





Risk-sensitive optimal foraging model. Imagine a shrew that must decide between patch 1 that always yields **8** pellets under the cover and patch 2 in which **half** the time there are no pellets and half the time there are **16** pellets. Both patches have the same mean (8), but the **variance** is greater in patch 2. If our forager takes **variance** into account, it is foraging in a **risk-sensitive** manner.

La recherche optimale de nourriture
Optimal Foraging Theory (OFT)

OFT cherche à expliquer le comportement alimentaire, nécessairement constitué de choix, car aucun animal ne peut manger toute la matière organique disponible. Elle se concentre sur les habitats où la nourriture elle-même n'est pas limitante. L'idée – clé est que les animaux recherche de la nourriture de façon à ce qu'ils maximisent leur gain d'énergie avec le moins de temps et effort dépensés.

E_i = énergie (k J) de l'item i

h_i = temps de manipulation de l'item i (capture, ingestion, digestion)

E_i / h_i = profitabilité de l'item i

S_i = temps passé à la recherche de l'item i

Le prédateur cherche à optimiser

$$\frac{\bar{E}}{\bar{h} + \bar{s}}$$

Quand un prédateur rencontre un item totalement nouveau, il peut choisir de le manger. Il devrait l'adopter dans son régime alimentaire si

1) s'il le reconnaît en tant que nourriture potentielle

2) $E_{\text{i nouveau}} / h_{\text{i nouveau}} + s_{\text{i nouveau}} > \frac{\bar{E}}{\bar{h} + \bar{s}}$

Optimal foraging and limitations of digestive rate

The Digestive rate model (DRM, Modèle du Taux de Digestion) of optimal foraging makes the same basic assumption as do other models, i.e. animals select food in order to maximize their rate of energy intake. However, it makes the point that some animals should select food on the basis of its 'digestive quality' (net energy content/digestive turnover time) rather than its 'profitability' (net energy content/handling time). Food or prey can come at the expense of indigestible bulk that takes up capacity in the digestive tract. The DRM states that animals under some circumstances will maximize their digestive rate instead of their food ingestion rate or foraging behaviour as predicted by the Contingency Model, the basic and most commonly used model of OFT. The DRM is a useful alternative to the foraging models that maximize ingestion rate only, as it explains some behaviour currently looked on as deviation from OFT.

This leads to a number of insights:

- Predators with short handling times relative to their search times should be generalists and include a wide range of items in their diet. They will hunt and eat every prey they encounter since the prey is far apart and the handling time is short.
- Predators with long handling times relative to their search time should be specialists and only include the most efficient items in their diet. They will maximize their efficiency by only eating prey with high energy contents since the handling time is long and the search time is short.
- Predators should be generalists in unproductive environments and specialists in productive environments.
- Predator-prey co-evolution often makes it non-profitable for a prey item to be included in the diet, since many anti-predator defenses increase handling time. Examples include porcupine quills, the palatability and digestibility of the poison dart frog, crypsis and other predator avoidance behaviors.
- The Digestive Rate Model suggests that species can avoid predation by increasing the amount of indigestible bulk, e.g. shell material for molluscs, seed husks or fibre content in plants.

If the predator forages unselectively, in T_s seconds it will obtain the following amount of food (E):

$$E = T_s(\lambda_1 E_1 + \lambda_2 E_2)$$

and this will take the following total time (T):

$$T = T_s + T_s(\lambda_1 h_1 + \lambda_2 h_2),$$

in other words searching time plus handling time.

The overall rate of intake of the predator (E/T) is therefore:

$$\frac{E}{T} = \frac{\lambda_1 E_1 + \lambda_2 E_2}{1 + \lambda_1 h_1 + \lambda_2 h_2}.$$

Note that T_s has cancelled out.

Now suppose that prey type 1 is more profitable than type 2 ($E_1/h_1 > E_2/h_2$). In order to maximize E/T the predator should eat only prey type 1 if

$$\frac{\lambda_1 E_1}{1 + \lambda_1 h_1} > \frac{\lambda_1 E_1 + \lambda_2 E_2}{1 + \lambda_1 h_1 + \lambda_2 h_2},$$

i.e. the rate of energy gain from prey type 1 alone is greater than that from both types. This equation can be rearranged as follows:

$$\frac{1}{\lambda_1} < \frac{E_1}{E_2}(h_2 - h_1) \qquad (4.1)$$

Table 4.1. Assumptions and predictions of the classical optimal diet model.

(a) Assumptions

1. Prey value is measurable net energy or some other comparable single dimension
2. Handling time is a fixed constraint
3. Handling and searching cannot be done at the same time. If this were not true there would be no need for the animal to forage selectively
4. Prey are recognized instantaneously and with no errors
5. Prey are encountered sequentially and randomly: the expected time to find the next prey of type i is always $1/\lambda_i$
6. Energetic costs per second of handling are similar for different prey
7. Predators are designed to maximize rate of energy (or other measure of value) intake

(b) Predictions

1. The highest ranking prey (in terms of profitability) should never be ignored
2. Low-ranking prey should be ignored according to the rule specified in equation 4.1
3. The exclusion of low-ranking prey should be all-or-nothing, depending on the direction of the inequality in equation 4.1
4. The exclusion of low-ranking prey does not depend on their own values of λ. Note that λ_2 does not appear in equation 4.1. This point can be grasped intuitively by realizing that when type 1 are sufficiently abundant 'time out' from search to handle type 2 is not profitable. In other words E_2/h_2 is less than what could be obtained by searching for and consuming the next type 1, so the abundance of type 2 is irrelevant

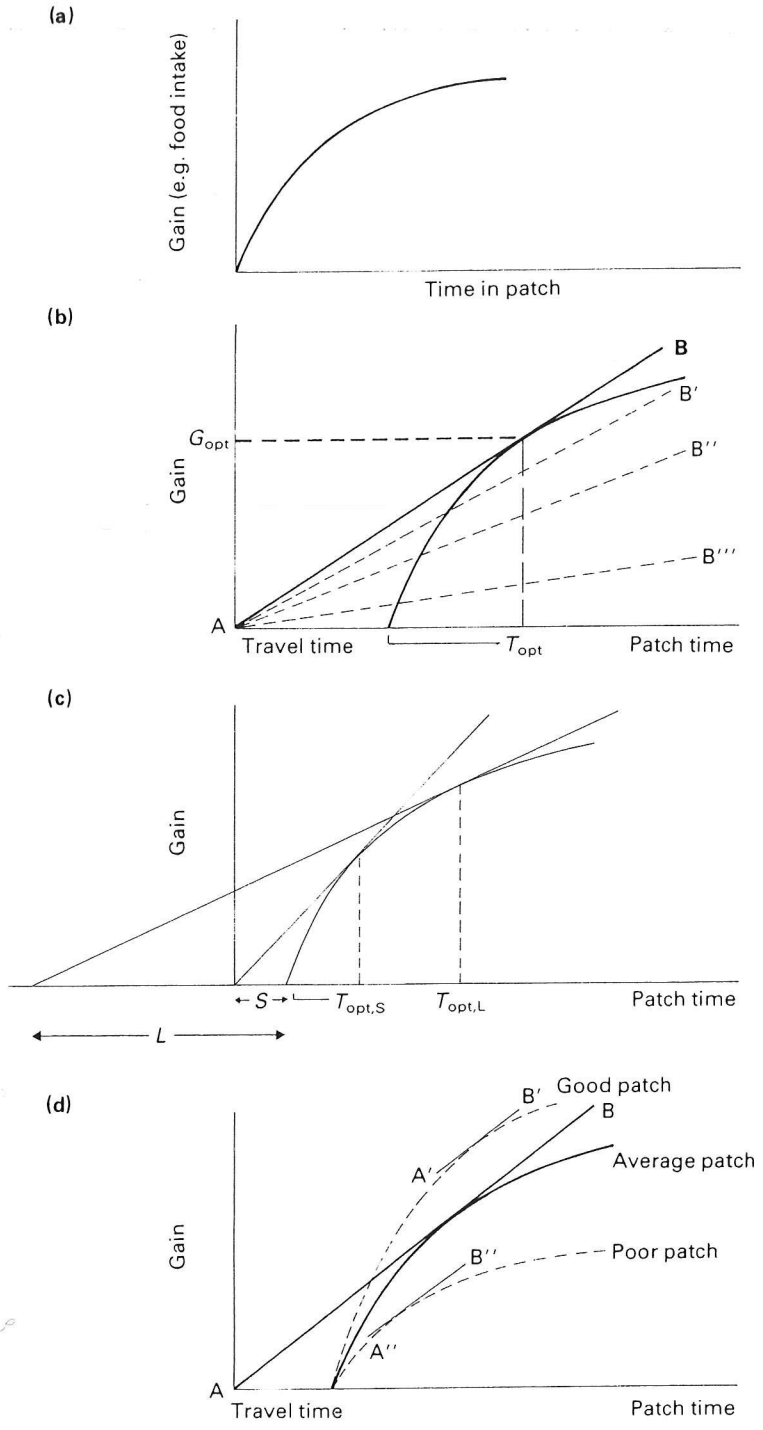


Fig. 4.2 The marginal value model. **(a)** An example of a gain curve arising from resource depression. **(b)** The 'tangent' method of finding the optimal patch resident time T_{opt}. The rays (AB) from the origin have slopes E/T and the steepest feasible line is the solid one. **(c)** As travel time increases, so does T_{opt}. For a short time (S), $T_{opt,S}$ is less than $T_{opt,L}$ for a long travel time (L). **(d)** When there are several different patch types, each should be exploited until the gain drops to the average for the environment (line AB).

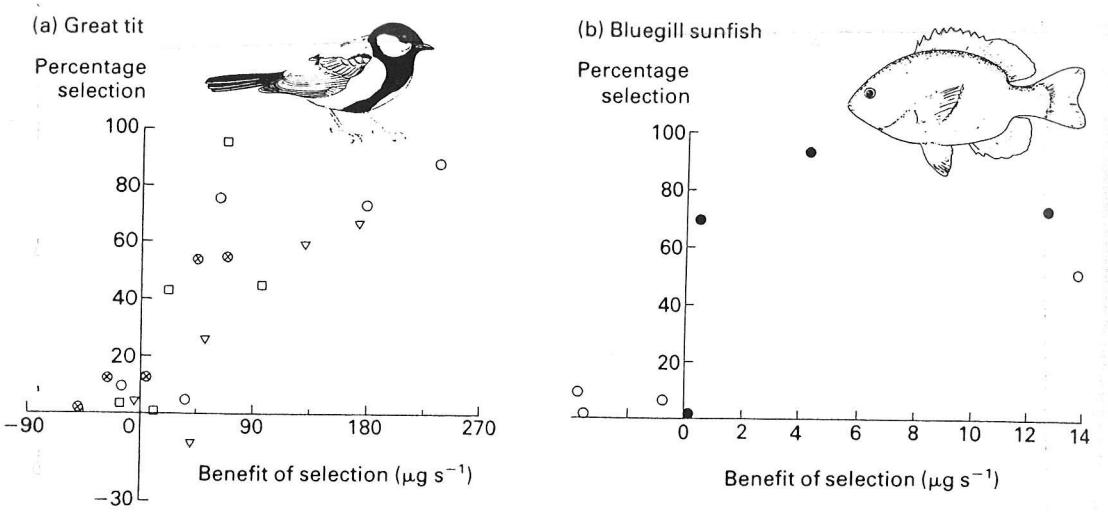


Fig. 4.1 (a) A test of the classical 'optimal diet model' with great tits. The graph shows on the *y*-axis the degree of selectivity for the larger (more profitable) of the two prey sizes, and on the *x*-axis the difference in intake rate obtained by selective and non-selective predation. To the right of the *y*-axis, 100% selection is predicted by the model. The four symbols are for four different animals, each tested at several values of λ_1 and λ_2. The birds become more selective to the right, as predicted, but they do not show a step function with all-or-nothing selection. The graph indicates that they take small prey when according to the model they should not. Another kind of possible error, rejecting large prey, is not shown on the graph. (b) Data from Werner & Hall 1974 for bluegill sunfish eating *Daphnia* plotted in a similar way. The data are for experiments with 20, 50, 200 and 300 large prey per tank, and corresponding variations in the density of medium and small prey. The closed circles refer to selection for large and medium prey (i.e. rejecting small ones) and the open circles refer to the selection of large only (rejecting medium and small). Data were taken from Tables 2 and 6 and Figure 4 of Werner & Hall 1974. As with the great tits, sunfish become more selective as they cross the threshold of equation 4.1, but they show partial preferences.

OPHELIA, 17(2): 239-246 (November 1978)

EFFICIENCY OF PARTICLE RETENTION IN 13 SPECIES OF SUSPENSION FEEDING BIVALVES

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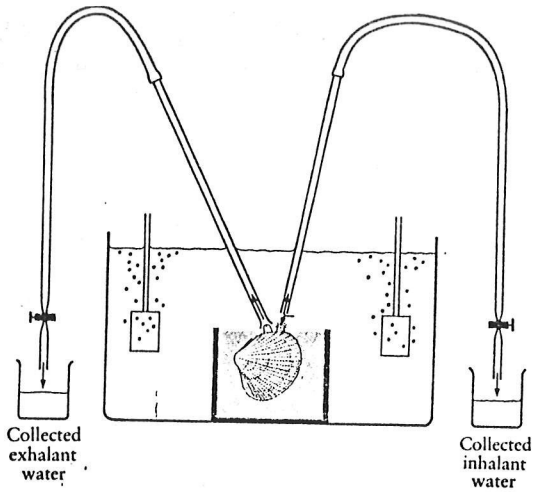


FIG. 2. Experimental set up. The glass-tubes collect water from the inhalant and exhalant currents by gravity.

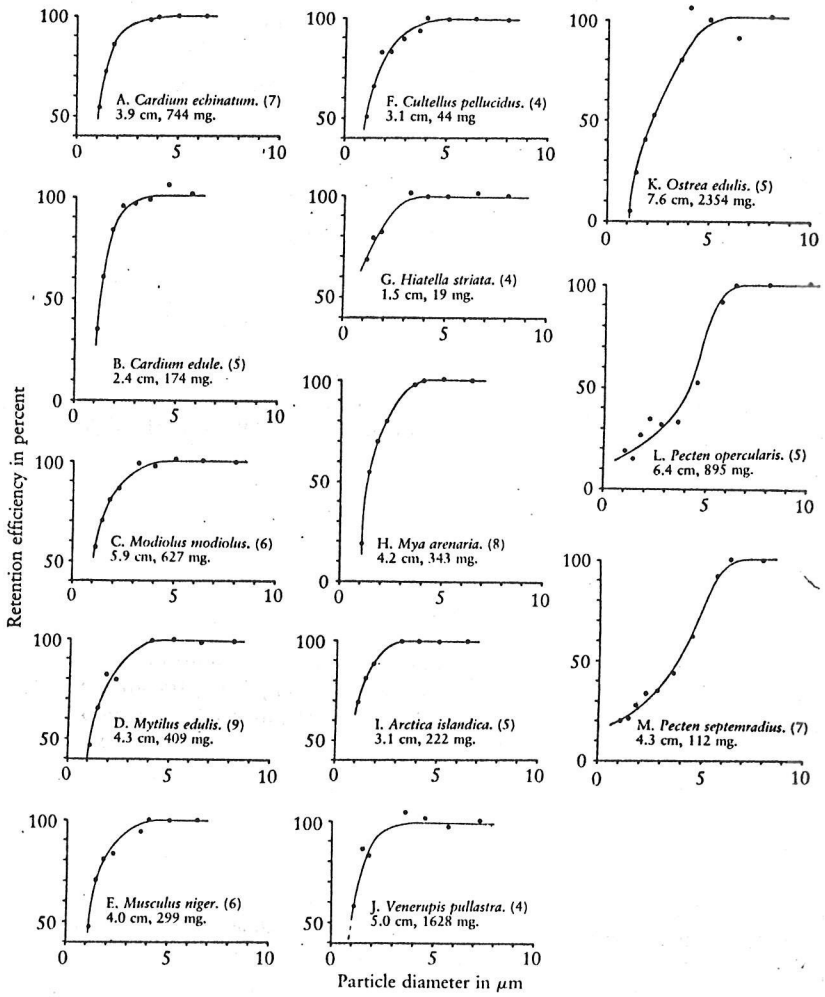


FIG. 3. Retention as a function of particle size in undisturbed bivalves. Each value is the mean of the number of measurements indicated by the figures in brackets. The figures given beneath the names of species are shell length and dry weight of soft parts, respectively.

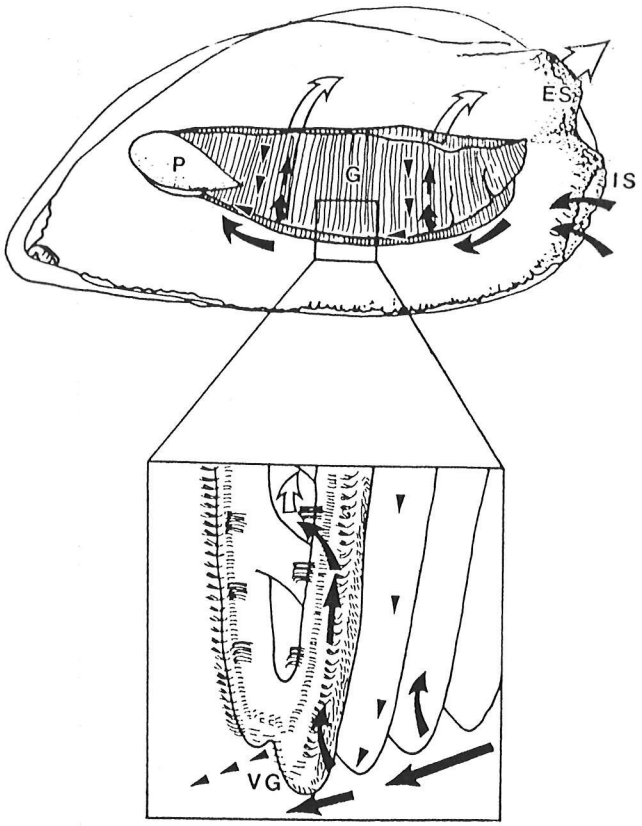


Fig. 3 Principal pathways of current flow and particle transport in a representative bivalve, *Mytilus edulis*. Water arrives via the incurrent siphon (*IS*), and the frontal surface of the gill (*G*) is exposed to a postero-anterior flow at the ventral margin, while the rest of the frontal surface is swept by a ventro-dorsal flow, with a progressive through component to the abfrontal region and out the exhalent siphon (*ES*). *Solid arrows* indicate current flow prior to passage across gills; *open arrows* show current flow after crossing through to abfrontal region and on to exhalent siphon. *Arrowheads* represent particle transport typical of the many species which possess homorhabdic gills and a ventral particle groove (*VG*) (*P* labial palp)

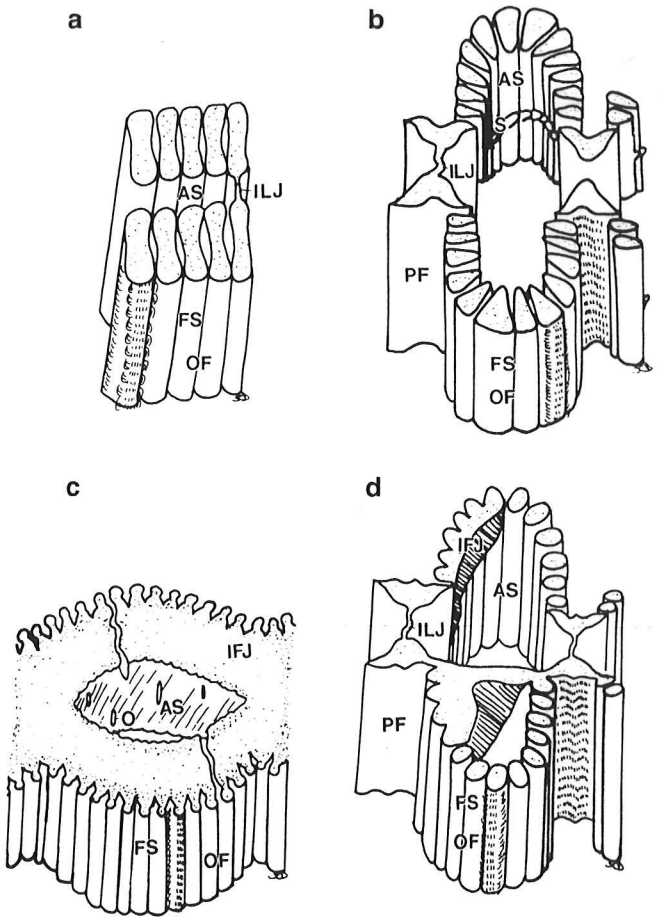


Fig. 1a–d Diagram of the principal gill types in suspension-feeding bivalves. Transversally sectioned demibranchs are illustrated; the *dotted surfaces* are those seen on transverse histological sections. **a** Homorhabdic filibranch. Ordinary filaments (*OF*) connected by interlamellar junctions (*ILJ*). **b** Heterorhabdic filibranch. Principal filaments (*PF*) and ordinary filaments, joined by interlamellar junctions and ciliated spurs (*S*). **c** Homorhabdic eulamellibranch. Ordinary filaments, joined by interfilamentar junctions (*IFJ*). O: ostia. **d** Heterorhabdic pseudolamellibranch. Principal filaments and ordinary filaments, joined by interlamellar junctions and interfilamentar junctions. *AS* Abfrontal surface, *FS* frontal surface

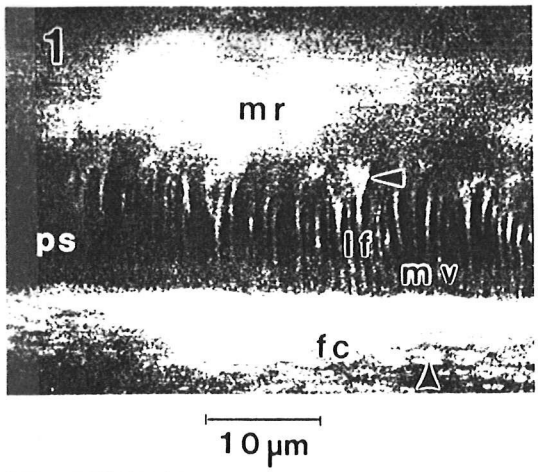
Mucociliary Transport in Living Tissue: The Two-Layer Model Confirmed in The Mussel *Mytilus edulis* L.

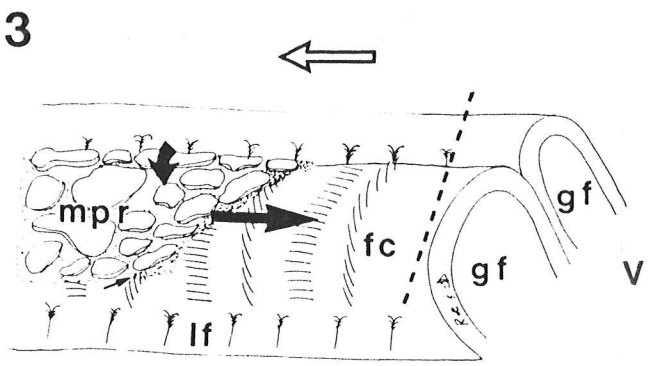
PETER G. BENINGER[1,*], JOHN W. LYNN[2], THOMAS H. DIETZ[2], AND HAROLD SILVERMAN[2]

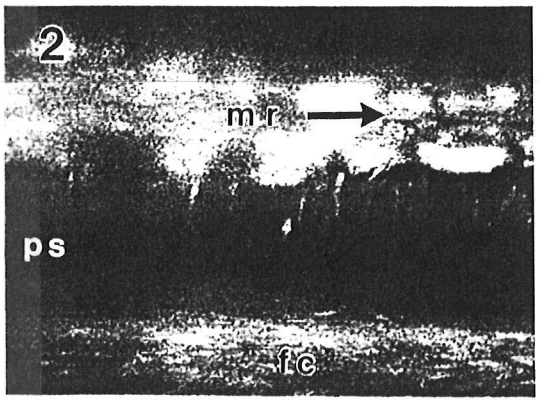
[1]*Département de Biologie, Université de Moncton, Moncton N.B. Canada E1A 3E9; and*
[2]*Department of Zoology and Physiology, Louisiana State University, Baton Rouge, Louisiana 70803*

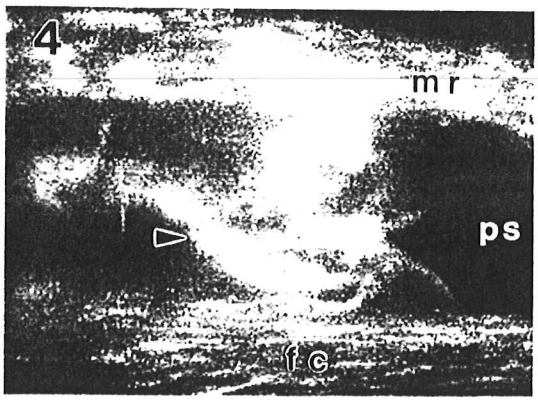
The present study combined video confocal laser microscopy (1) and tissue reflectance and autofluorescence to visualize mucus position and mucociliary transport in excised living gill tissue from the blue mussel Mytilus edulis. *Rafts of mucus and embedded particles were transported atop a periciliary space traversed by frontal cilia, which engaged the mucus layer and moved it during the effective stroke, disengaging and completing the cycle during the recovery stroke. These results confirm the two-layer model for mucociliary transport in the mussel gill. Given the conservative nature of ciliated epithelial structure and function (2, 3), and the structural similarity of mucociliary surfaces as diverse as terrestrial vertebrate respiratory epithelium and molluscan gill, the two-layer mechanism of mucociliary transport may be a general feature of Metazoan biology.*

Reference: *Biol. Bull.* **193**: 4–7. (August, 1997)









Figures 1–2. Lateral view of frontal surface of *Mytilus edulis* gill filament. The two-layer structure is evident, comprising the periciliary space (ps), within which the frontal cilia (fc) beat, and a mucus raft (mr), propelled by the frontal cilia (arrow). The latero-frontal cirri (lf) are visible when within the plane of focus. The splayed cirral 'V' (upper arrowhead) belongs to one of the shorter cirri, which does not extend over the raft surface. The V width, nuclei (bottom arrowhead) and the height of the microvillar layer (mv) were used for scale calibration.

Figure 3. Interpretive drawing (not to scale) showing two transversally-sectioned gill filaments (gf), latero-frontal cirri (lf), frontal cilia (fc), position and direction of movement of mucus raft with included particle masses (mr, straight solid arrow), direction of latero-frontal cirral beat (curved solid arrow), direction of pallial fluid flow over filaments (hollow arrow), and direction of frontal cilia effective stroke (small arrow). Dotted line represents plane of view in Figs. 1, 2, 4. V, ventral orientation.

Figure 4. Live crustacean larva (arrowhead) trapped within the periciliary space (ps), between frontal cilia (fc) and mucus raft (mr).

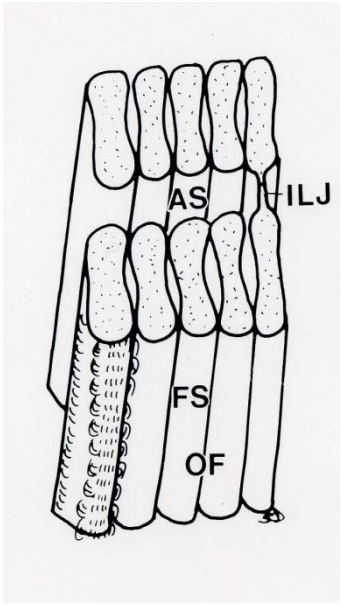
Scale bar valid for Figs. 1, 2, 4.

(WHAT I HAVE LEARNED ABOUT) PRINCIPLES OF PARTICLE PROCESSING IN BIVALVES

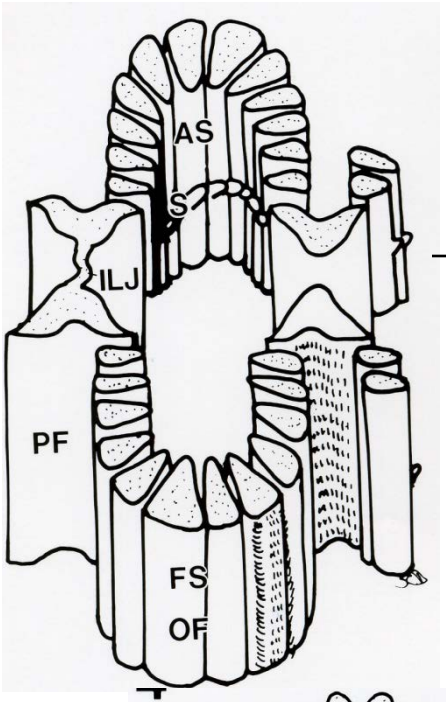
Peter G. Beninger
Université de Nantes
France

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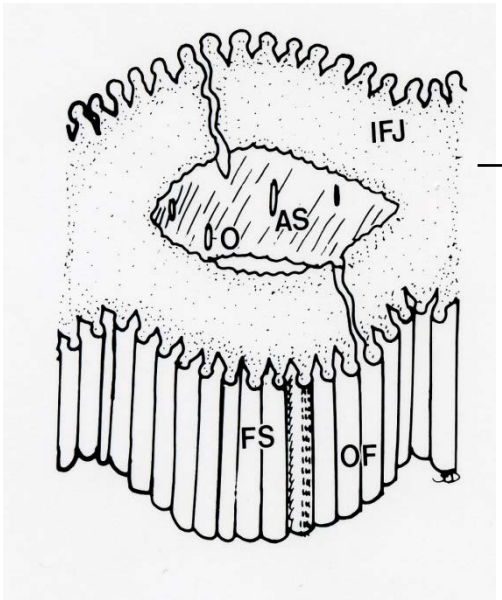
4 principal bivalve gill types (processing systems)



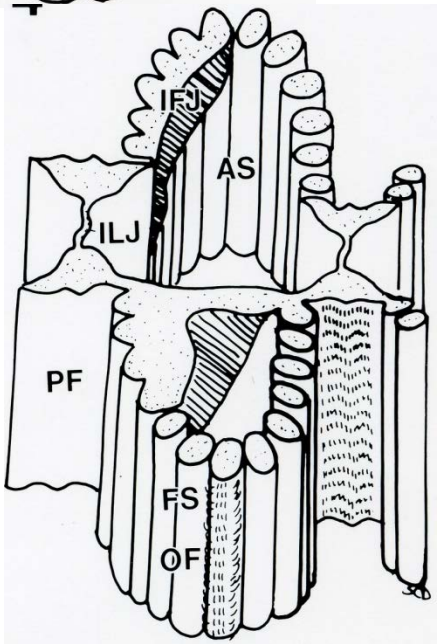
Homorhabdic filibranch (Mytilidae)



Heterorhabdic filibranch (Pectinidae)

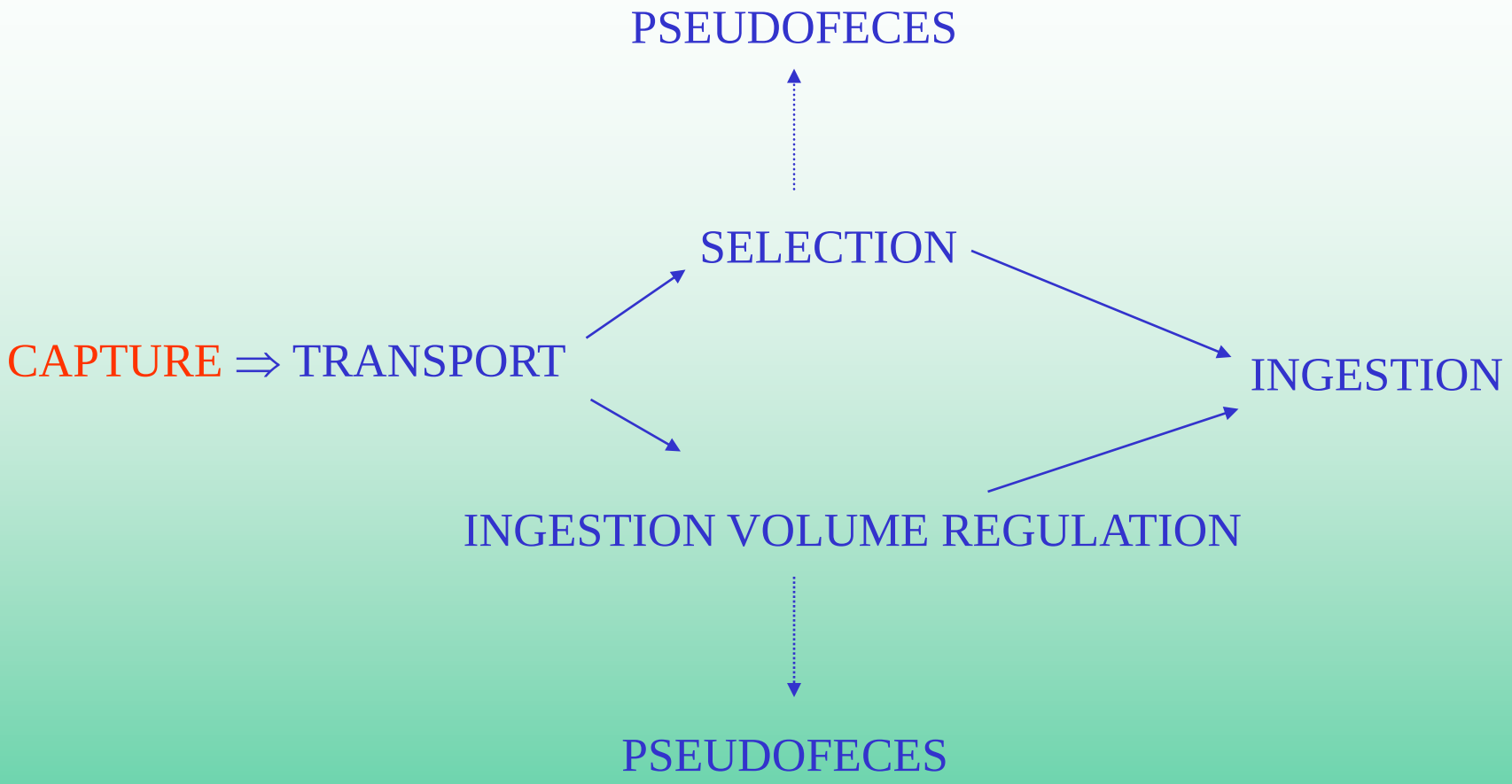


Eulamellibranch (Heterodonta)



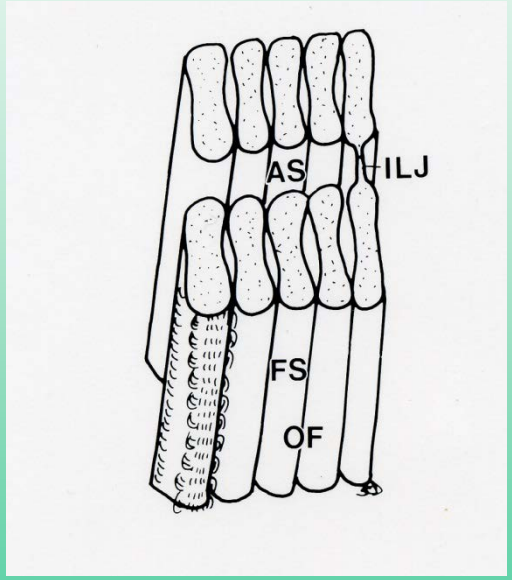
Pseudolamellibranch (Ostreidae)

V

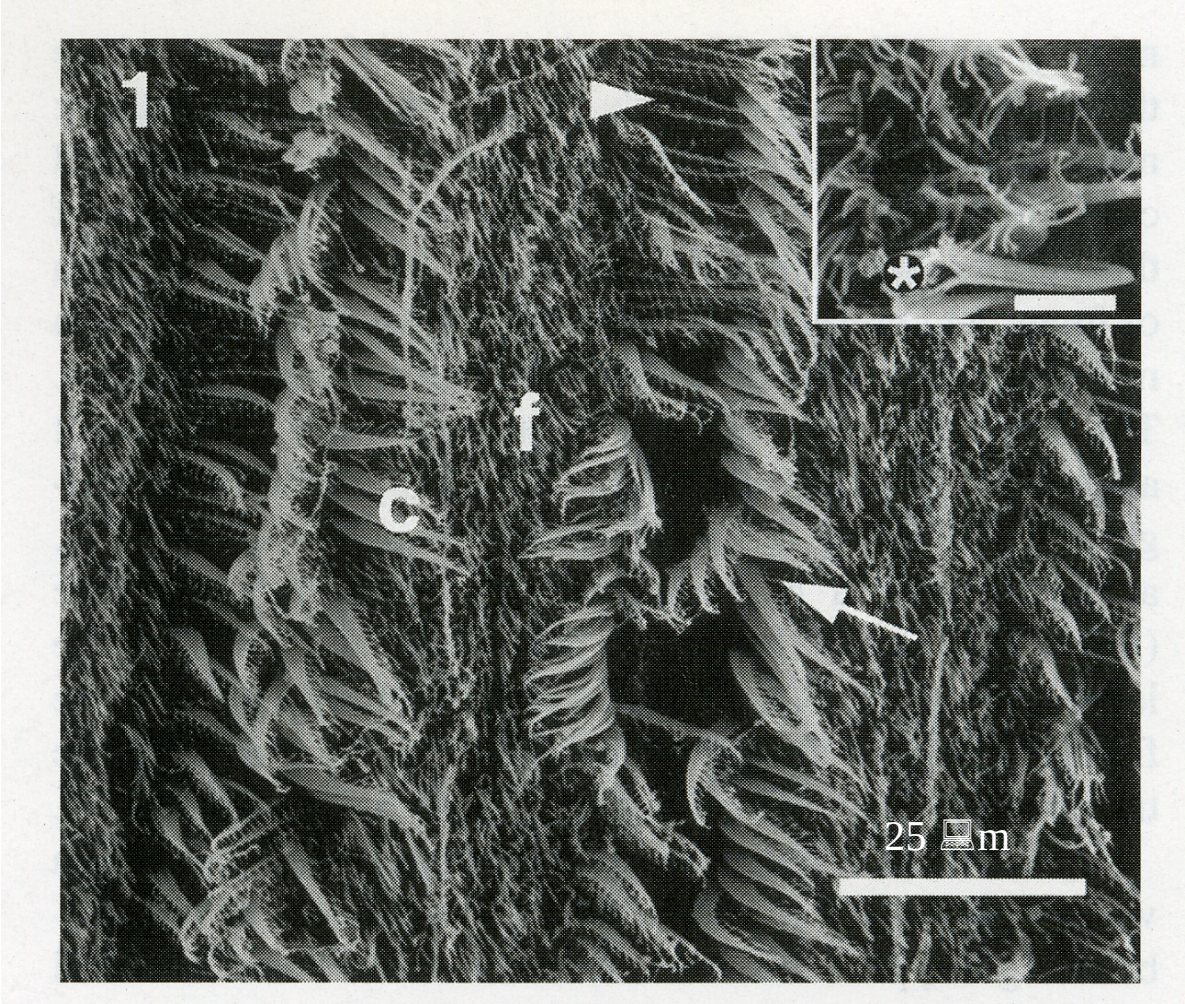


BASIC STEPS IN PARTICLE PROCESSING

1. THE HOMORHABDIC FILIBRANCH SYSTEM

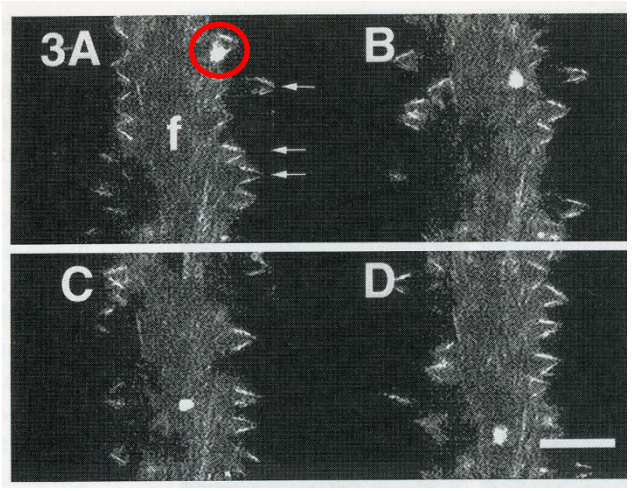


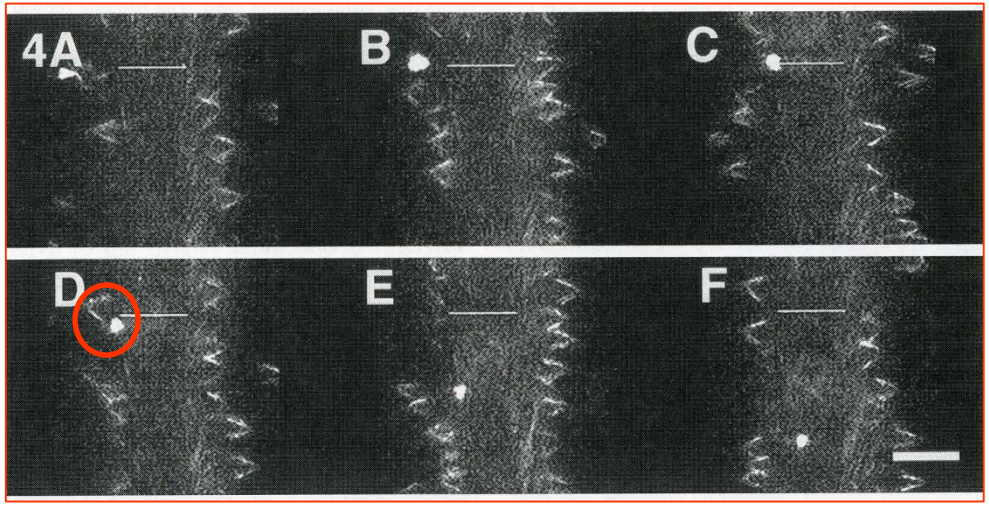
CAPTURE

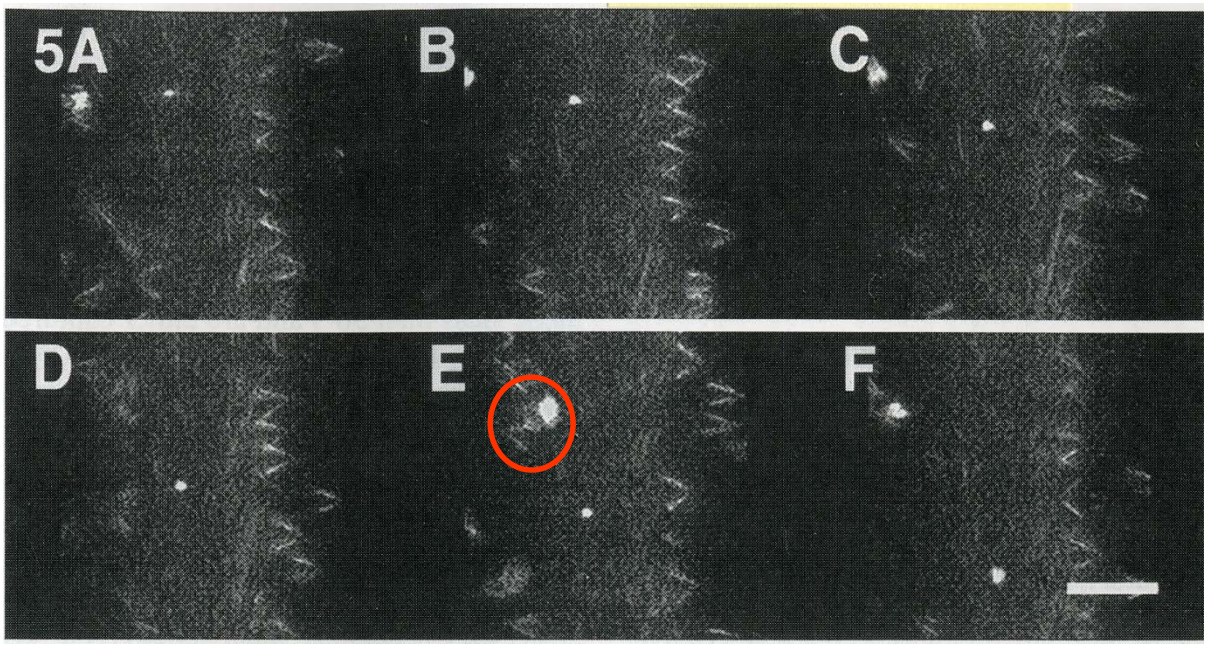


Capture equipment - Mytilus edulis latero-frontal cirri
Silverman et al Biol. Bull. 1997

CAPTURE

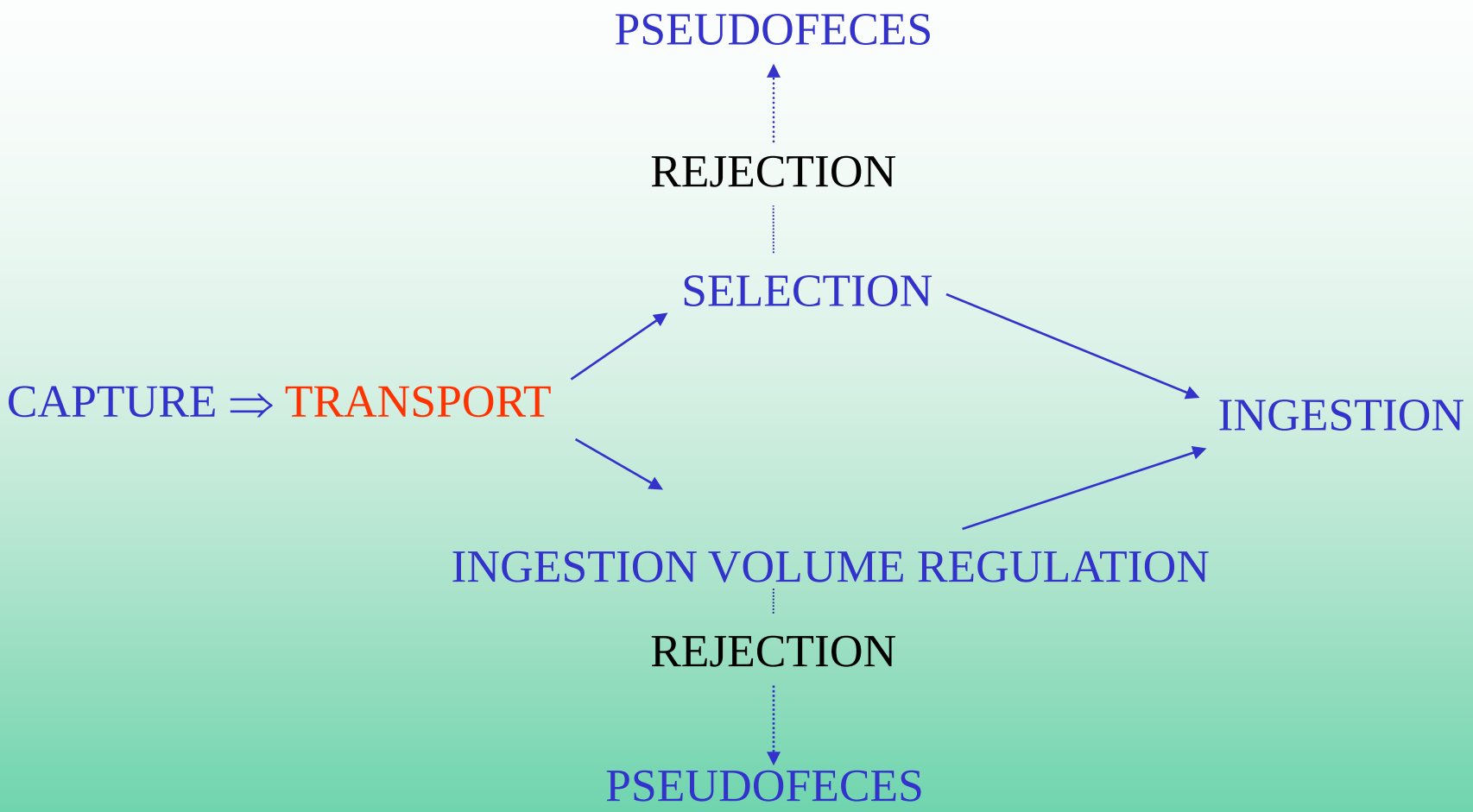






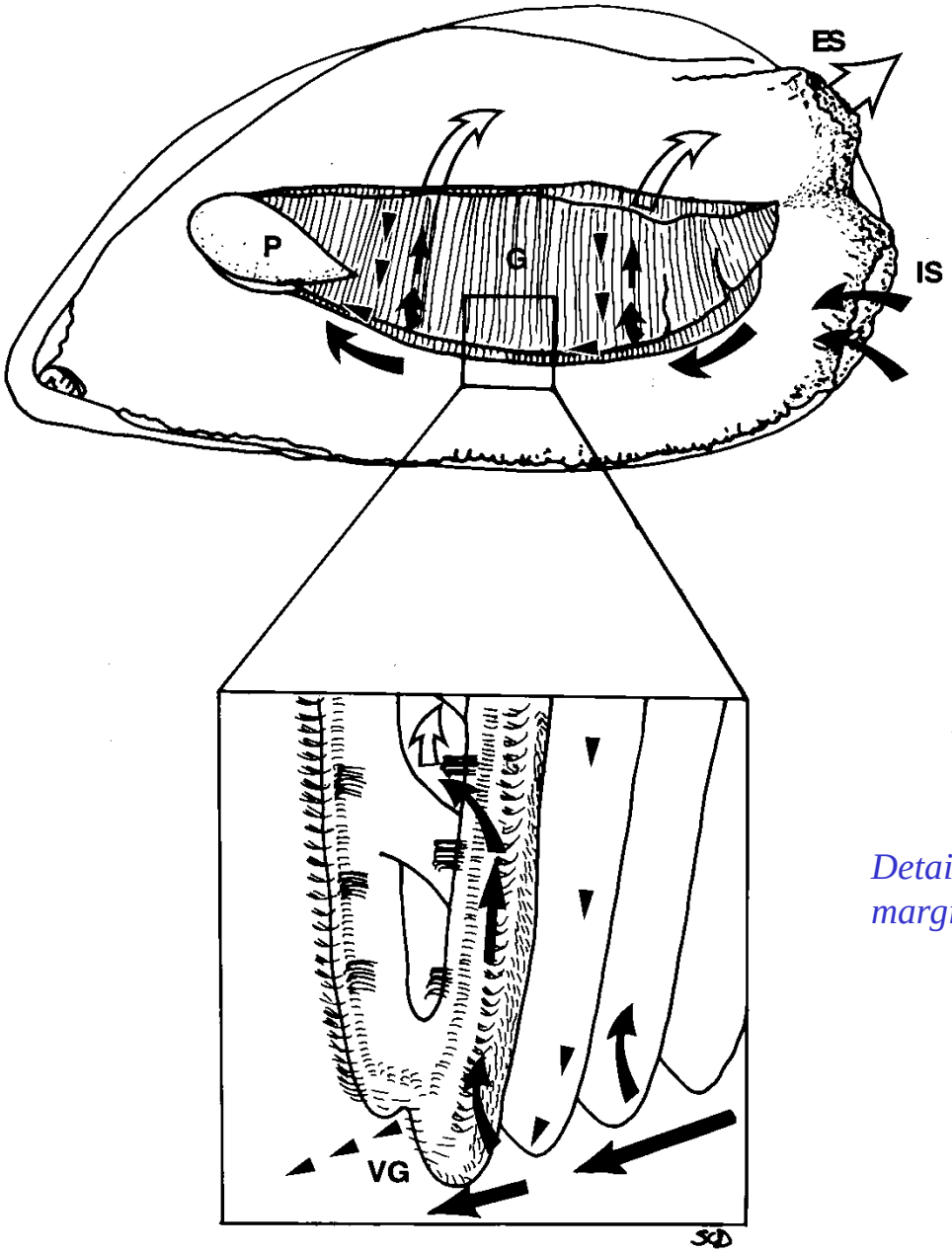
*Particle capture in a bivalve with latero-frontal cirri (*Mytilus edulis*): close-range events. Confocal laser high-speed video microscopy of live gill filaments with fluorescent particles.*

Intimate contact with cirri, not just baffle effect.



BASIC STEPS IN PARTICLE PROCESSING

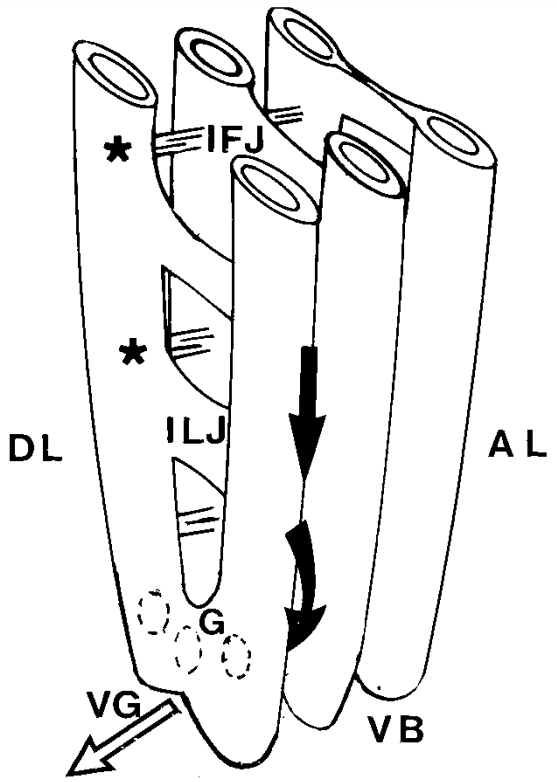
TRANSPORT

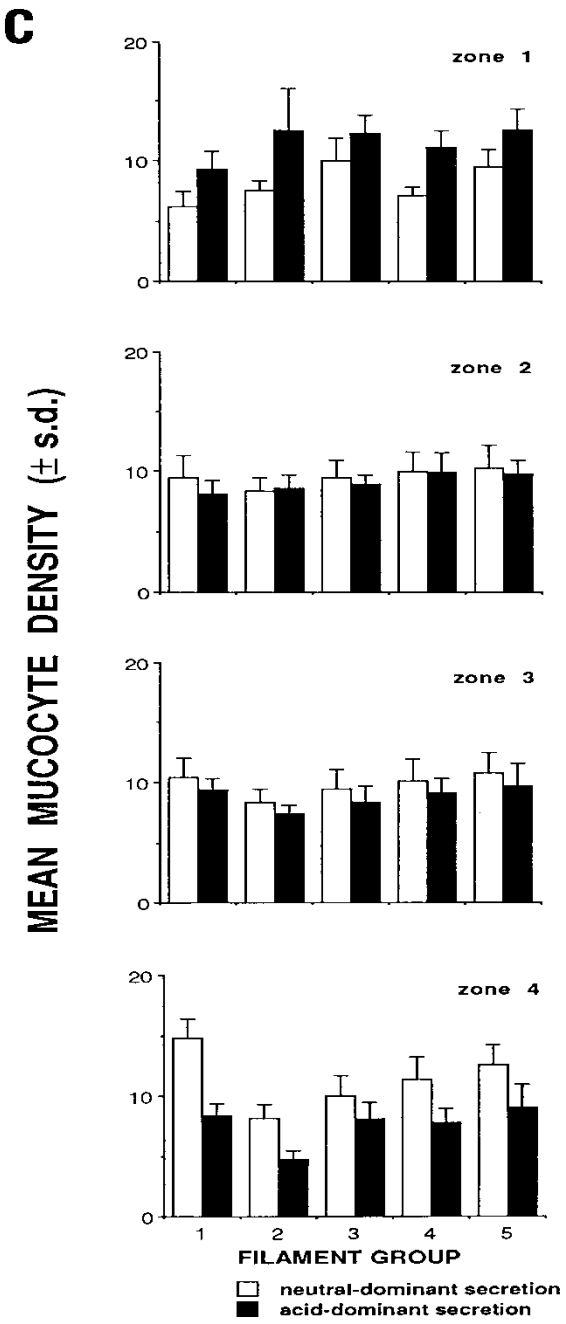


Homorhabdic filibranch (Mytilus edulis): water flow in pallial cavity, particle transport ventralward on gill, then anteriorly in ventral particle groove

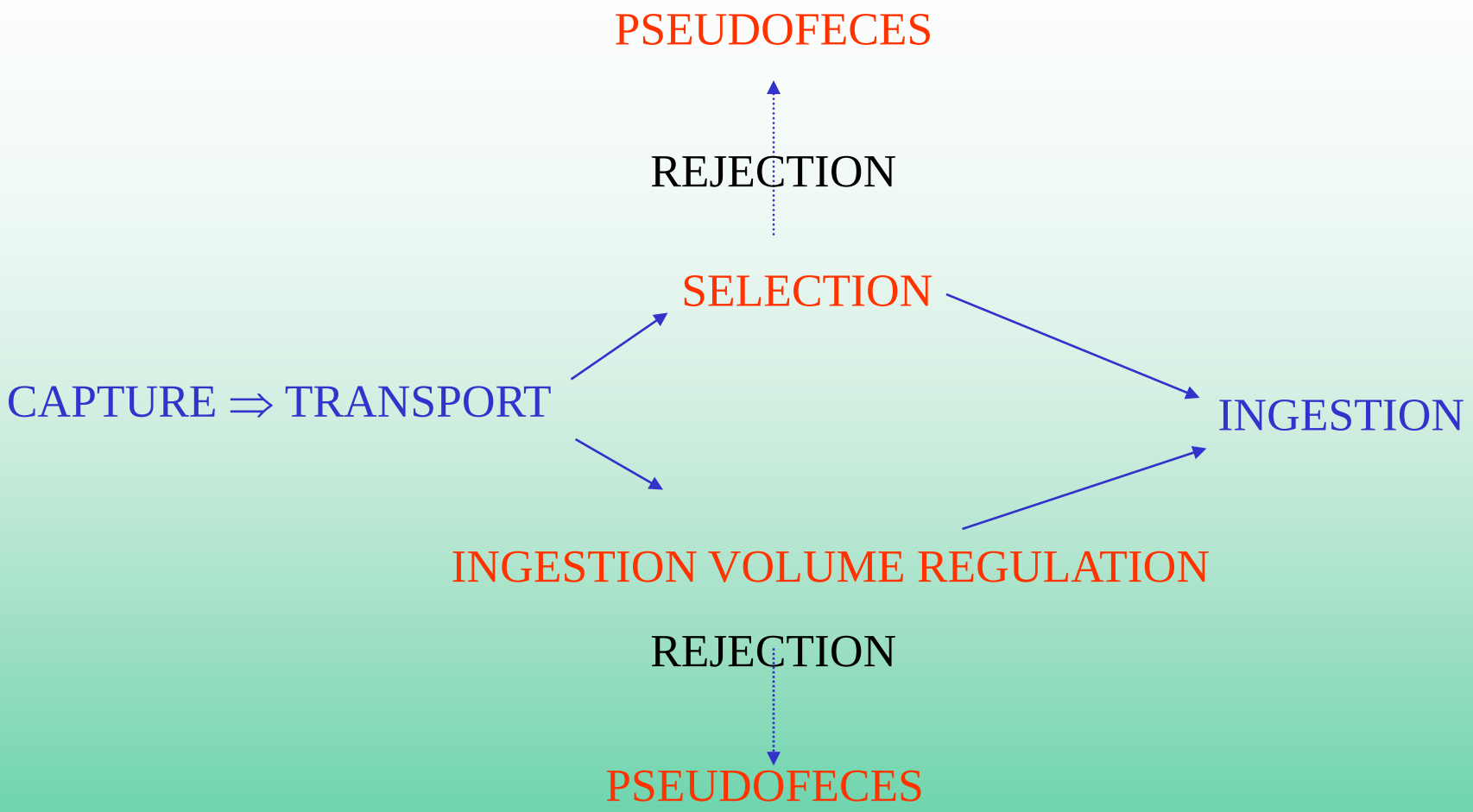
Detail of water flow and particle transport at ventral margin of gill

TRANSPORT



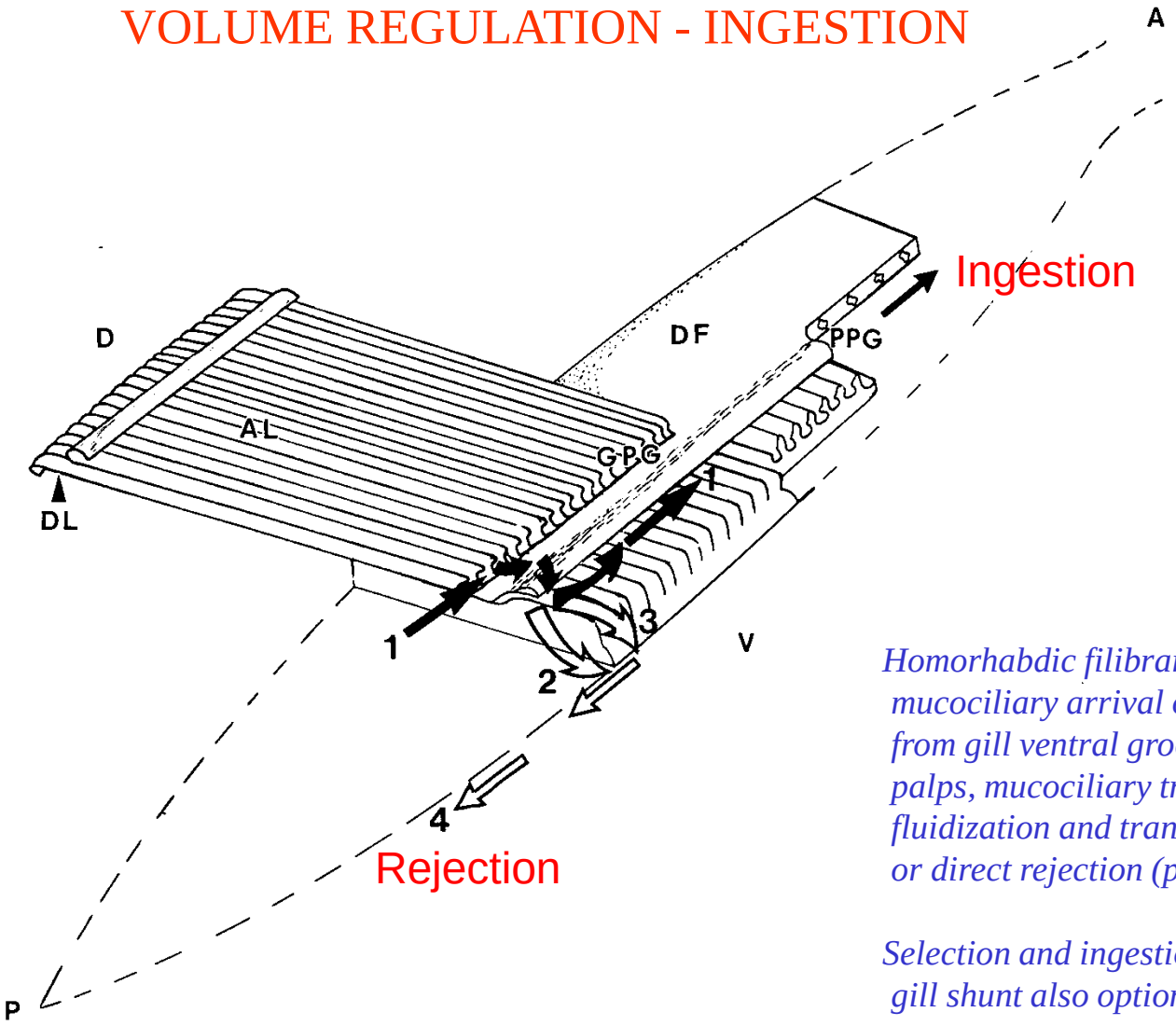


Homorhabdic filibranch (Mytilus edulis) gill and mucocyte distribution. All particles transported ventrally in relatively viscous MPS mixture.



BASIC STEPS IN PARTICLE PROCESSING

TRANSPORT – SELECTION – INGESTION
VOLUME REGULATION - INGESTION

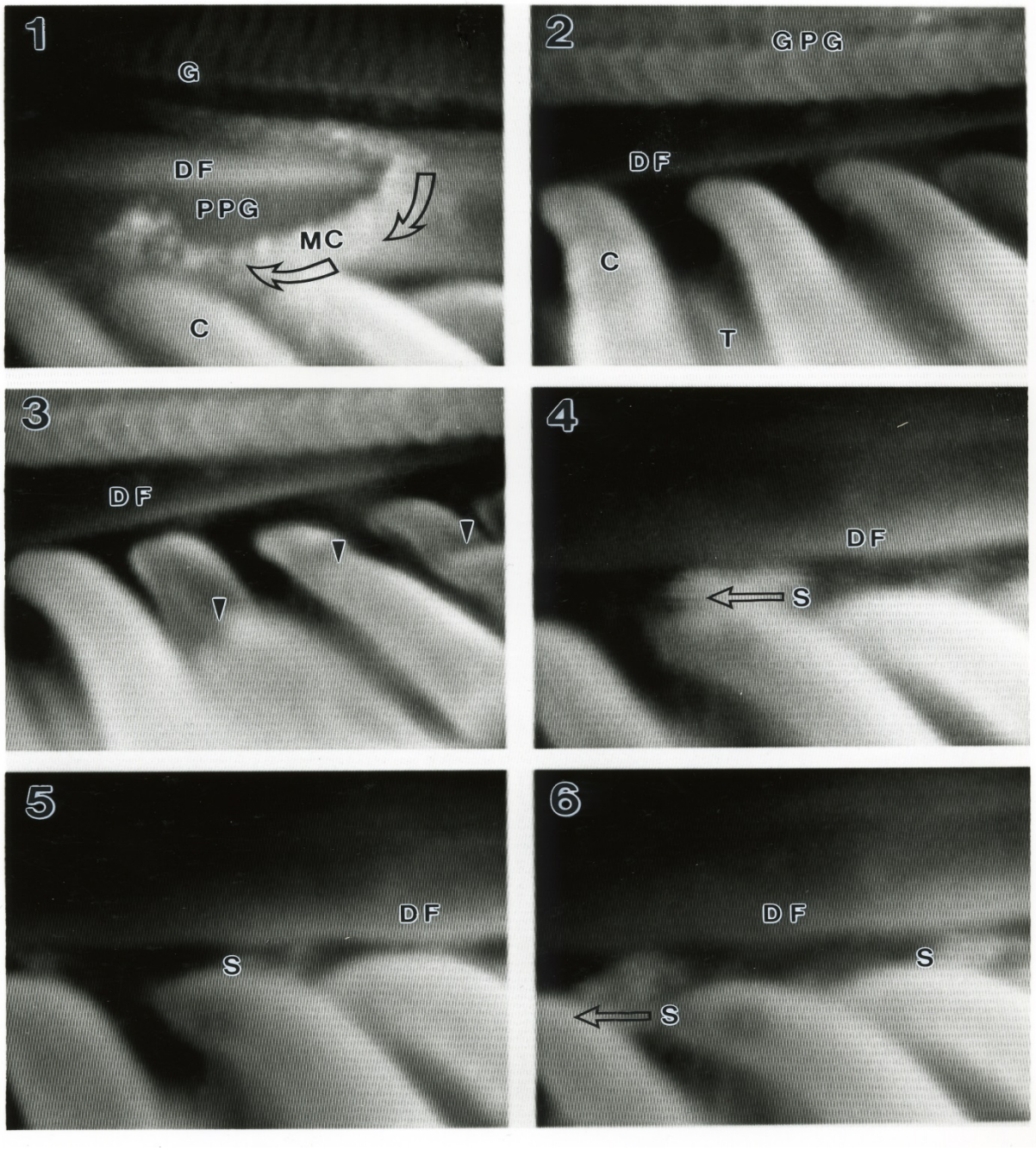


*Homorhabdic filibranch (*Mytilus edulis*): mucociliary arrival of mucus-particle cords from gill ventral groove to dorsal fold of labial palps, mucociliary transfer to palp particle groove, fluidization and transport to mouth (anterior) or direct rejection (posterior).*

Selection and ingestion volume regulation on palps; gill shunt also option for IVR..

Beninger and St-Jean MEPS 1997

TRANSPORT – SELECTION - INGESTION

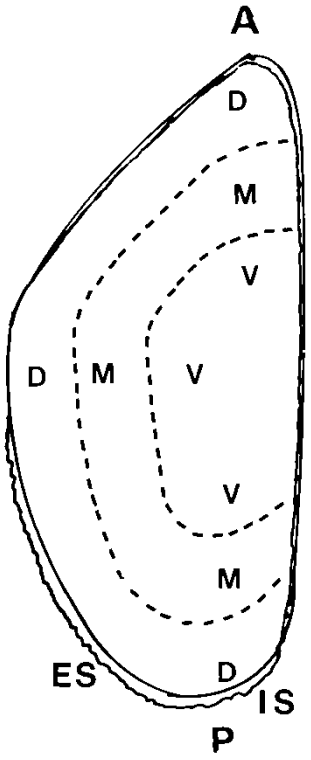


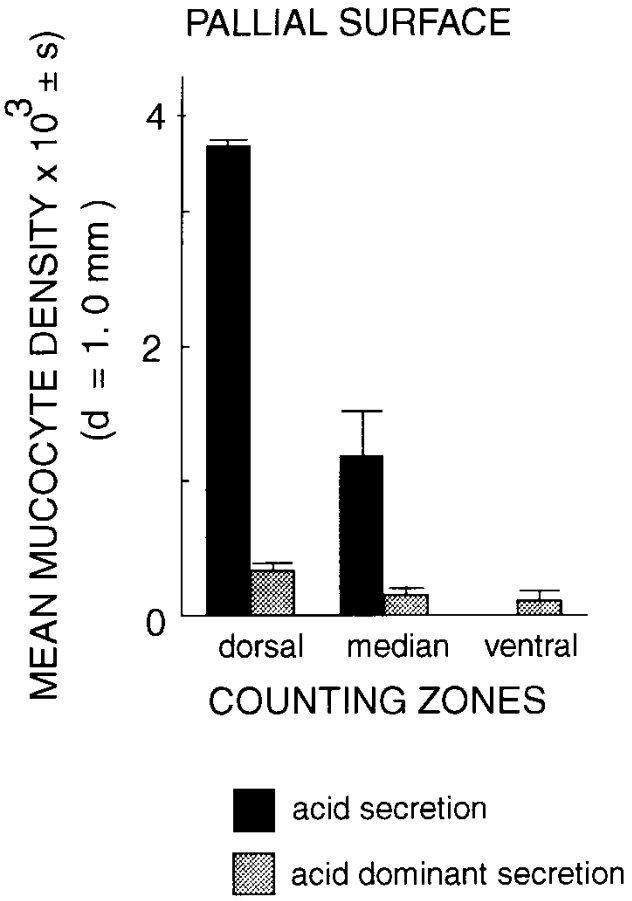
*Homorhabdic filibranch (*Mytilus edulis*): arrival of mucus-particle cord from gill particle groove, transfer over dorsal fold to palp particle groove, and anteriorward movement toward mouth (1) Movement of palp ridges effecting mechanical fluidization (2 – 6). Neutral MPS also added, effecting biochemical fluidization.*

Fluidization enables qualitative selection and determines state of ingested matter

Beninger & St-Jean MEPS 1997

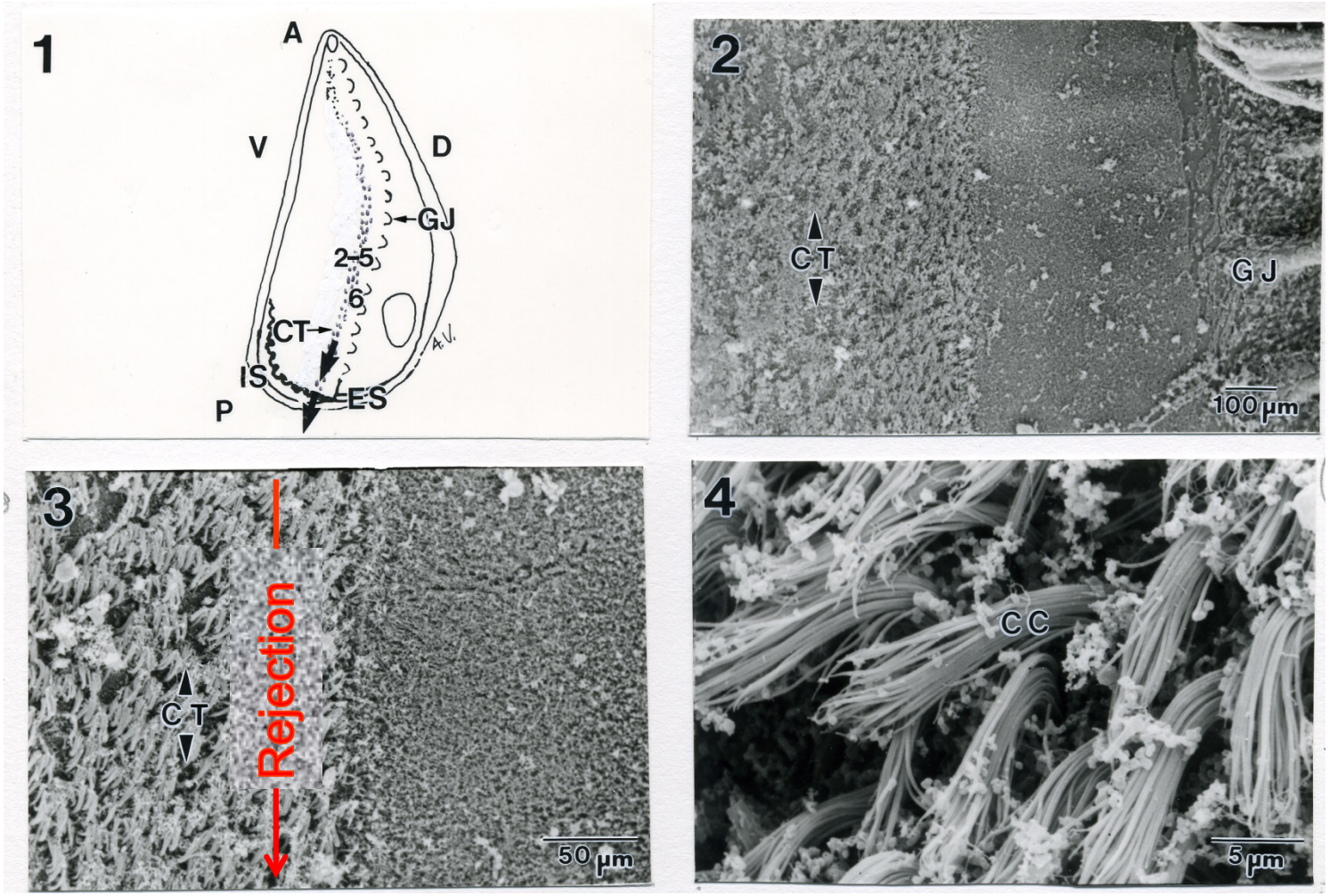
TRANSPORT - REJECTION



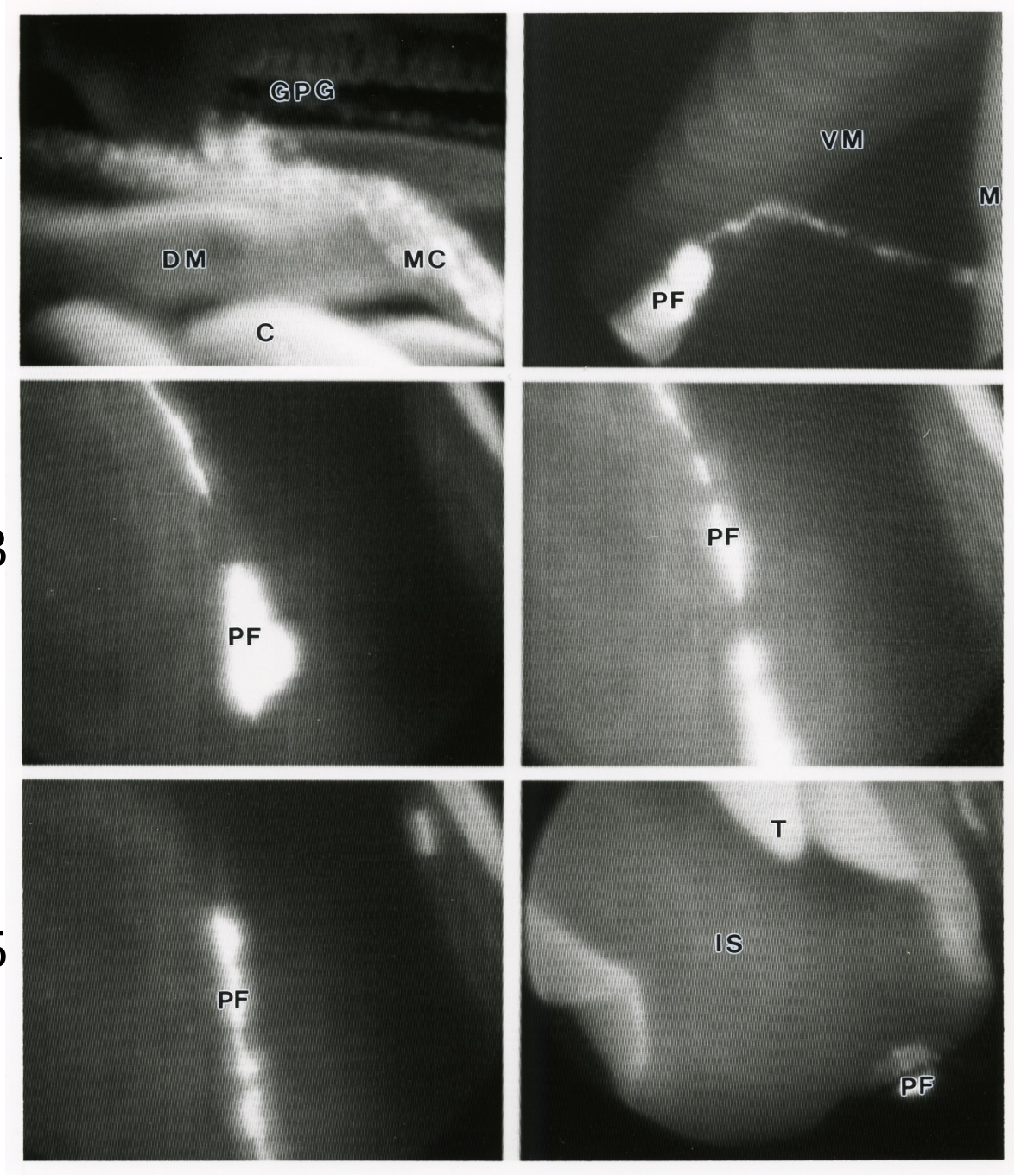


Homorhabdic filibranch (Mytilus edulis): distribution of mucocyte types on the mantle, showing dominance of high-viscosity AMPS on dorsal rejection tract. Beninger & St-Jean Mar Biol 1997

TRANSPORT - REJECTION



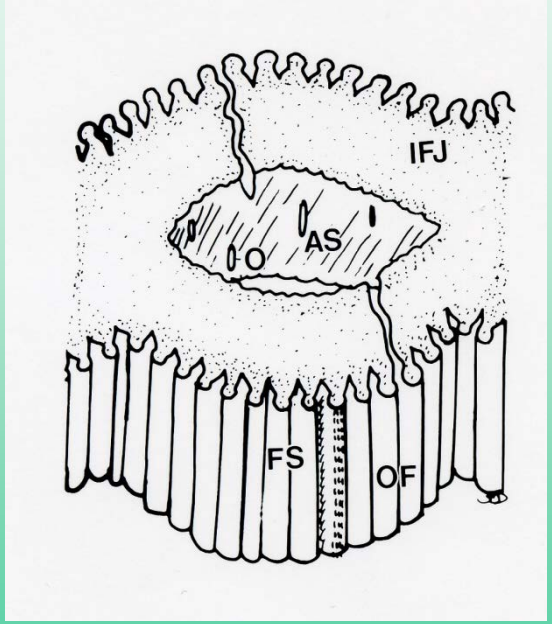
*Homorhabdic filibranch (*Mytilus edulis*): cilia mapping to show location of mantle rejection tract (1 – 3), and composite cilia which form the tract (4) . Beninger et al MEPS 1999.*



Homorhabdic filibranch (Mytilus edulis): rejection of mucus cord from gill particle groove to palp (1) , from palp ventral margin to mantle rejection tract via mucus bridge(2), along mantle rejection tract (3 – 5), and finally out inhalent siphon (6). Cord and secretions of rejection tract rich in AMPS.

Beninger & St- Jean, Mar Biol 1997

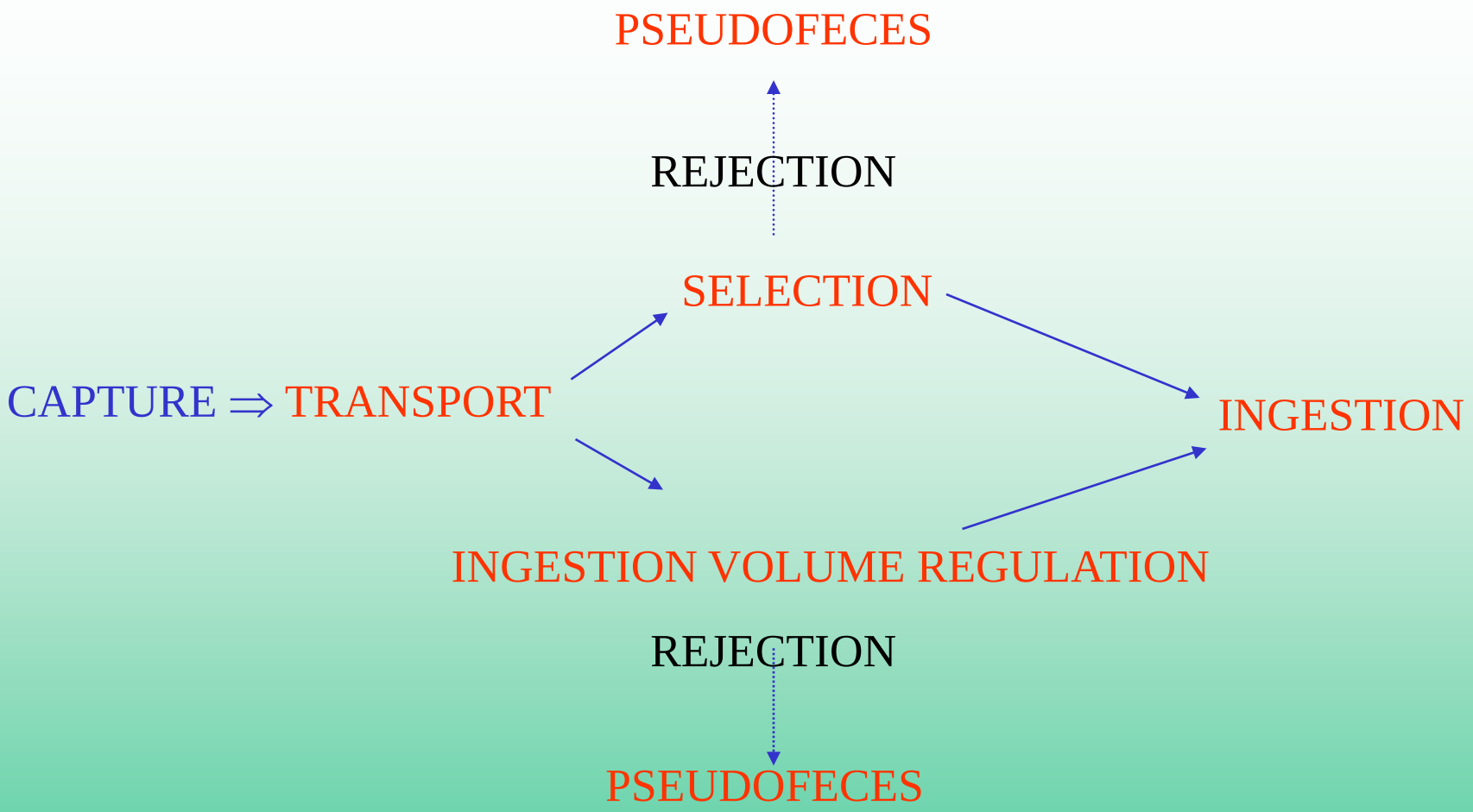
THE EULAMELLIBRANCH SYSTEM



The eulamellibranch system: capture

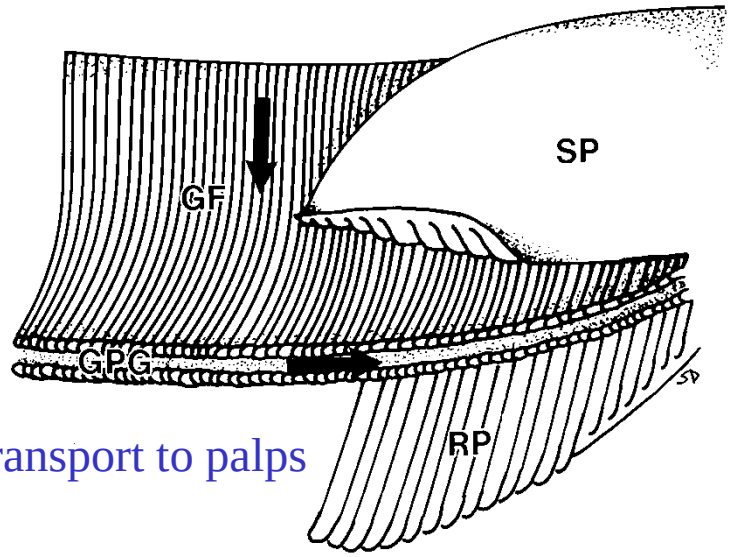
Near-field events same as for homorhabdic filibranchs

Silverman et al Biol Bull 1996



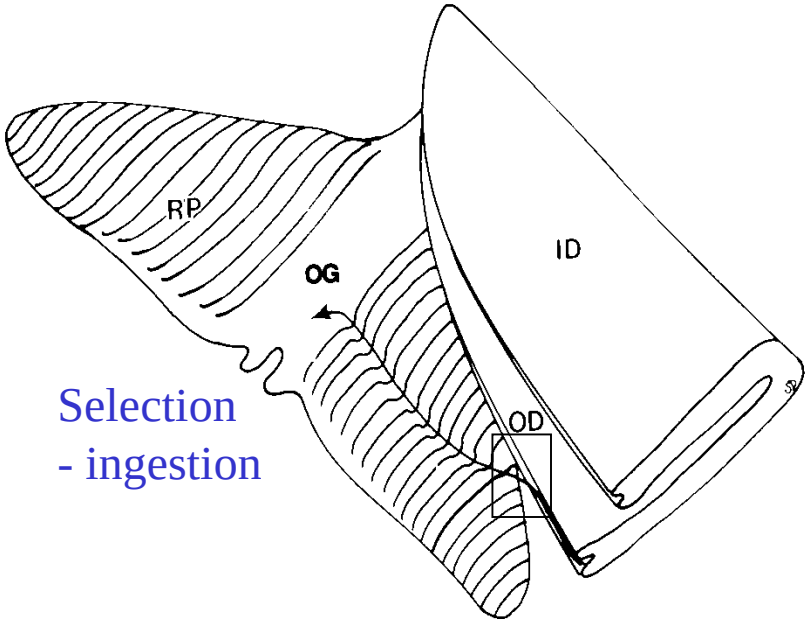
BASIC STEPS IN PARTICLE PROCESSING

TRANSPORT – SELECTION – IVR
INGESTION - REJECTION



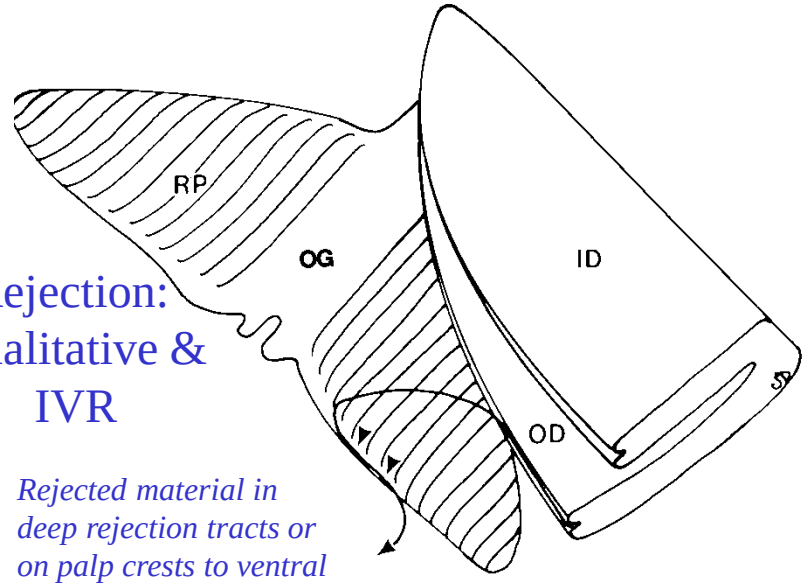
Transport to palps

Captured particles transported ventrally in viscous mucus (AMPS) to particle groove, then anteriorward to palps



Selection - ingestion

Transfer from gill to palp via mucus bridge. Accepted material in temporary tracts to oral groove

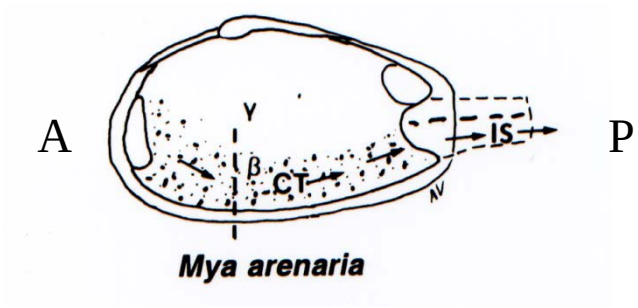


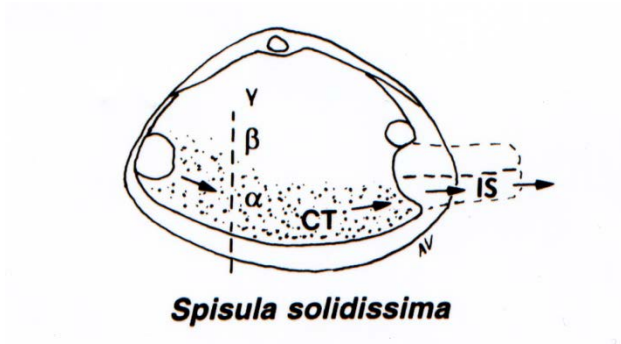
Rejection: Qualitative & IVR

Rejected material in deep rejection tracts or on palp crests to ventral , ventroposterior margin

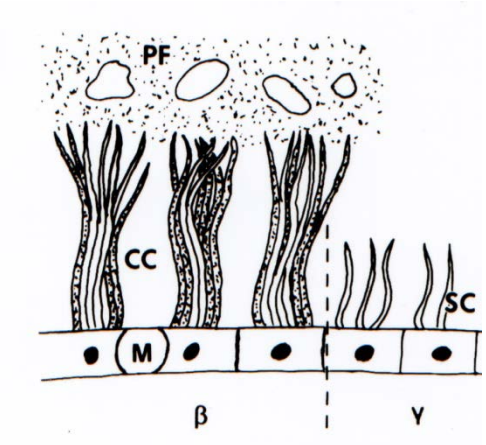
Particle processing in a eulamellibranch (Mya arenaria) – Beninger et al MEPS 1997

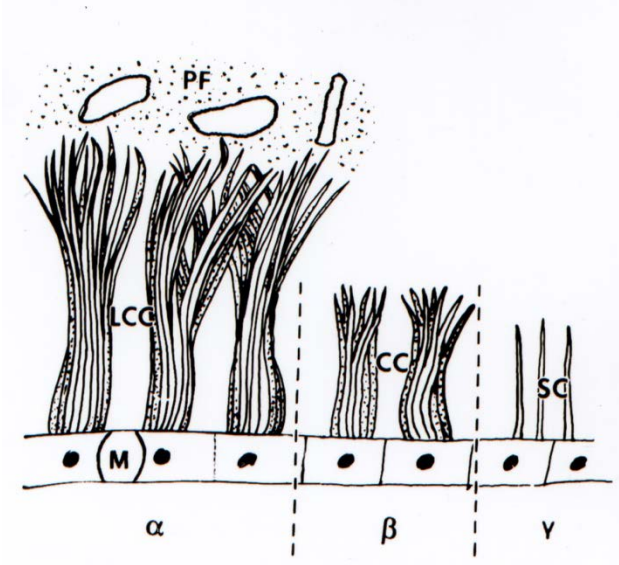
REJECTION - Continued



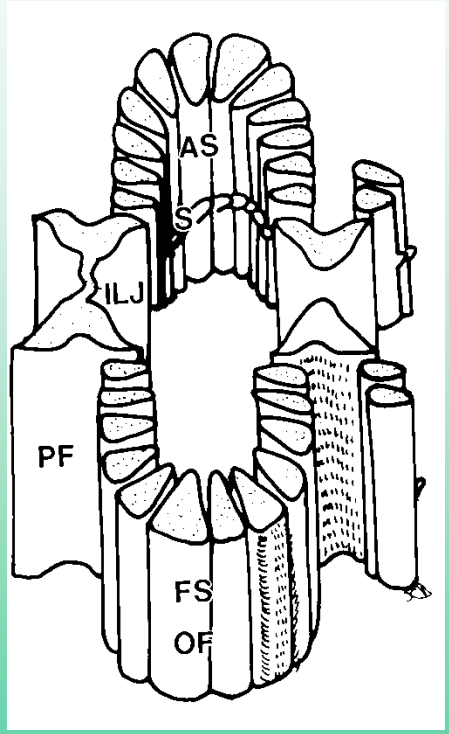


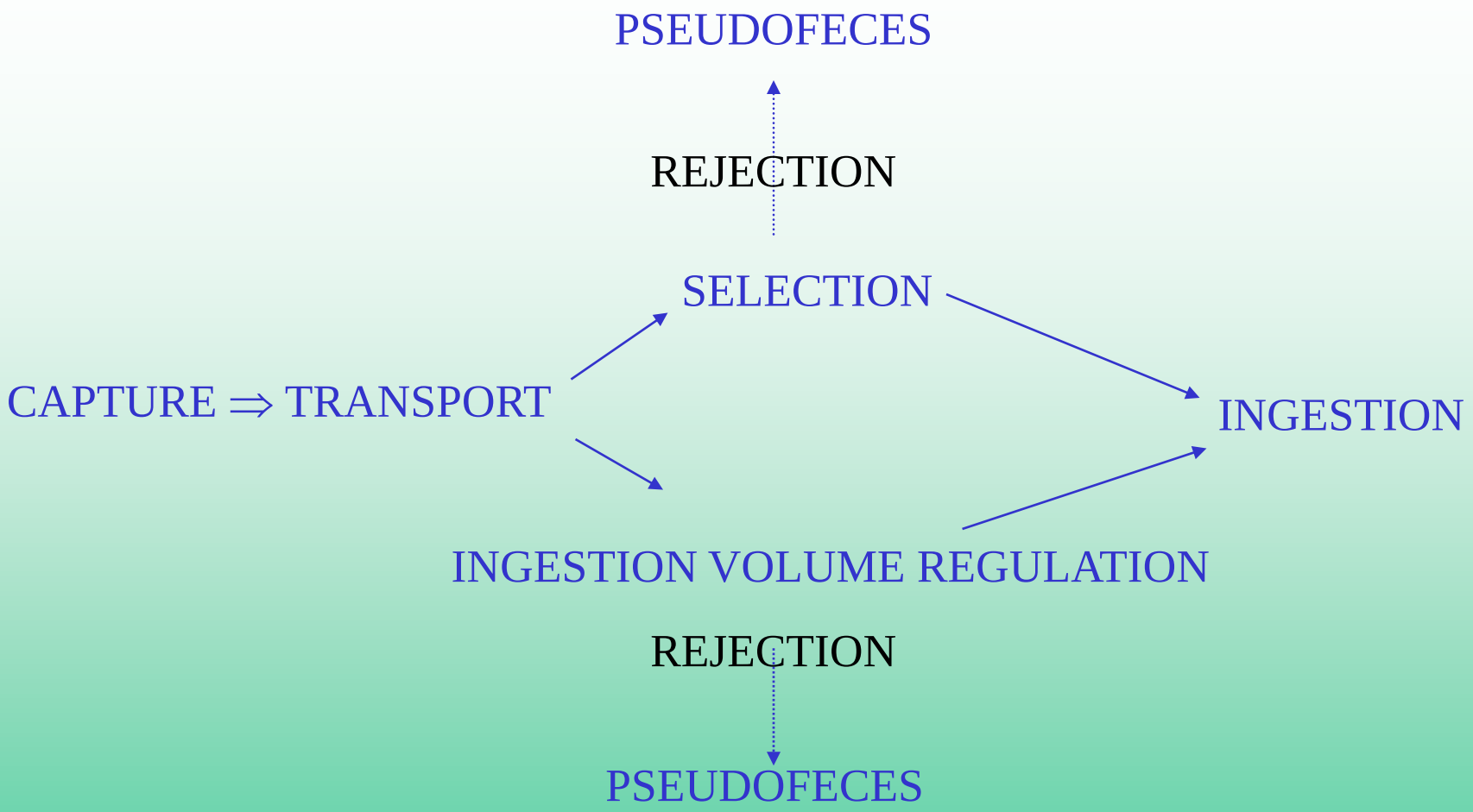
Pseudofeces from labial palps (Ant) to inhalent siphon (Post), counter-current, on exposed mantle surface, via ciliated mantle rejection tract and AMPS secretions. Cila are grouped, allowing elevation above general mantle surface





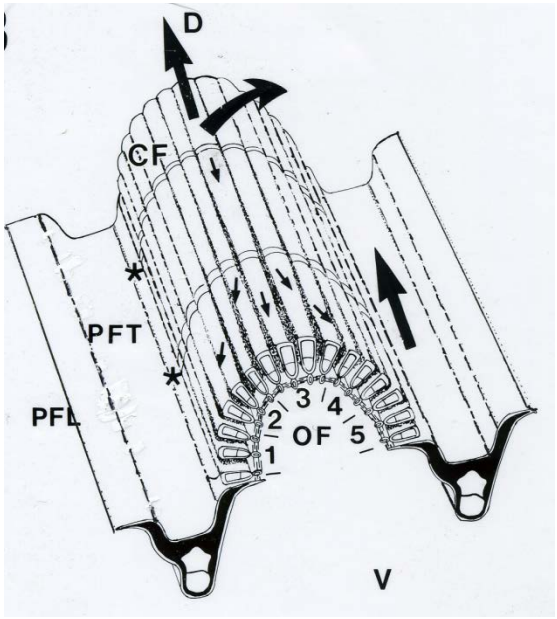
HETERORHABDIC FILIBRANCH SYSTEM



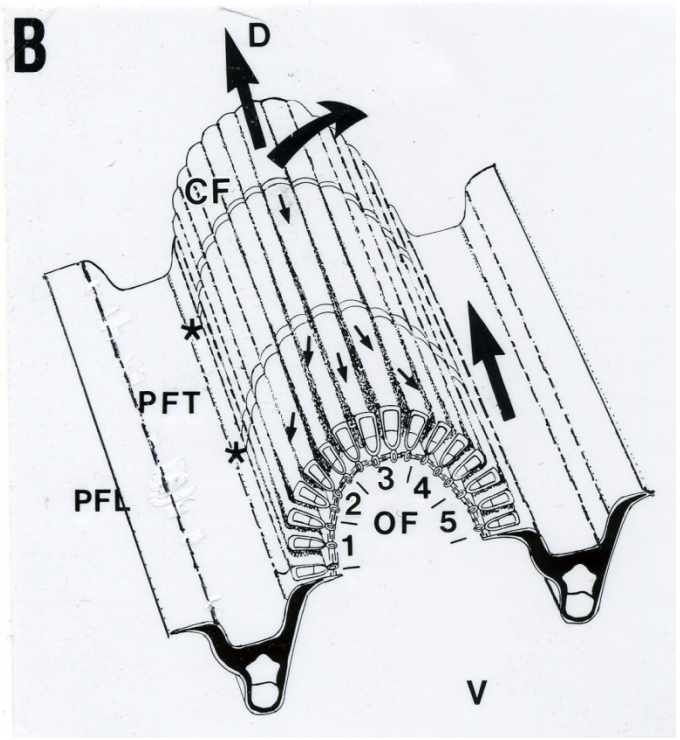


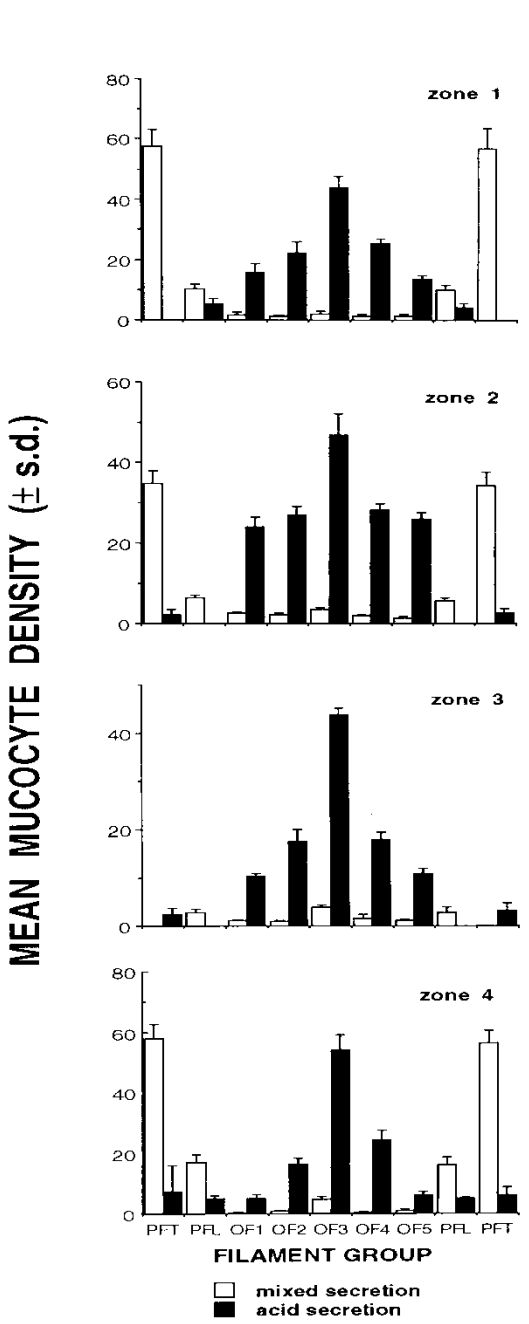
BASIC STEPS IN PARTICLE PROCESSING

CAPTURE – no LF cirri, but markedly plicate, heterorhabdic gill and bi-directional flow; hydrodynamic interception via deflection of current ???



TRANSPORT - REJECTION

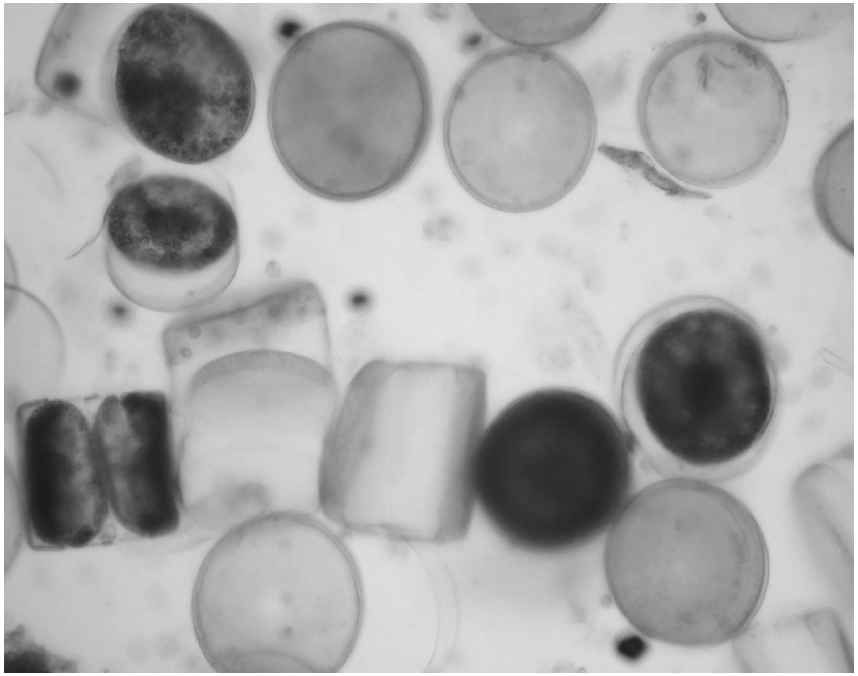


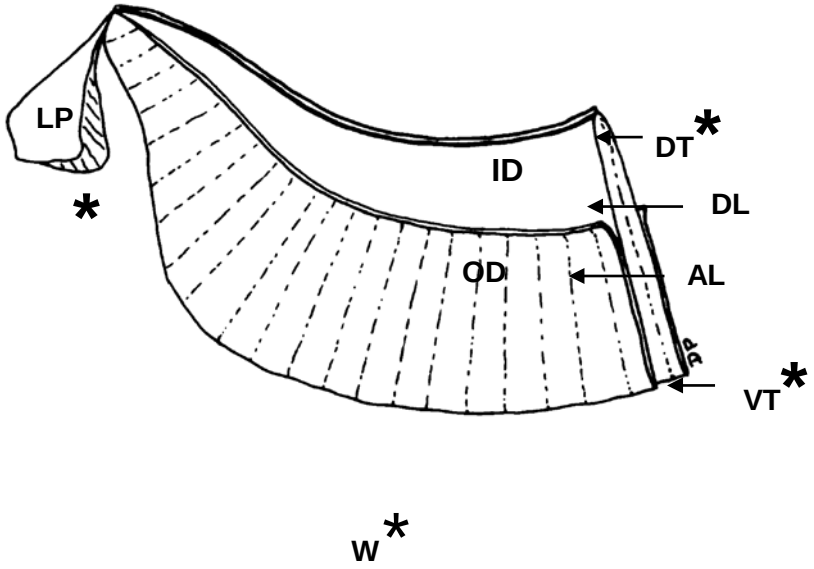


Heterorhabdic filibranch (Placopecten magellanicus) gill and mucocyte distribution: relatively low – viscosity secretion in dorsal trajectory (acceptance) tracts, high viscosity in ventral trajectory (rejection) tracts

Beninger et al MEPS 1993

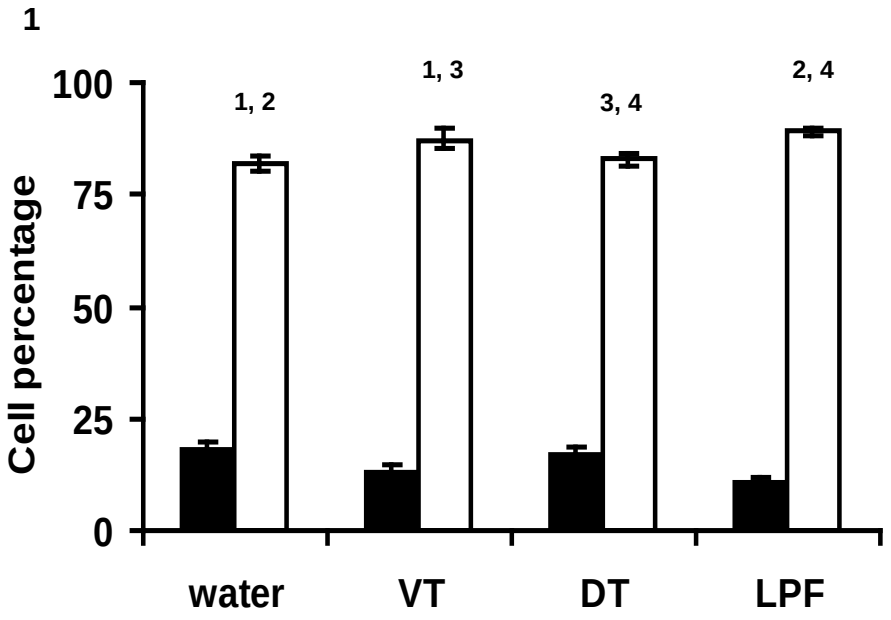
SELECTION: (*Pecten maximus*)*: in vivo endoscope-directed sampling in the water and at strategic processing sites (gills, palp pseudofeces), using large (74x96 µm), intact and empty, cleaned versions of naturally-occurring Coscinodiscus perforatus.*

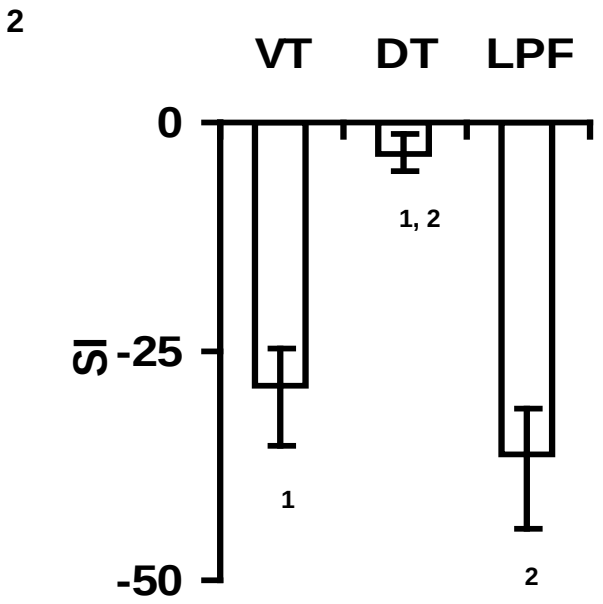




Beninger et al MEPS in press

SELECTION (Pecten maximus)

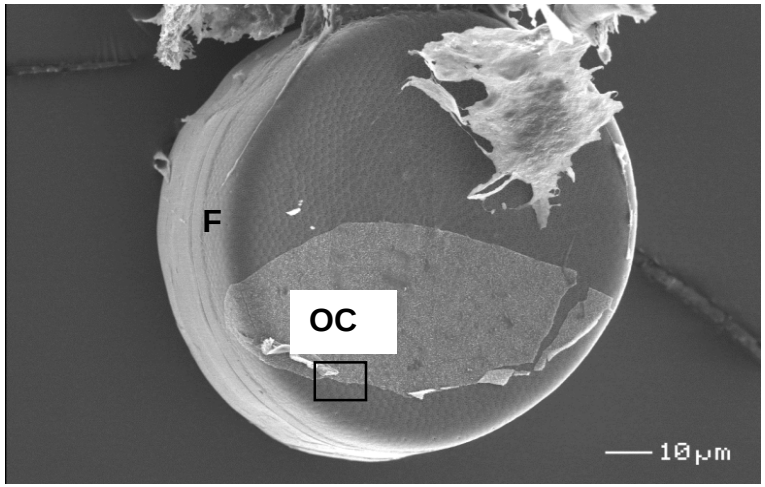


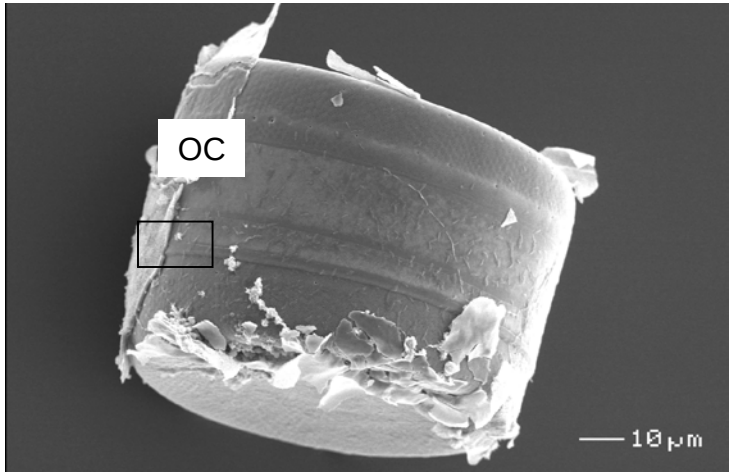


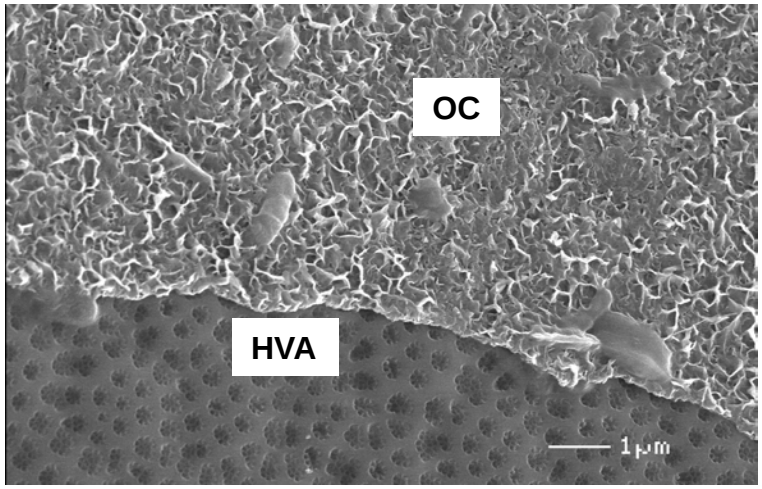
Percentages of empty and intact ❺ *Coscinodiscus at sampling sites: qualitative selection on gill and labial palps*

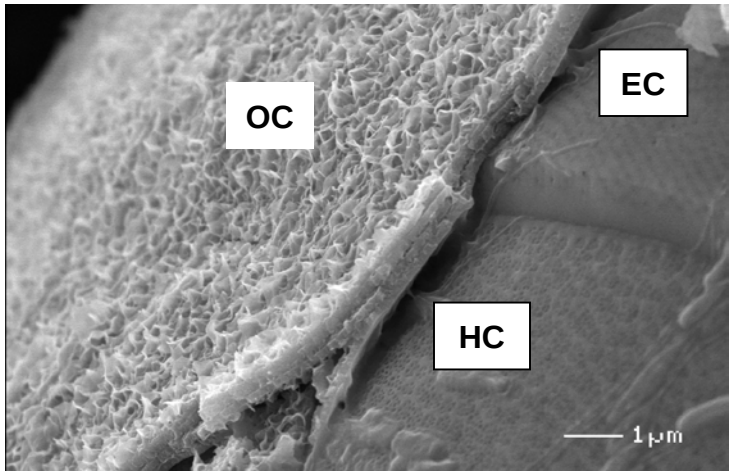
Progressive selection indices confirm selection first on gill, then at palps

Beninger et al MEPS in press



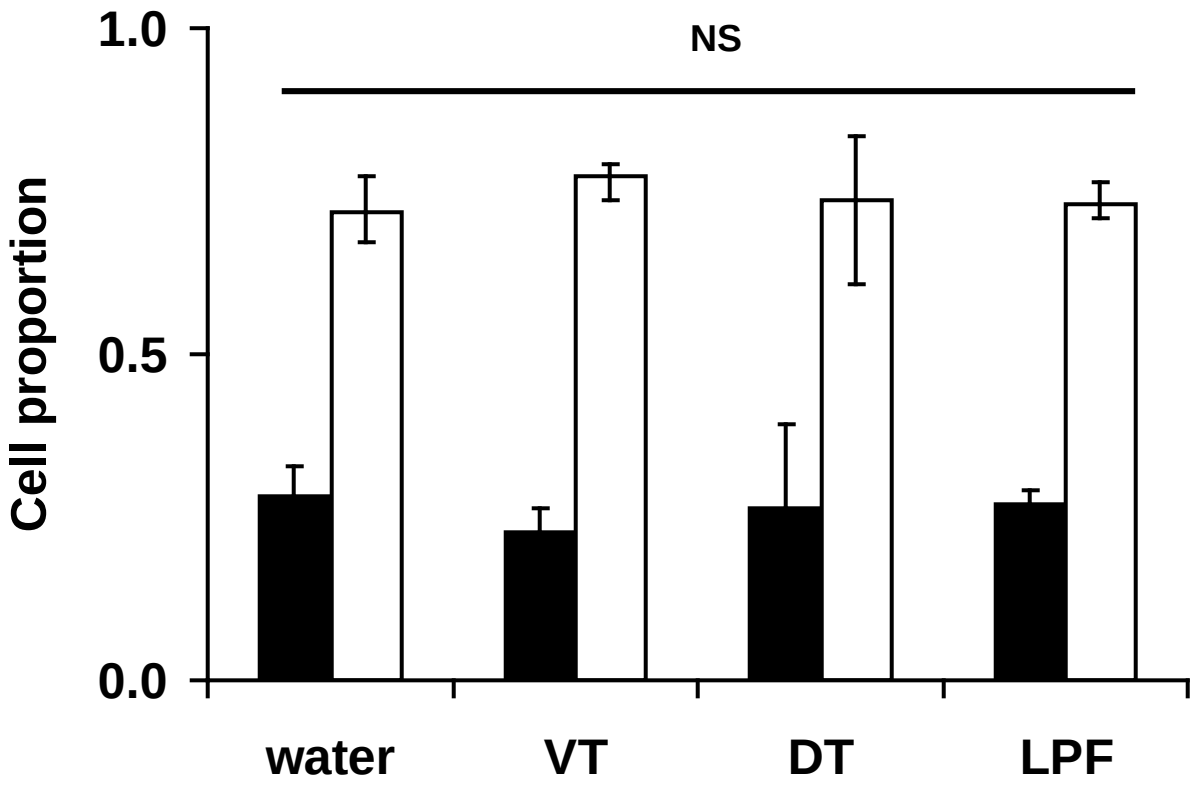






SELECTION

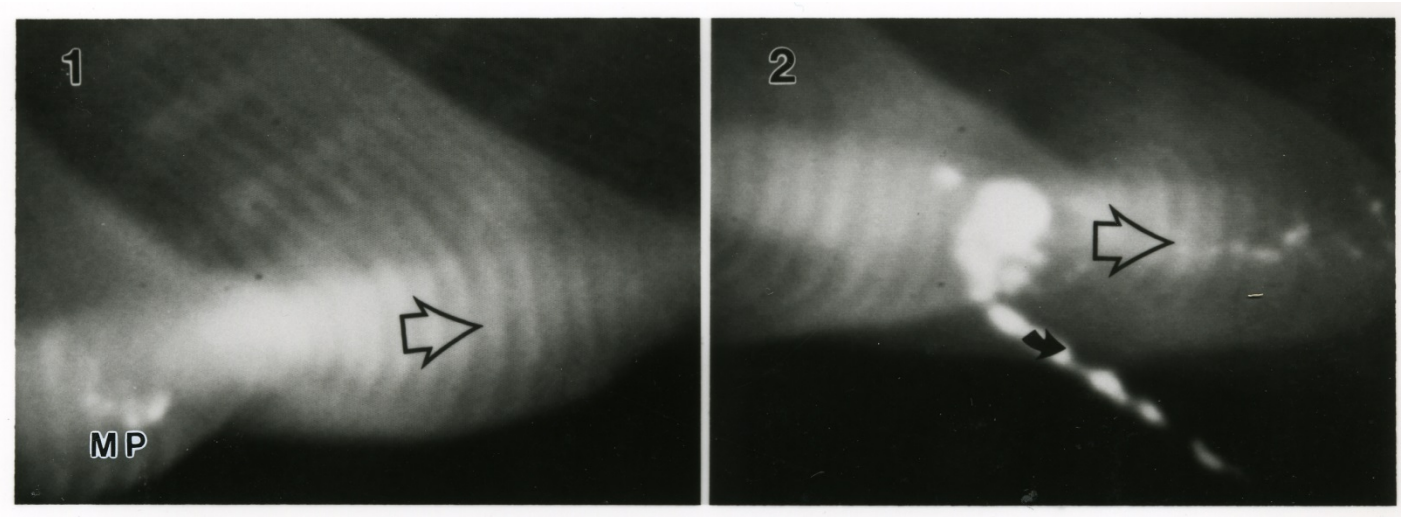
Role of organic casing in selection: casing on C. perforatus empty and intact cells fed to Pecten maximus.
Beninger and Decottignies, submitted



SELECTION: *Intact and empty, uncleaned diatoms are treated the same way by Pecten maximus: importance of organic casing*

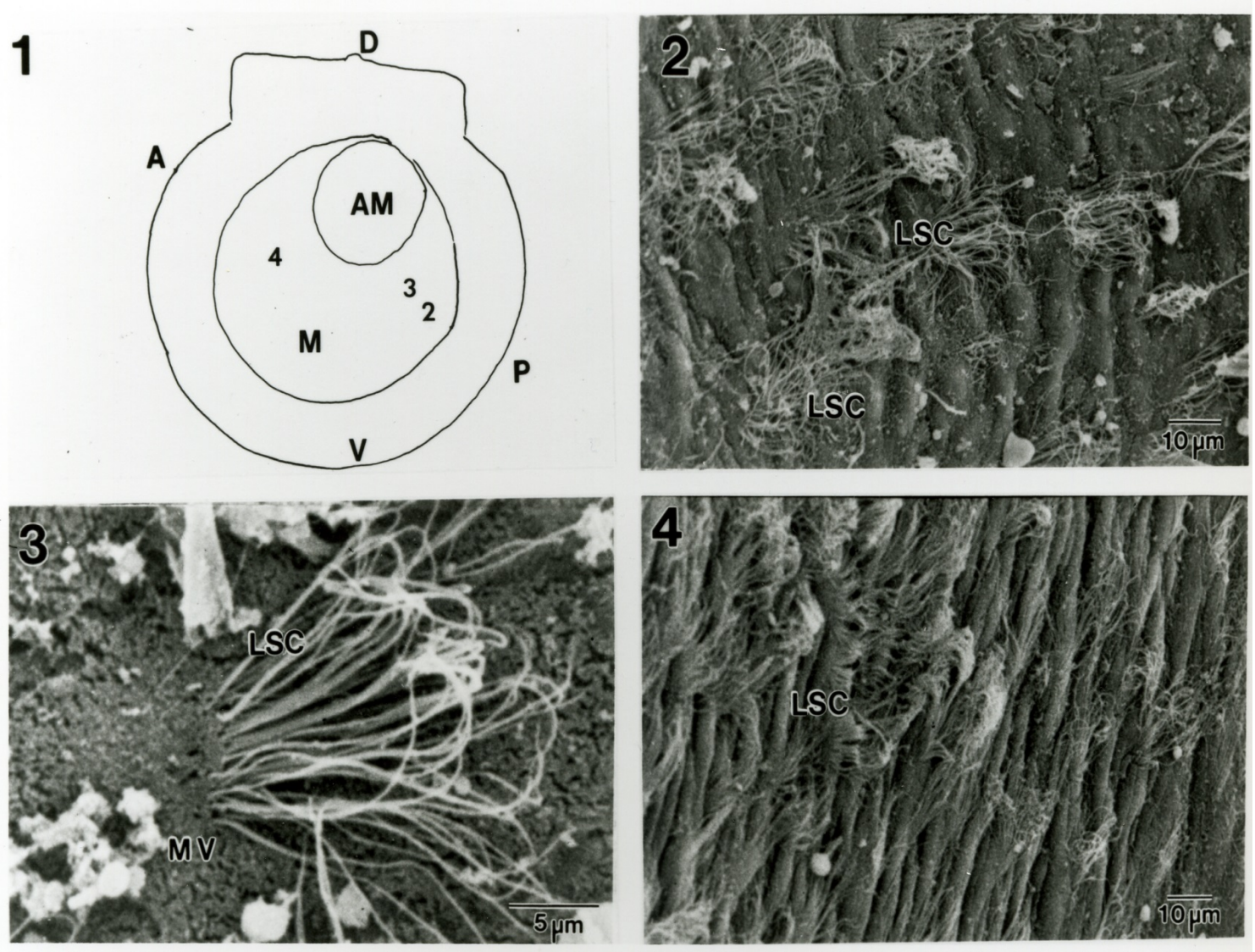
Beninger and Decottignies, submitted

TRANSPORT - REJECTION



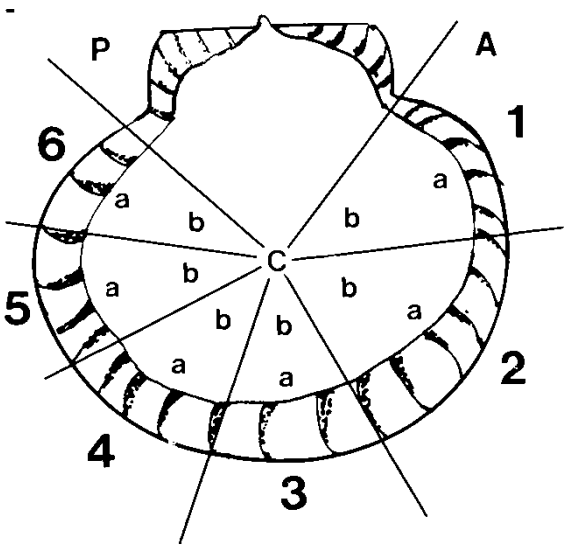
Placopecten magellanicus : rejection directly from gill ventral bend to pallial cavity
⇗valve clapping (Beninger et al Mar Biol 1992)

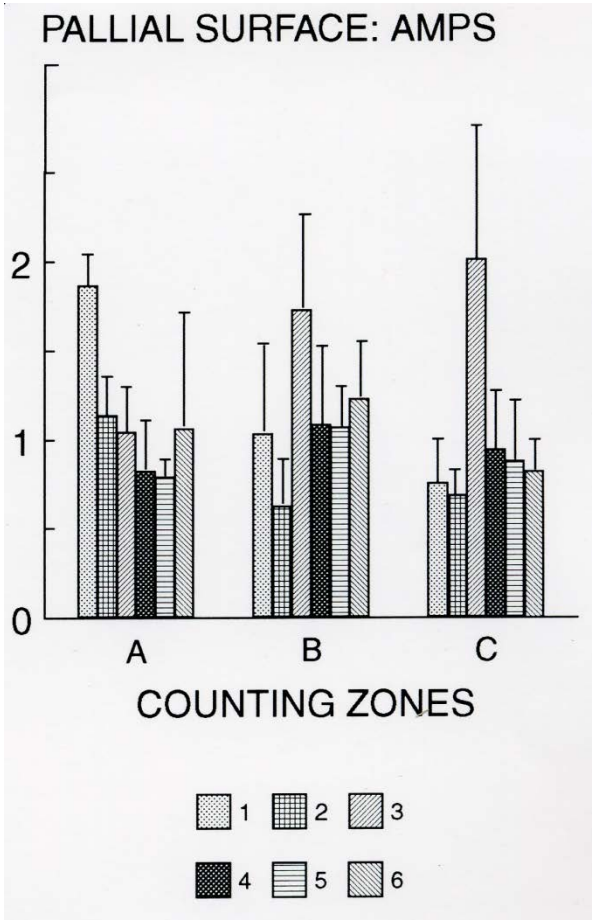
TRANSPORT - REJECTION



*Homorhabdic filibranch (*Placopecten magellanicus*): cila mapping showing lack of a mantle rejection tract.*
Beninger et al MEPS 1999

TRANSPORT - REJECTION

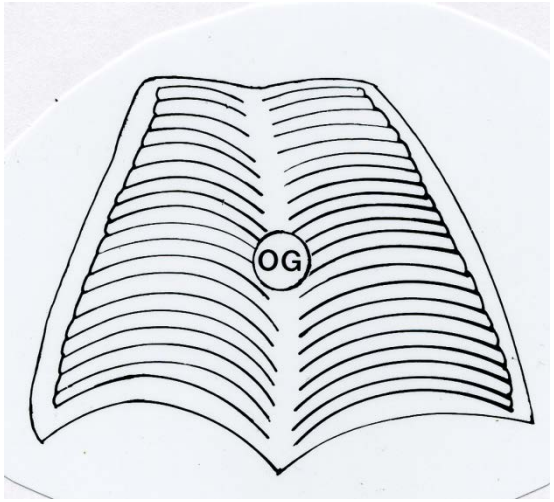




Heterorhabdic filibranch (Placopecten magellanicus): distribution of mucocyte types on mantle surface, showing lack of pseudofeces transport tract.

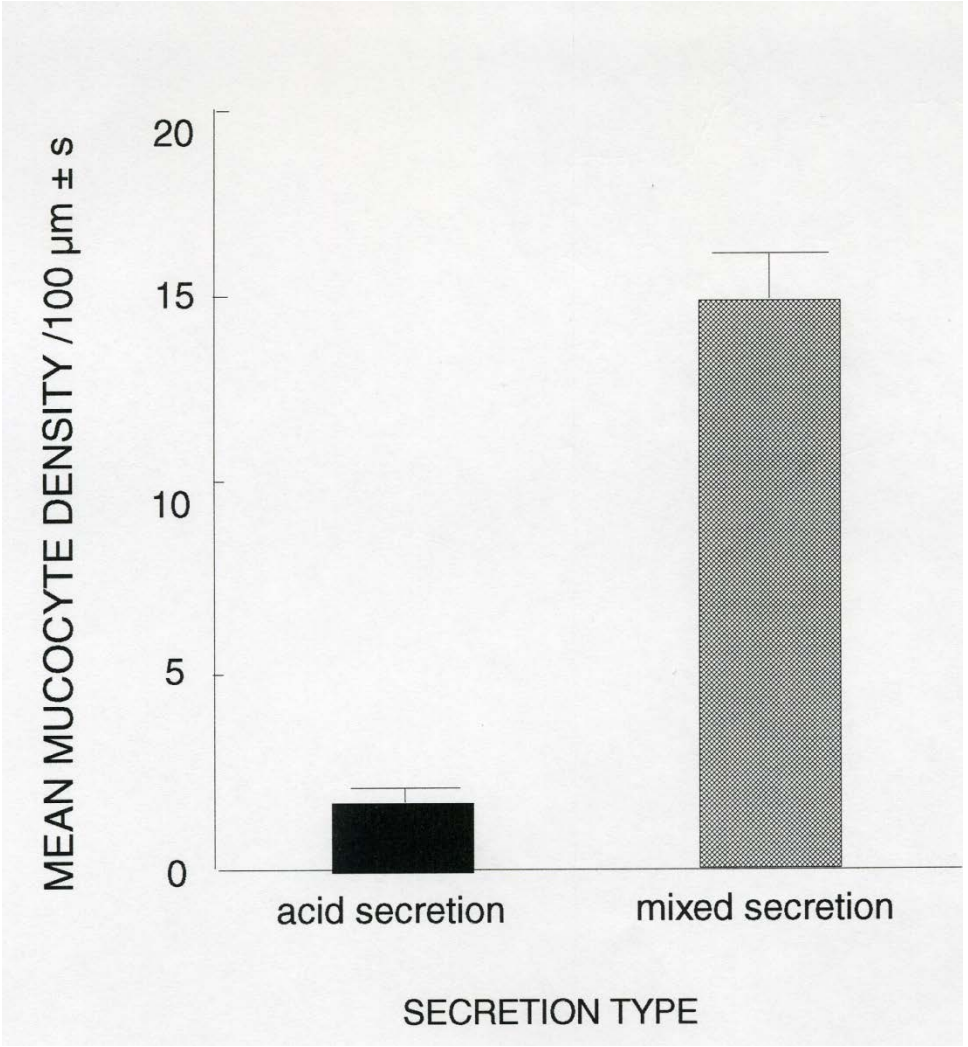
Beninger and St-Jean Mar Biol 1997

TRANSPORT - INGESTION

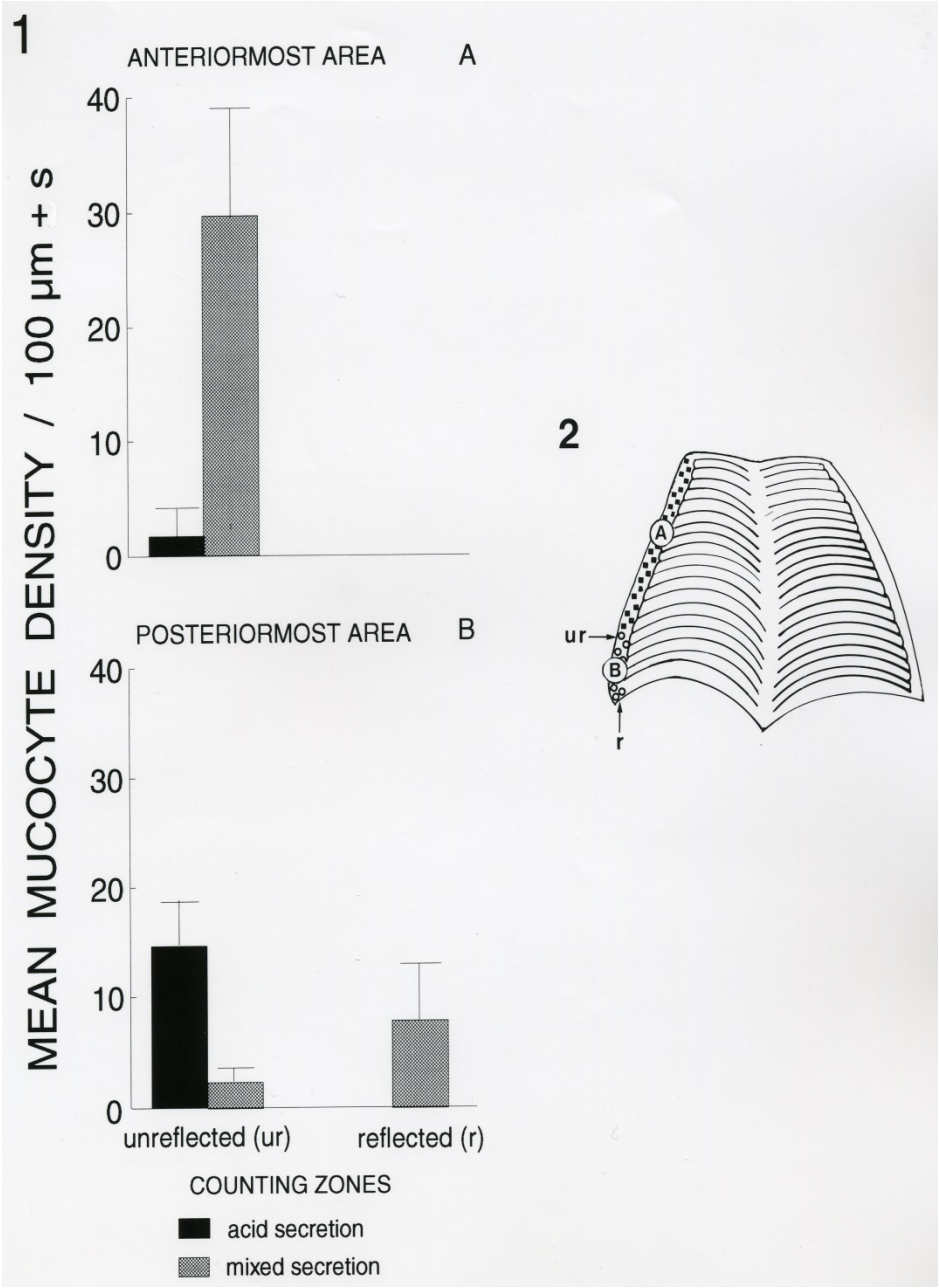


*Heterorhabdic filibranch (*Placopecten *magellanicus): mucocyte types and distribution in palp oral groove, showing preponderance of intermediate-viscosity secretions (MMPS) used for ingestion*

Beninger & St-Jean MEPS 1997



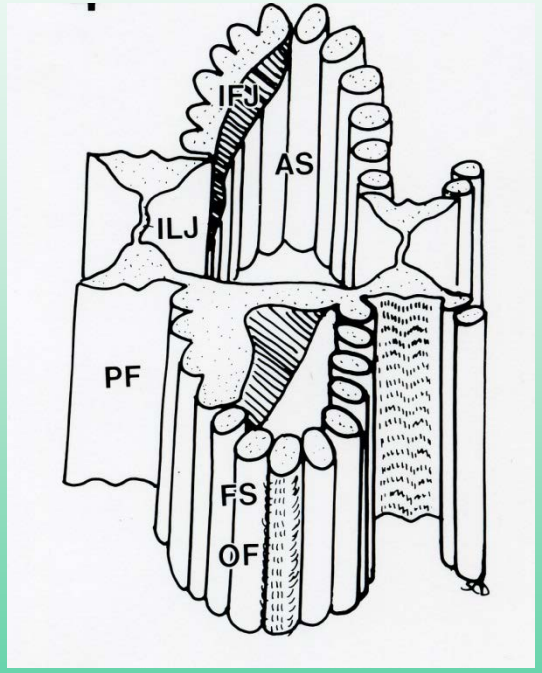
TRANSPORT - REJECTION



Placopecten magellanicus: *mucocyte distribution on palps. Intermediate – viscosity to postero-ventral margin, then high viscosity for transfer via mucus bridge to pallial cavity, counter-current.*

Beninger and St-Jean MEPS 1997

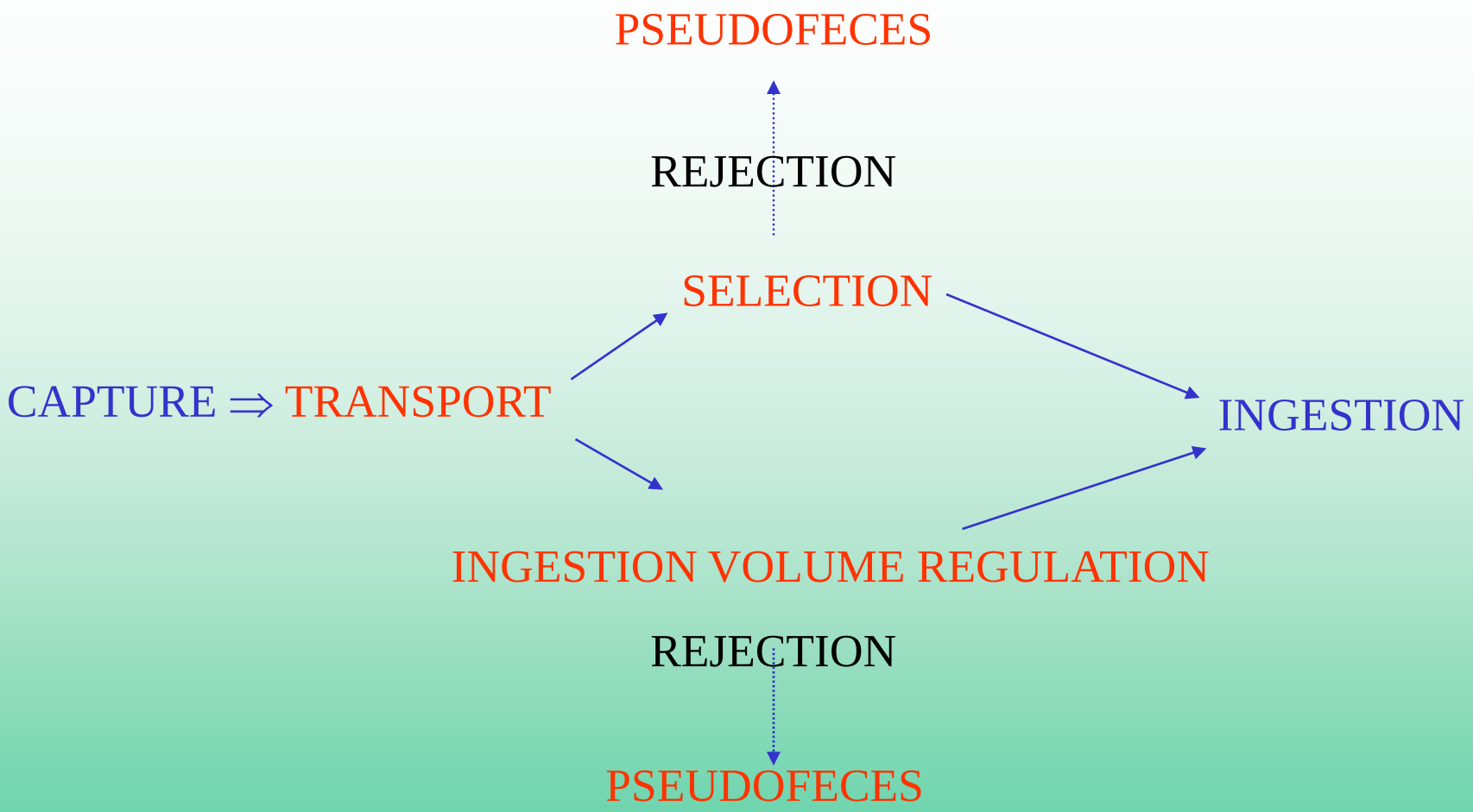
PSEUDOLAMELLIBRANCH SYSTEM



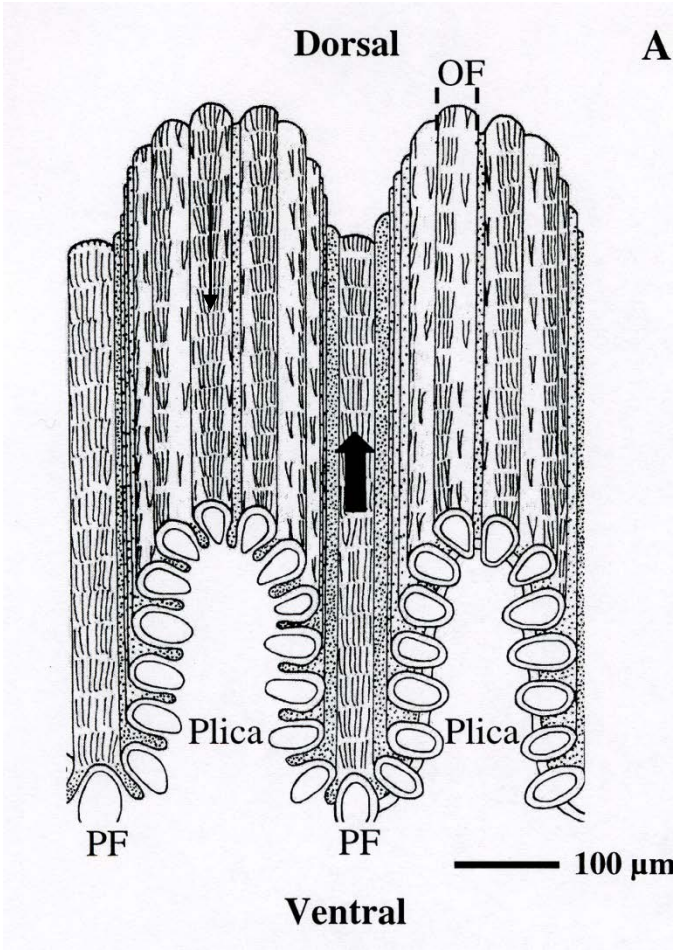
The oyster gill is the most quirky and most mysterious. Superficial but misleading resemblances on all major points.



Capture – hydrodynamic as in heterorhabdic filibranchs? Role of LF cirri – composite, not compound??



BASIC STEPS IN PARTICLE PROCESSING



Transport : *dorsalward = initial acceptance*

ventralward = initial rejection

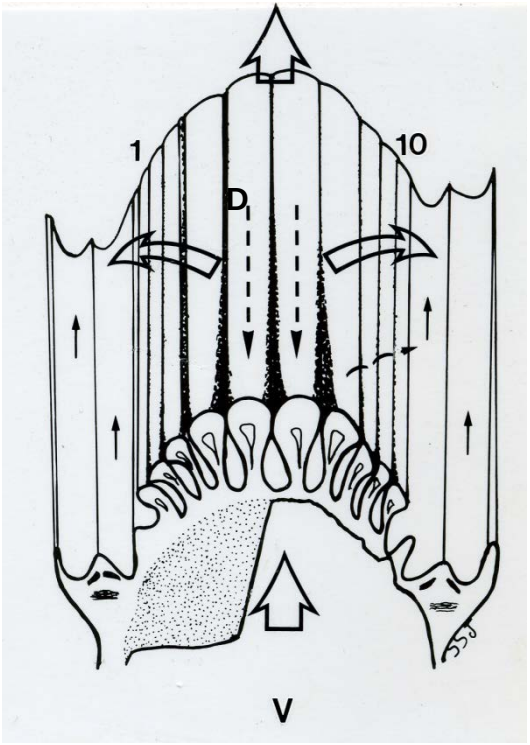
Ward et al Mar Biol 1998

For particles small enough to enter principal filament.

Larger particles all directed ventrally, then to palps, where selection takes place.

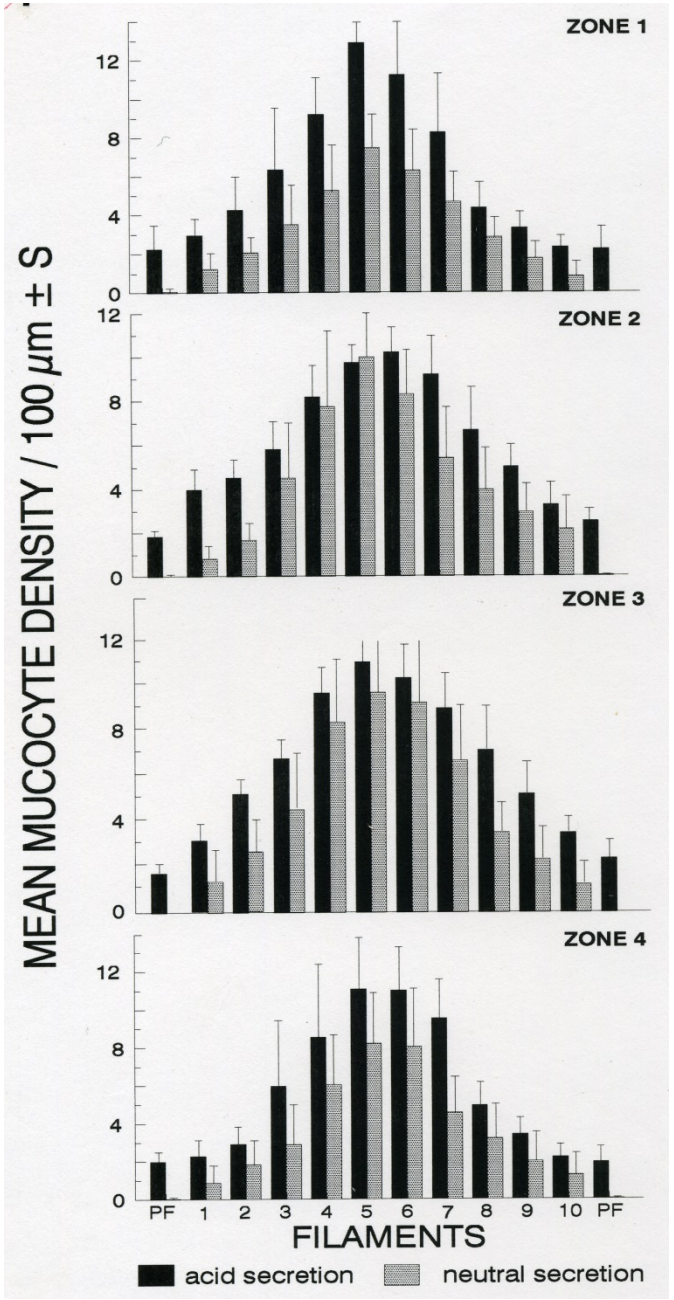
Cognie et al MEPS 2003

TRANSPORT



Pseudolamellibranch (Crassostrea virginica) mucocyte distribution: resulting mixture on plicae = acid-dominant, for transport on exposed surface, counter-current.

Beninger & Dufour MEPS 1996

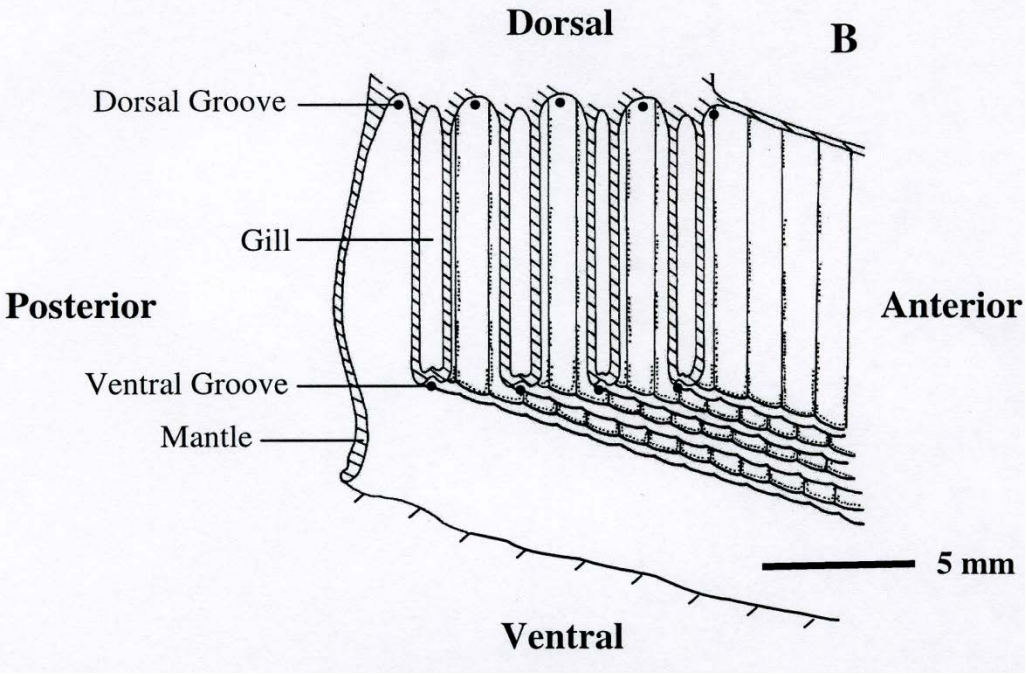


SELECTION

Initial demonstration of oyster gill as selection site (Ward et al Mar Biol 1998); however: (1) particle types differed in both quality and shape (2) all particles fit into the small oyster PF opening.

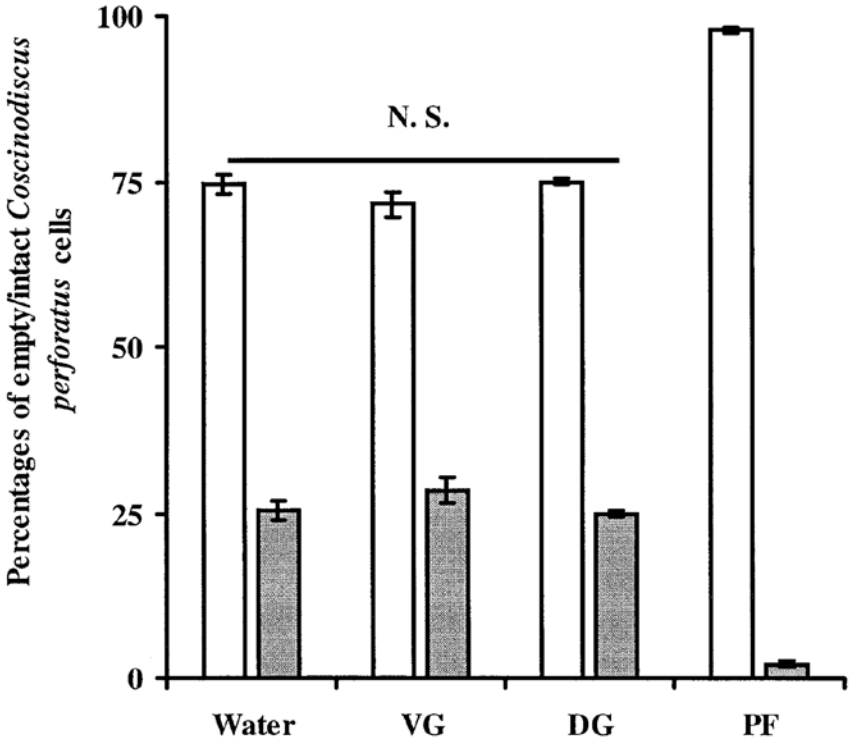
Unanswered questions: Selection due to quality or to shape??? Palps and selection???

Plan: use empty and intact algae of same species and shape, of size > PF opening to render selection at the gill impossible



SELECTION

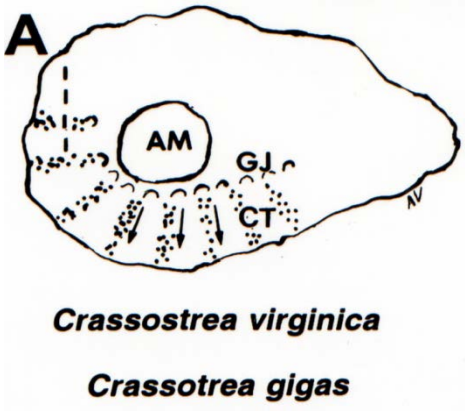
Qualitative selection in a pseudolamellibranch: endoscope-directed sampling in water column and strategic sites (gills, pseudofeces), using large (150x250 □m), intact and empty, cleaned versions of naturally-occurring Coscinodiscus perforatus.

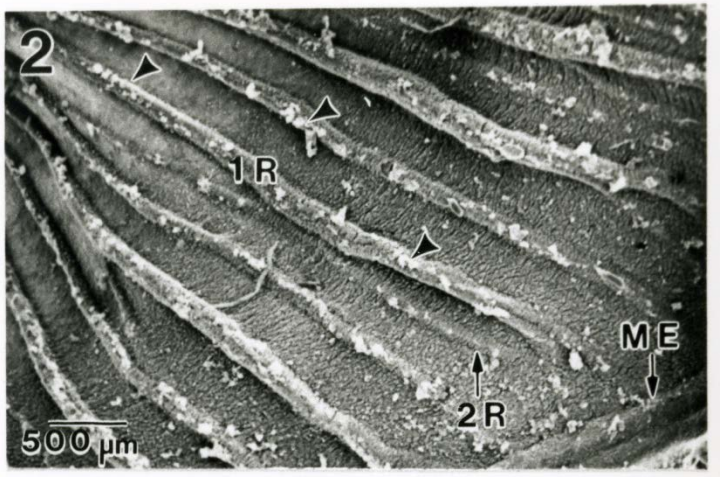


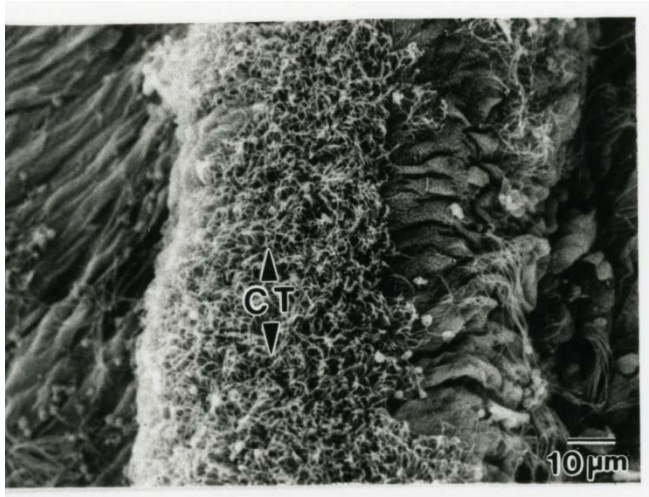
Selection on the gill impossible due to size constraint of oyster principal filament opening (75 □m), compared to diatom size (150x250 □m). Qualitative selection of larger particles performed by the palps.

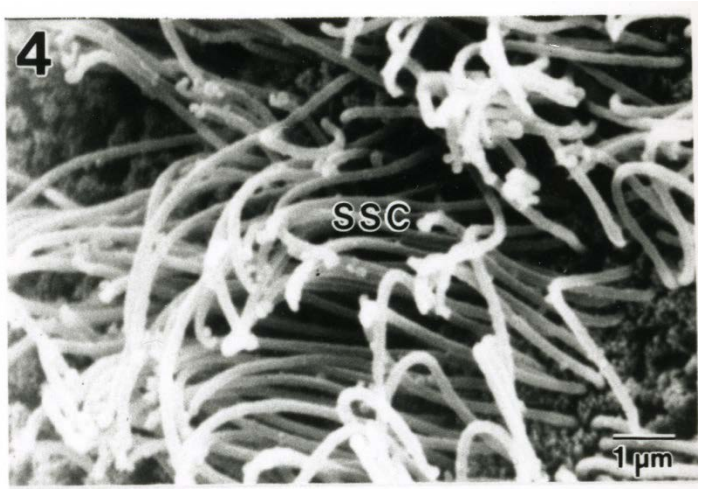
Cognie et al MEPS 2003

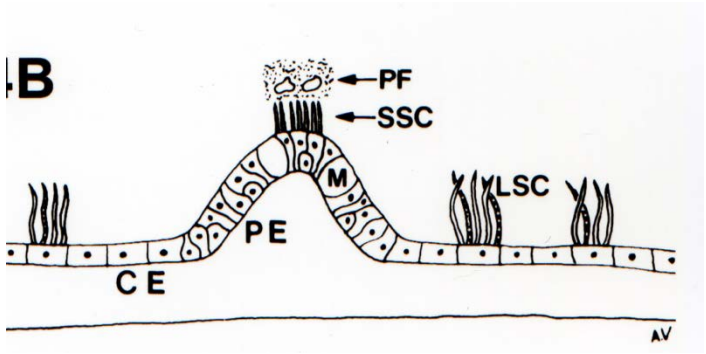
TRANSPORT - REJECTION











Pseudofeces rejection on mantle via mantle folds and simple cilia; elevation above mantle surface

UNIFYING PRINCIPLES
(What all this boils down to)

1. Gill type determines basic characteristics of particle processing (eg. capture via lfc, transport to ventral and /or dorsal tracts, type of mucus used.

2. Type of mucus determined by physical environment: exposed or not, counter-current or not.

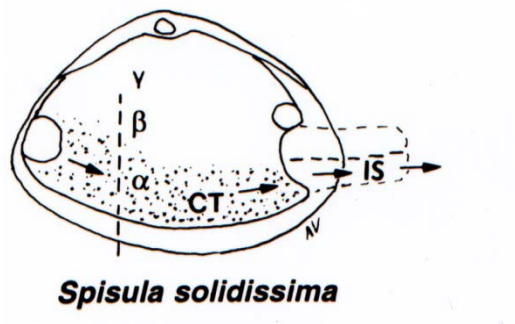
3. Specialized types of cilia used for appropriate functions: simple, compound, composite.

4. Thank you.

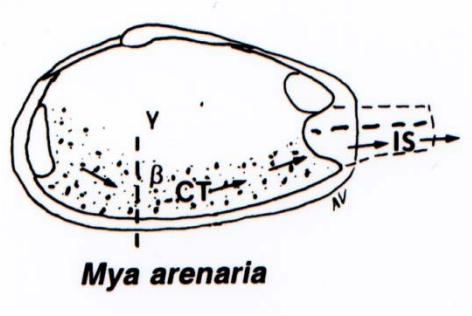
Unifying principles: types of mucopolysachharide used in different contexts and functions

Viscosity	**Low** →	→	→	**High**
Secretion type	NMPS	MMPS	ADMPS	AMPS
Surface type	various	enclosed/ semi-enclosed	open	open
Current flow	forward	forward	counter	counter
Function	dilution of AMPS and ADMPS to reduce viscosity	ingestion	- indiscriminate transport for later "decision" - rejection of pseudofeces	

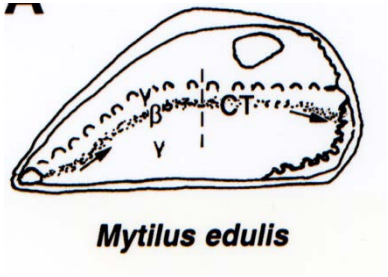
Unifying principle: elevation of pseudofeces on mantle rejection tract, by either long composite cilia (Category 1),



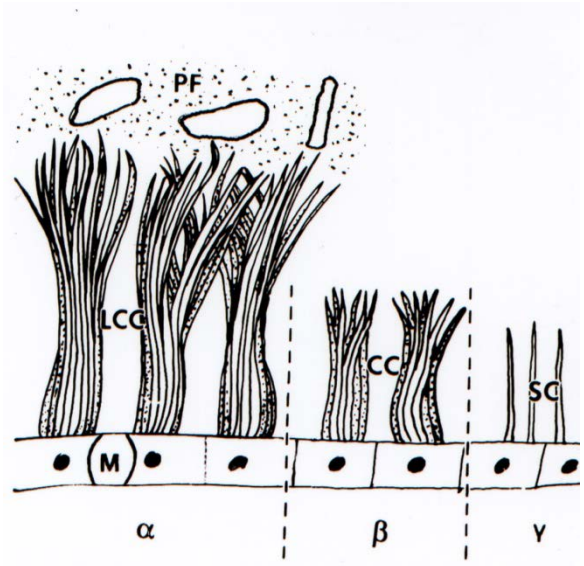
Spisula solidissima

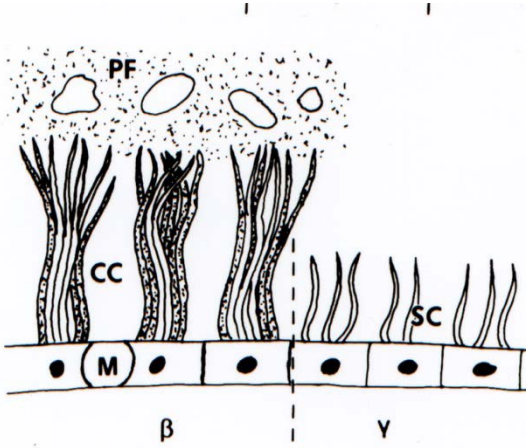


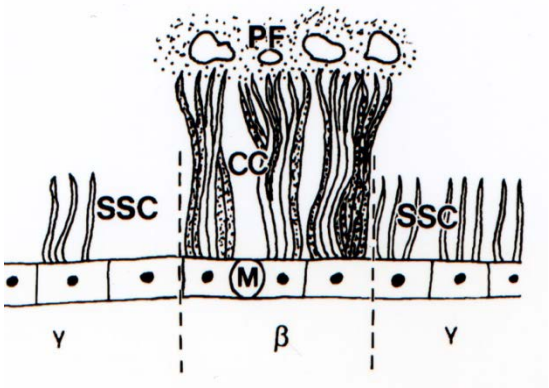
Mya arenaria



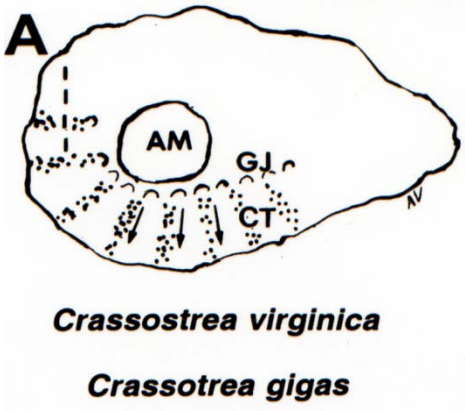
Mytilus edulis

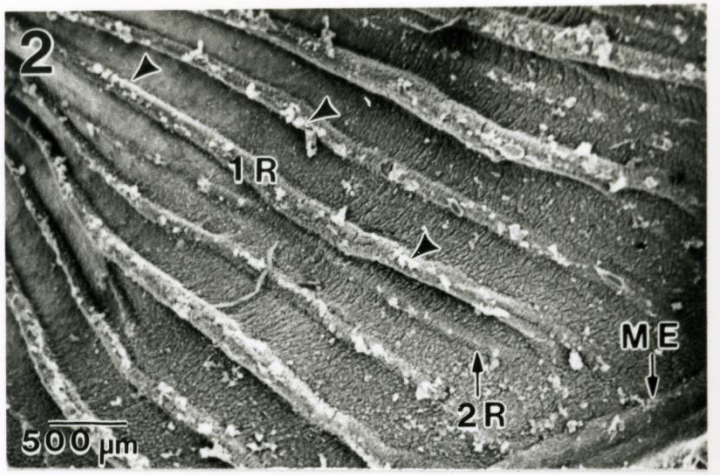


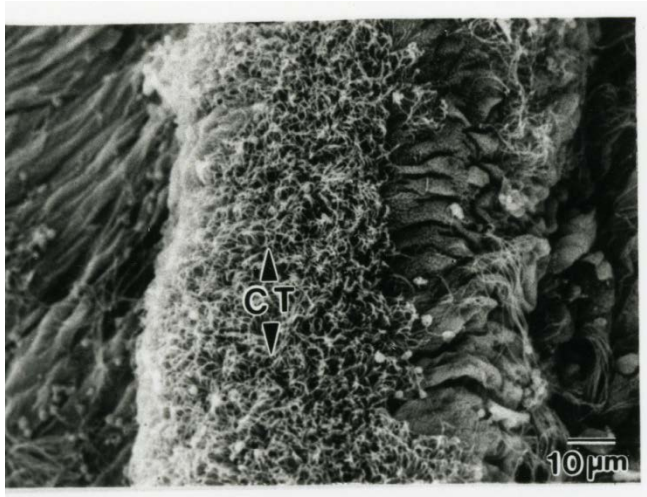


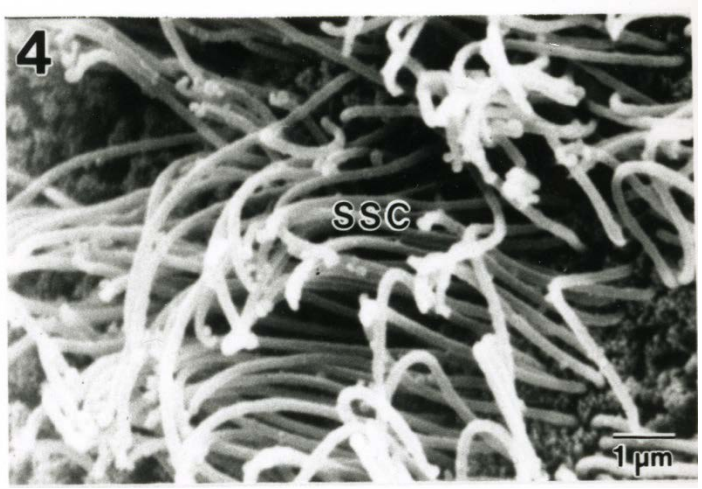


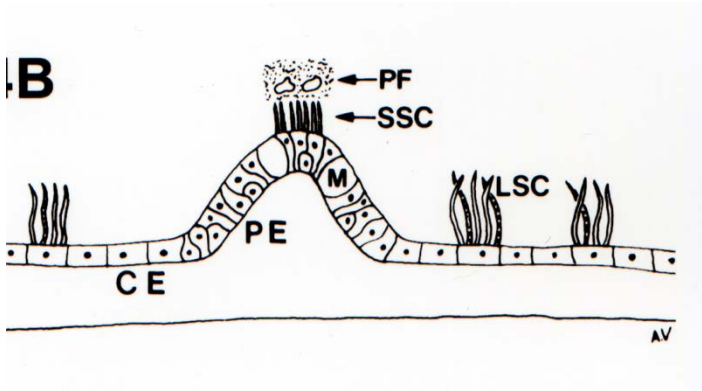
…or by means of simple cilia atop specialized mantle folds











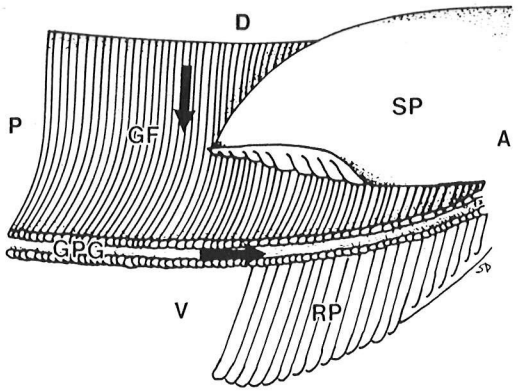


Fig. 5. *Mya arenaria*. Natural configuration of pallial organs and particle trajectories on gill. Arrows indicate direction of mucus-particle masses along gill filaments (GF) and in gill particle groove (GPG). Dominant pallial currents postero-anterior (P–A) and ventro-dorsal (V–D). RP: ridged palp surface; SP: smooth palp surface

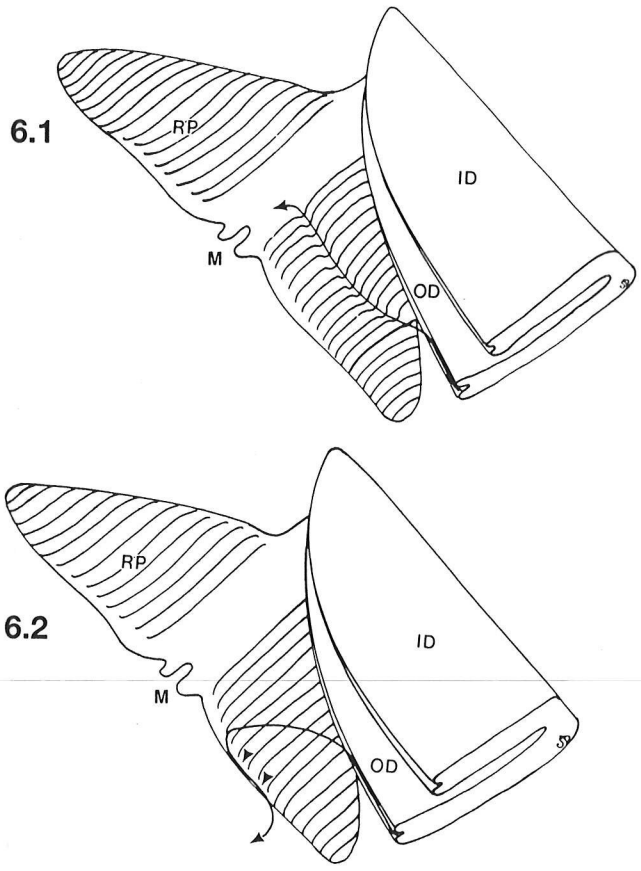


Fig. 6. *Mya arenaria*. Diagrams of pallial organs with mucus-particle trajectories during ingestion and rejection. Labial palps spread to reveal ridged surface. (6.1) Direction of mucus-particle masses destined for ingestion along ridged palp surface (RP). (6.2) Direction of rejected mucus-particle masses along ridged palp surface (RP). Arrowheads represent small mucus-particle masses exiting troughs and joining mucus cord on the palp ventral margin. ID: inner demibranch; M: mouth; OD: outer demibranch

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Particle processing on the labial palps of *Mytilus edulis* and *Placopecten magellanicus* (Mollusca: Bivalvia)

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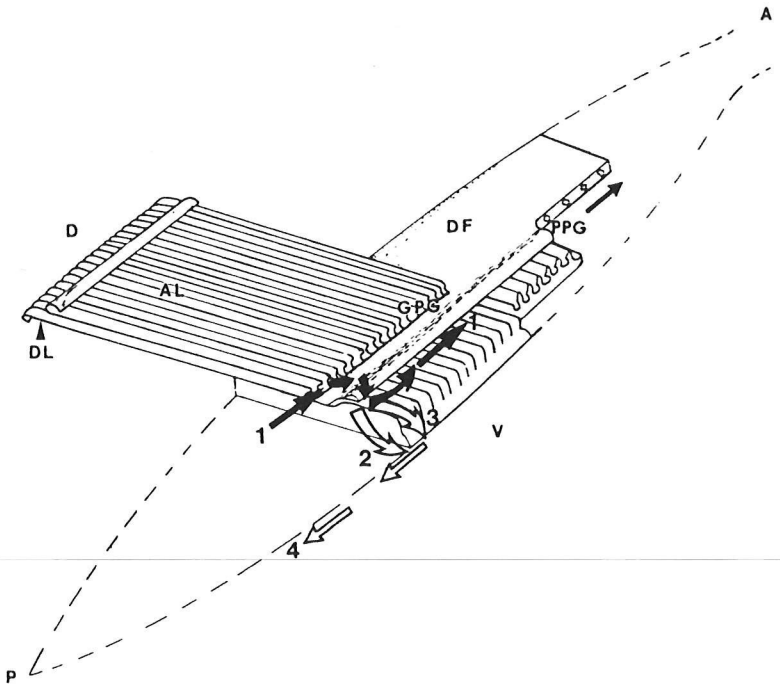


Fig. 4. *Mytilus edulis*. Summary of particle processing on the labial palp. Mucus-particle cord arriving in gill particle groove (GPG) is pulled out by cilia on dorsal fold (DF) and directed ventrally to the palp particle groove (PPG). Three trajectories are possible: if the ingestive/handling capacity is not overloaded, fluidization of the mucus-particle cord creates a flocculent slurry (see text), from which selected particles proceed anteriorly for ingestion (1); rejected particles are deviated into the palp troughs and transported to the ventral margin (2), where they begin to move posteriorly; if the ingestive/handling capacity is overloaded, the mucus-particle cord is shunted directly to the ventral margin without fluidization, where it begins to move posteriorly (3). The common rejection pathway is a posterior movement on the ventral margin (4), toward the posteriormost tip of the palp. Dominant pallial current postero-anterior. A: anterior; AL: ascending lamella of gill; D: dorsal; DL: descending lamella of gill; P: posterior; V: ventral

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The role of mucus in particle processing by suspension-feeding marine bivalves: unifying principles

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Abstract Contemporary research on bivalve suspension-feeding has revealed a diversity of particle processing mechanisms depending on the anatomy and functioning of the pallial organs involved. On the biochemical level, however, some evidence of homogeneity has emerged concerning the types of mucopolysaccharide associated with particle processing. The present study uses both previous data and original research combining video endoscopy and mucocyte mapping to further explore the relationships between pallial organ topography, functional correlates, direction of current flow, and mucocyte secretion type. Five species representing five different families and all four major gill types are represented: *Mytilus edulis*, *Placopecten magellanicus*, *Crassostrea virginica*, *Mya arenaria*, and *Spisula solidissima*. Viscous acid or acid-dominant mucopolysaccharides are used when particle transport occurs on an exposed surface, or on a structure leading directly to such a surface, counter to the prevailing current flow. Associated functions are indiscriminate transport in gill ventral particle grooves and rejection of pseudofeces. Lower-viscosity mixed mucopolysaccharides are used when particle transport is on an enclosed or semi-enclosed surface, leading to other such surfaces, and with the current flow. Associated functions are transport of particles destined for ingestion, and ingestion itself. Low-viscosity neutral mucopolysaccharides are found in regions where reduction of mucus viscosity is important, such as the areas of the labial palps responsible for fluidization of the high-viscosity mucus-particle cord of the gill ventral particle groove prior to particle extraction. There thus appears to be a specialization of mucus type corresponding to functional specialization of the various pallial organs in suspension-feeding marine bivalves.

Table 5 Summary of mucus secretion types used in particle processing on bivalve pallial cavity organs (*NMPS* neutral mucopolysaccharides; *MMPS* mixed mucopolysaccharides; *ADMPS* acid-dominant mucopolysaccharides; *AMPS* acid mucopolysaccharides)

	Viscosity:			
	Low	→	→	High
Secretion type	NMPS	MMPS	ADMPS	AMPS
Surface type	Various	Enclosed/ semi-enclosed	Open	Open
Current flow	Forward	Forward	Counter	Counter
Function	Dilution of AMPS and ADMPS to reduce viscosity	Ingestion	– Indiscriminate transport for later "decision" – Rejection of pseudofeces	

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Localization of qualitative particle selection sites in the heterorhabdic filibranch *Pecten maximus* (Bivalvia: Pectinidae)

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ABSTRACT: Particle-processing, and specifically qualitative particle selection, was studied in *Pecten maximus* using intact and empty versions of the naturally occurring diatom *Coscinodiscus perforatus*. Endoscope-directed sampling allowed comparison of particle proportions or numbers in the experimental medium, dorsal and ventral gill tracts, and labial palp pseudofeces. The general processing of particles was closely similar to that previously described for the more primitive scallop *Placopecten magellanicus*, despite the large phylogenetic distance between these 2 species. The gill and the labial palps of *Pecten maximus* each effected particle selection (rejection of empty, cleaned frustules), both in the presence of intact *C. perforatus* particles, and when only empty, cleaned frustules were presented. Particle selection operated even when small proportions (approx. 10%) of empty, cleaned *C. perforatus* frustules were present in the particle mixture. This represents the first direct experimental demonstration of the sites of qualitative particle selection in a heterorhabdic filibranch bivalve, and shows the process to be located in both the gills and the labial palps. The qualitative selection capacity of the heterorhabdic gill could be the result of either early evolution in turbid environments, or an adaptation which avoids overloading qualitative selection of the palps in this gill type.

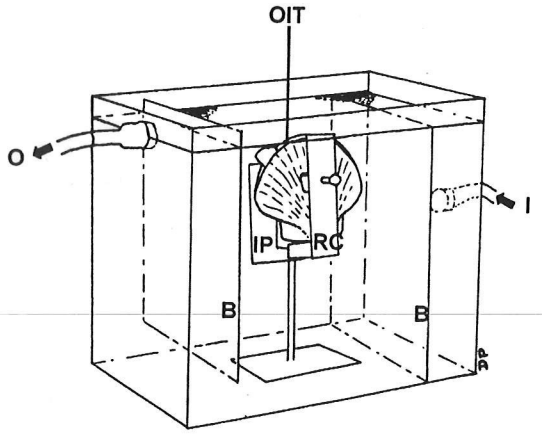


Fig. 1. Diagram of experimental chamber, showing *Pecten maximus* specimen with optical insertion tube (OIT), inclined platform (IP) and restraining collar (RC). B: baffles; I: inflow; O: outflow

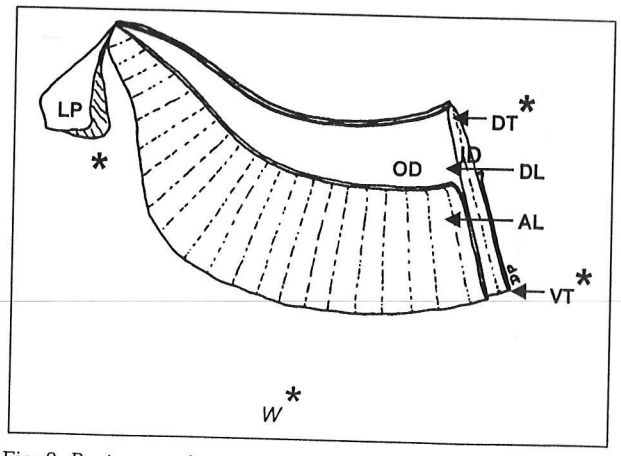


Fig. 2. *Pecten maximus*. Diagram of anterior portion of left gill and left labial palps (viewed from left side), showing location of sampling sites (*). AL: ascending lamella; DL: descending lamella; DT: dorsal tract; ID: inner demibranch; LP: labial palps; OD: outer demibranch; VT: ventral tract; *W*: water in experimental chamber

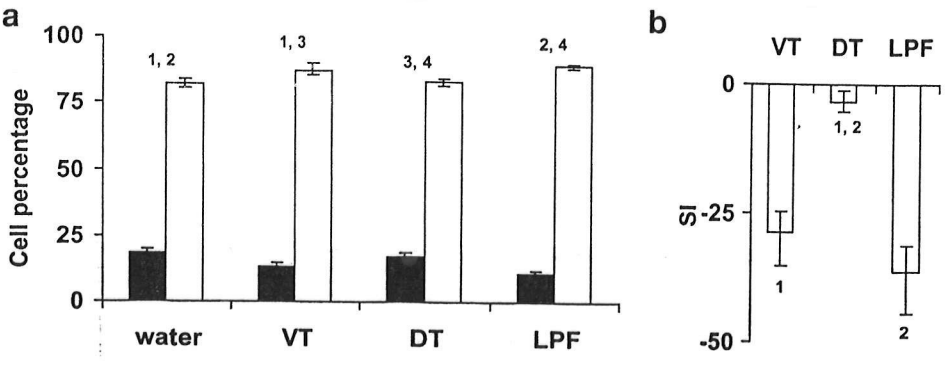


Fig. 4. *Coscinodiscus perforatus*, Condition 1 (mixed suspension). (a) Mean percentages of intact (black bars) and empty (white bars) cells for the different sampling sites: ambient water, ventral tracts (VT), dorsal tracts (DT) and labial palp pseudofeces (LPF); (b) Mean selection index (SI) at the different sites. Error bars represent range of values (n = 3). Bars with same number are significantly different from each other ($p < 0.05$)

What makes diatoms attractive for suspensivores? The organic casing and associated organic molecules of *Coscinodiscus perforatus* are quality cues for the bivalve *Pecten maximus*

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A major question in the study of seston particle dynamics is the properties of particles which induce selection in suspension feeders. In order to determine whether the diatom organic casing and associated organic molecules (herein termed 'perifrustular envelope') influences selection by the Great Scallop Pecten maximus L., a mixed culture containing intact and naturally dead (empty frustules) Coscinodiscus perforatus was presented to normally feeding scallops. The presence of the envelope on all cells of the culture was verified using scanning electron microscopy. Proportions of intact and dead cells were determined via endoscopy-assisted sampling at strategic points in the particle processing chain. The use of C. perforatus as a food item was verified by microscopic examination of faeces at the end of the experiment. Selection indices were calculated to quantify eventual enrichment of empty, dead cell proportions at the rejection sites, in relation to the previous steps in the particle-processing chain. No significant difference was observed in the proportions of intact and dead cells at any of the sites, nor in the selection indices, and the large P-values suggest that both cell types were treated in the same manner by these actively feeding scallops ($P = 0.760$ and 0.839 for proportions and selection indices, respectively). Together with previous studies which have shown that empty, cleaned (perifrustular envelope absent) diatom frustules provoke rejection; these results strongly support the hypothesis that the presence of the external organic components is a major selection cue. This has implications for phytoplankton dynamics, notably with respect to the potential fates of species captured by this suspension feeder.

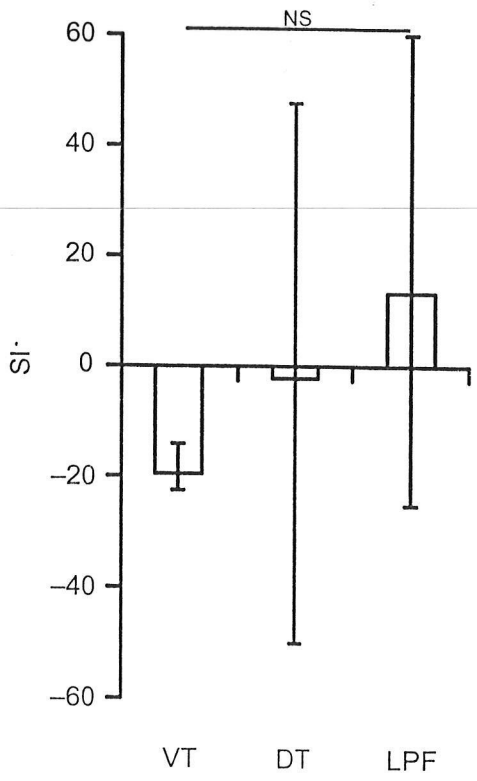


Fig. 4. Mean selection index (SI) at the different sites: dorsal tracts (DT), ventral tracts (VT) and labial palp pseudofaeces (LPF). Error bars represent the range of values ($n = 3$). NS: not significant ($P \gg 0.05$).

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The oyster proves the rule: mechanisms of pseudofeces transport and rejection on the mantle of *Crassostrea virginica* and *C. gigas*

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ABSTRACT: The pseudolamellibranch is the only main bivalve particle processing system whose mechanisms of pseudofeces rejection on the mantle have not been elucidated. To this end, the entire mantle surface and underlying tissue characteristics were investigated in the oysters *Crassostrea virginica* and *C. gigas*, using scanning electron microscopy and histology, as well as observations of particle transport on half-shell preparations. Pseudofeces transport is effected by short simple cilia atop specialized radial ridges, which extend from the gill-mantle junction near the adductor muscle to the mantle edges. The histology of the ridges is characterized by a densely-ciliated pseudostratified epithelium and numerous acid mucopolysaccharide (AMPS)-containing mucocytes, and differs markedly from that of the inter-ridge regions which present a cuboidal epithelium containing few mucocytes and isolated tufts of long simple cilia. In contrast to the pseudofeces transport (infrabranchial) region of the mantle, the suprabranchial mantle region is uniformly and densely ciliated, and devoid of ridges. The sparse ciliation of the inter-ridge region and the counter-current beat of the ridge cilia (presumably isolated to varying degrees from the pallial flow by the mucus-particle rafts involved in mucociliary transport) indicate that the maintenance of pallial flow is accomplished almost exclusively by the gill and the suprabranchial mantle ciliation in these 2 species. Pallial pseudofeces rejection mechanisms to date are summarized for the 4 main bivalve gill systems, which may be grouped into 3 categories: (1) pallial transport with vertical elevation accomplished via composite cilia (homorhabdic filibranchs and eulamellibranchs), (2) pallial transport with vertical elevation accomplished via mantle ridges (pseudolamellibranchs), and (3) voidance via valve clapping (heterorhabdic filibranchs).

KEY WORDS: Oyster · Feeding · Mantle · Rejection · Pseudofeces

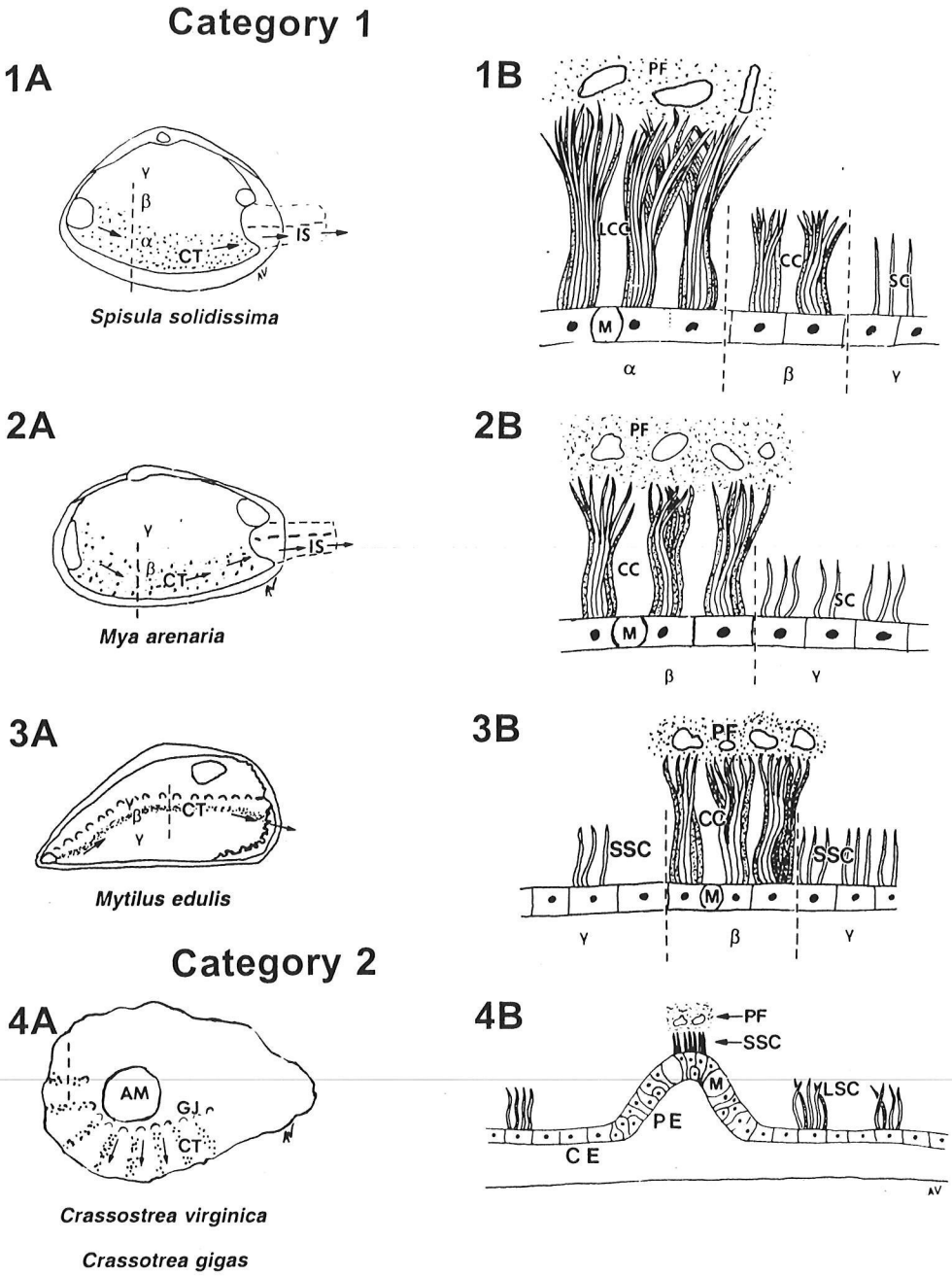


Fig. 4. Schematic drawings to compare the characteristics of *Crassostrea* spp. with those of species previously studied from the standpoint of pseudofeces transport and rejection (Beninger et al. 1999). (4.1–4.3) bivalves possessing a gill ventral particle groove and pallial siphons/apertures. (4.1A) *Spisula solidissima*. Three ciliary bands are arranged in a ventro-dorsal sequence: α: long composite cilia (rejection tract); β: composite cilia; γ: simple cilia. M: mantle. Arrows show direction of pseudofeces transport, dashed line shows plane of section for Fig. 1B. (4.1B) Cilia types of each band and transport of pseudofeces (PF) atop the LCC of the rejection tract. (4.2A) *Mya arenaria*. Two ciliary bands are found: β (composite cilia: CC), and γ (simple cilia: SC). Arrows show direction of pseudofeces transport, dashed line shows plane of section for Fig. 4.2B. (4.2B) Cilia types of each band and transport of pseudofeces atop the CC of the rejection tract. (4.3A) *Mytlius edulis*. Three parallel ciliary bands extend dorso-ventrally: short simple cilia (γ), compound cilia (β), and short simple cilia (γ). Arrows show direction of pseudofeces transport, dashed line shows plane of section for Fig. 4.3B. (4.3B) Cilia types of each band, and transport of pseudofeces atop the CC of the rejection tract. (4.4A) *Crassostrea virginica, C. gigas*. Ciliated tracts of mantle pseudofeces rejection ridges, showing direction of pseudofeces transport (arrows). AM: adductor muscle; GJ: gill junction with mantle. Dashed line shows plane of section for Fig. 4.3B. (4.4B) Cilia types on mantle: short simple cilia (SSC) on rejection ridge, isolated tufts of long simple cilia (LSC) in inter-ridge region. In all cases, pseudofeces are transported above the general pallial surface, either atop composite cilia longer than the cilia of the general pallial surface (Figs. 1–3), or atop short simple cilia, which are themselves elevated with respect to the general pallial surface via specialized rejection ridges (Fig. 4, *Crassostrea* spp.)