Topic 1 - Feeding and digestion - invertebrates

Introduction

In this lecture we will examine feeding and digestion of the two groups of invertebrates that are the most significant in aquaculture: decapod crustaceans and bivalve molluscs. We will also examine the storage of digested nutrients: their composition, location and seasonal variations. The nutrient composition of food will be referred to (e.g., peptides, amino acids, lipids, triacylglycerols, amylose and amylopectin), this forming a necessary part of understanding digestion and assimilation. These nutrients will be discussed in much greater detail in Topic 3, so you may wish to re-read this lecture in the light of knowledge gained later.

CRUSTACEANS

The most cultured group of decapod crustaceans are shrimp. These can be filter feeders, scavengers and predators; they are classified as herbivores, carnivores and omnivores. In the wild, or in extensive and semi-intensive aquaculture, shrimp eat other species of crustaceans, annelids, molluscs, echinoderms, nematodes, fish tissue, insects, seeds, algae, macrophytes (an aquatic plant that grows in water) vegetable matter and detritus (Figueiredo & Anderson, 2009; Focken *et al.*, 1998). In extensive and semi-intensive pond-cultured shrimp, the naturally available food organisms can dominate over the externally applied feed (Nunes *et al.*, 1997; Nunes & Parsons, 2000).

1. Feeding

The external feeding appendages, the pereiopods and the mandibles (jaws) are involved in feeding behaviour (Maynard & Sallee, 1970) (Fig. 1).

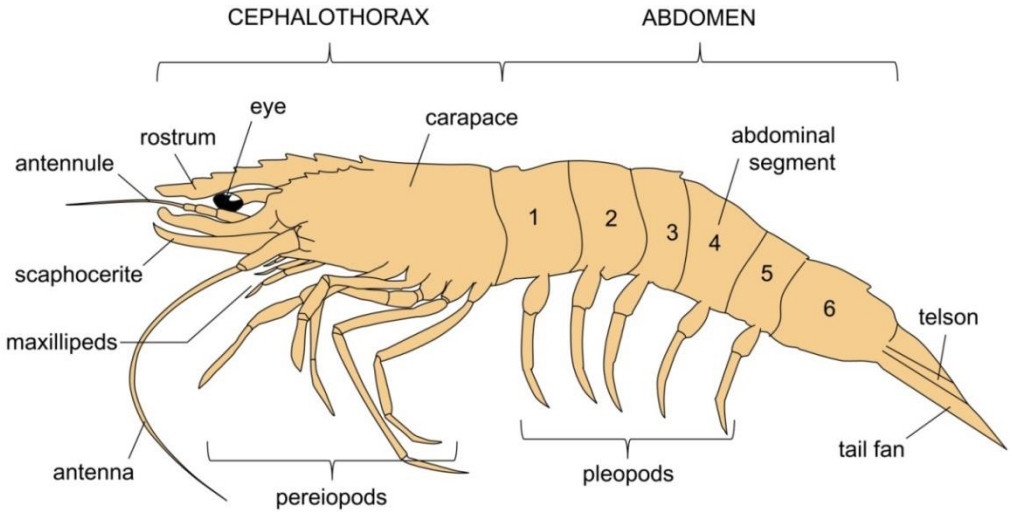

Fig. 1. General anatomy of a penaeid prawn.

The antennae and antennules are used as feelers or sensory feelers. The maxillipeds are used to rip food apart before it is moved into the mandible where it is crushed and devoured.

Crustaceans detect chemicals produced by food using small cuticular sense organs called sensilla. Two types of sensilla are involved in this process: bimodal sensilla and unimodal sensilla called aesthetascs (Schmidt & DeForest Jr., 2011). Bimodal sensilla contain both mechanoreceptor and chemoreceptor neurones whose dendrites terminate in a pore at the tip of a seta. These organs are present on the body and all appendages. Aesthetascs contain numerous neurones whose branched dendrites fill the lumen of a tube-like seta. They are found only on the outer flagellum of the antennules and provide input into the olfactory lobes of the brain.

Once food has been identified remotely and the animal has moved adjacent to it, the pereiopods and the mouthparts locate the food via their own chemo- and mechanosensory setae and then grasp, manipulate, crush and transport it to the mouth (Derby, 1982; Derby & Atema, 1982a; 1982b; Derby & Harpaz, 1988). Postlarvae may also use their mouthparts to capture suspended particulate food.

The sense of taste (gustation) is present in most, if not all, of the crustacean appendages. A substance is tasted by a hair-like hollow structure, the sensillum, that houses gustatory neurones (Reinhard, 2010). What distinguishes gustation from olfaction is that in the former, the material requires to be in direct contact with the sensory organ. The mouthparts provide the final check of food quality before ingestion since food that has been accepted by the pereiopods may be subsequently rejected after handling by the mouthparts (Derby *et al.*, 1984).

Food is masticated by the external, toothed maxillipeds and mandibles before being swallowed. It then enters the cardiac pocket of the stomach via peristaltic waves of the relatively short oesophagus.

2. Digestive system

The alimentary canal of decapods is a tubular structure, which begins with an anteroventral mouth, runs dorsally along the body and terminates in the anus, located in the base of the telson Fig. 2 and Fig. 3.

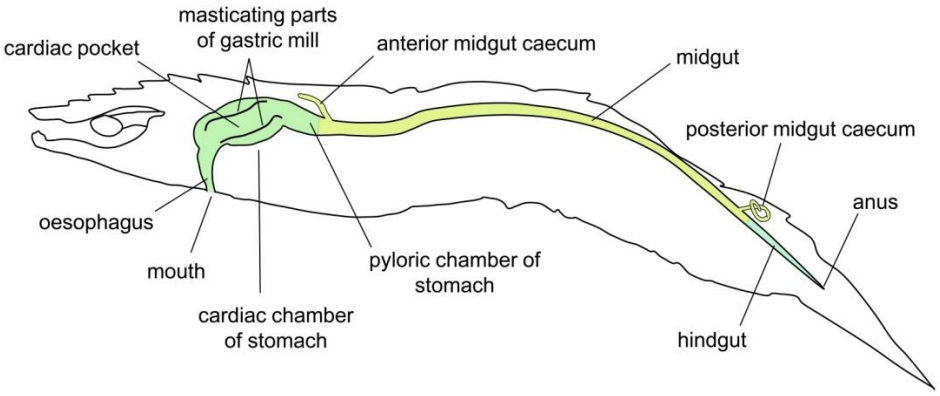

Fig. 2. The digestive system of a penaeid prawn. The hepatopancreas is not shown.

The digestive system is divided into three distinct regions: foregut, midgut and hindgut (Felgenhauer, 1992; Icely & Nott, 1992). The foregut is lined with a thin cuticle and comprises the mouth, the oesophagus and the stomach. The midgut comprises the intestine and associated organs: the hepatopancreas (digestive gland or midgut gland) and the midgut caeca. The relative length of the midgut varies considerably between decapod groups (Smith, 1978). The hindgut, like the foregut, is lined with cuticle and forms a muscular rectum that leads to the anus.

Fig. 3

Whiteleg shrimp, *Litopenaeus vannamei (*formerly *Penaeus vannamei*). The anatomy of the digestive tract can be seen due to the relative transparency of the body tissue.

2.1 The foregut

When the sensory receptors within the oesophagus are stimulated, they cause a rhythmic contraction and relaxation of the oesophageal muscles which move the food into the anterior chamber of the stomach (the cardiac stomach or pocket) (Robertson & Laverack, 1979). The cardiac stomach serves as a storage area, its relative size varying between species. Food is passed from this storage area to the masticating parts of the gastric mill (Fig. 4).

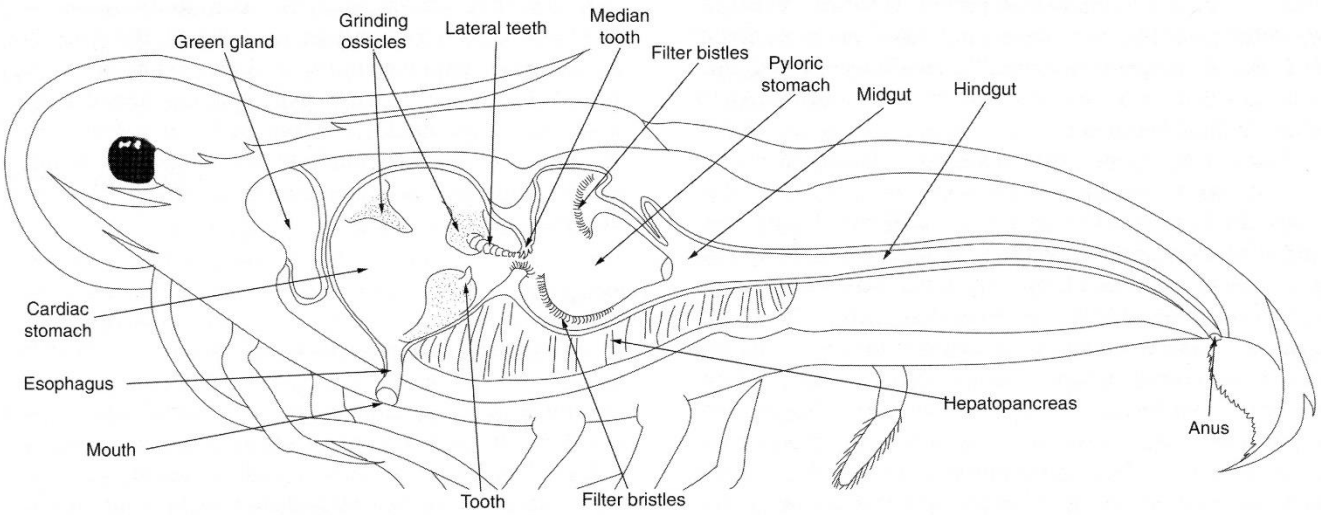

Fig. 4. The digestive system of a penaeid prawn. Detail of foregut.

The epidermis of the cardiac pocket is associated with the exoskeleton and participates in the periodic moulting process. The epidermis secretes sclerotized, cuticular structures which are often calcified. These structures are moved by individual muscles located outside the stomach wall and receive their own specific innervation. The gastric mill of the cardiac pocket grinds the food into a soup of fine particles. Particles are circulated and undergo further mastication until they are fine enough to pass into the pyloric stomach via the cardiopyloric valve.

The pyloric stomach squeezes material through the more dorsal aspects of the pyloric filtering apparatus and final filtration is performed by the more ventral gland filters. This filtration ensures that the hepatopancreas does not become clogged by large particles. In the lobster, such particles need to be less than 1 µm diameter (Bayer *et al.*, 1979).

The lining of the stomach, particularly in the pyloric region, is invaginated suggesting that some digestion may take place although transit time is short (under a minute). Macerated food is passed to the midgut where it is further digested and most nutrients are absorbed.

2.2 The midgut

The intestine of the midgut consists of a simple columnar epithelium which is folded longitudinally and its surface area is increased by the presence of microvilli. Three organs are associated with the midgut: the anterior and posterior midgut caeca and the hepatopancreas (or digestive gland). The midgut caeca consist of blind-ending tubules that connect to the lumen of the midgut. Decapod crustaceans usually possess a pair of anterior caeca that arise laterally, one on either side of the anterior midgut and a single posterior caecum that arises dorsally close to the junction between the midgut and the hindgut (Smith, 1978). These caeca appear to play only a minor role in digestion and osmoregulation (Holliday *et al.*, 1980) although Mykles (1980) has reported that they are significant in the uptake of water during ecdysis. Recent evidence suggests that the caeca are involved in endocrine regulation of feeding behaviour (Christie *et al.*, 2007).

The midgut is connected to the hepatopancreas, so named because it combines the functions of the vertebrate liver and pancreas. The hepatopancreas forms a pair of glands on either side of the stomach where it occupies a large proportion of the cephalothorax (Fig. 5).

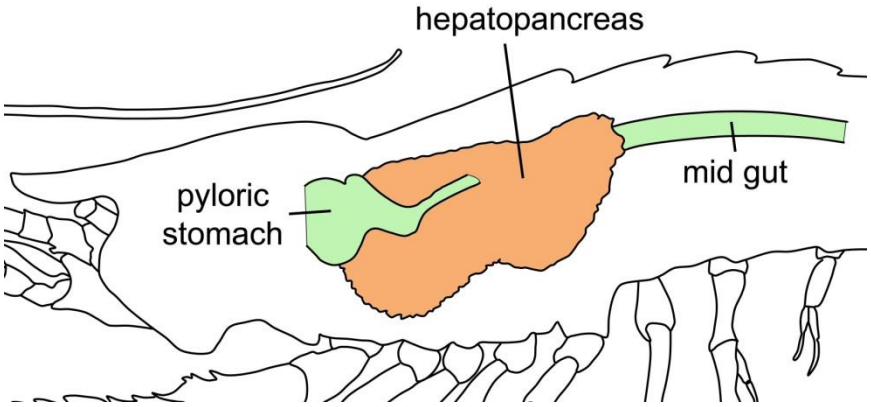

Fig. 5

Position of the hepatopancreas in a lobster.

The hepatopancreas is also known as the midgut gland or digestive gland. This organ is particularly large in decapod crustaceans, where it makes up 2-6% of the body mass (Brockerhoff & Hoyle, 1967; Stewart *et al.*, 1967). The colour of the hepatopancreas is variable (e.g., brown, red, green, yellow) depending on the pigments that it contains. Each half of the hepatopancreas consists of a cluster of ducts that subdivide into small tubules that make up the glandular mass. The primary duct of each gland opens into the midgut. Digested food and secretions exchange between the gland and the midgut as a result of the peristaltic action of circular and longitudinal muscles.

The hepatopancreas is responsible for the secretion of digestive enzymes, the absorption of nutrients, the storage of energy reserves and the transport of monovalent and divalent ions. Digestive enzymes are synthesised in the F-cells (fibrillar cells) and accumulate in the B-cells (blister-like cells) (Sousa *et al.*, 2005; Vogt *et al.*, 1989).

The products of digestion are absorbed and stored by the hepatopancreas. This tubular system has a single layer of epithelial cells that facilitate rapid trans-cellular nutrient transport to the haemolymph. Two of the cell types of the hepatopancreas, the R- (resorptive cells) and F-cells, are equipped with microvilli, indicating absorptive functions. Epithelial cells of the intestinal ceca, present in several shrimp species, also have well organized microvilli (Ceccaldi, 1997). The midgut intestine is capable of absorbing nutrients such as amino acids and glucose but this is relatively minor compared to the role played by the hepatopancreas (Ahearn, 1974; Ahearn & Maginniss, 1977; Gibson & Barker, 1979b).

Significant sodium-dependent glucose and glutamate uptake and non-sodium-dependent alanine and lysine uptake has been demonstrated in crustacean hepatopancreas; transport rates are several orders of magnitude greater than those of the intestine (Ahearn & Clay, 1987; Ahearn *et al.*, 1985; Verri *et al.*, 2001).

Experiments on penaeids have revealed that R- (resorptive cells) and B-cells of the hepatopancreas possess different types of glucose transport mechanisms with B cells showing a Na^+-dependent sugar transport process and R cells showing a Na^+-independent sugar carrier system (Blaya *et al.*, 1998; Verri *et al.*, 2001; Vilella *et al.*, 1998). Simmons *et al*. (2012) found that, in the hepatopancreas of the White shrimp, *Litopenaeus setiferus*, sodium, potassium, and metallic cations such as zinc and manganese may stimulate the absorption of D-glucose, L-leucine and L-histidine by independent carrier-mediated transport processes. These mechanisms use the gradient of one solute's concentration to force the other molecule or ion against its gradient. Such mechanisms are known as cotransporters.

The activities of several membrane transporters in the hepatopancreas are pH-dependent (Verri *et al.*, 2001). This has been demonstrated for the Na^+-D-glucose cotransporter, the Na^+/Cl^-/L-alanine cotransporter, the $Na^+/2Cl^-$/L-

leucine cotransporter and the Na^+/Cl^-/L-glutamate cotransporter. The low pH in the hepatopancreatic lumen facilitates nutrient influx into the epithelial cells.

Undigested residues from the hepatopancreas are passed back to the midgut intestine and combine with material that was diverted away from the hepatopancreas opening. This material is compacted in the intestine where water and ions are removed and transported across the epithelium.

The hepatopancreas also releases an insulin-like factor in lobsters and probably other decapod crustaceans (Sanders, 1983a). This factor increases glycogenesis in lobster muscle (Sanders, 1983b) but is not released in response to a rise in glucose in the haemolymph nor does it reduce haemolymph glucose levels (Sanders, 1983c).

In some decapods, such as the marine *Carcinus maenas* and *Cancer pagurus*, and the freshwater crab *Paratelphusa hydrodomous*, calcium and phosphate are accumulated by the hepatopancreas during the pre-moult period and stored as calcium phosphate granules (Greenaway, 1985).

2.3 The hindgut

Digestion is completed in several hours and indigestible matter passes into the peritrophic membrane of the midgut. The faecal pellet is contained in this tube, which is grasped by the rectal pads and extruded at intervals in lengths equal to that of the midgut. Infolding of the basal membrane in the hindgut epithelial cells as well as folding of the entire epithelium allow for stretching of the hindgut lining under faecal loading.

The cells lining the short hindgut appear to be specialized for cation transport (Mykles, 1980), but their specific functional role in nutrition has yet to be defined. Since the hindgut is lined with cuticle, it is unlikely to absorb digestive materials (van Weel, 1955). It may be involved in water transport (see the section on osmoregulation), but again its role must be limited due to the cuticular lining. The rectum is probably also the means by which the external medium is pumped into the midgut but, again, the function of this rectal pumping has not been firmly established.

3. Digestive enzymes

The pH of digestive fluids is acidic (about pH 5) (Hoyle, 1973) which favours the activities of the various enzymes involved in digestion. The shrimp stomach, which is covered in a chitin layer, does not secrete acid or enzymes, although its contents often show digestive enzyme activities, some of which may derive from the hepatopancreas and some from food animals.

The midgut contains several enzymes including protease, amylase and lipase which are secreted by the hepatopancreas (Ceccaldi, 1989). The proportions of these enzymes can vary with development (Biesiot & Capuzzo, 1990). Digestive enzymes are synthesised in the F-cells (fibrillar cells) and accumulate in the B-cells (Sousa *et al.*, 2005; Vogt *et al.*, 1989).

Normally, secretion is thought to be by merocrine (by exocytosis) or apocrine (by enclosing in plasma membrane to form vesicles) discharge; however, the stimulation of food intake following a period of starvation may induce holocrine secretion (by rupture of the cell membrane) (Gibson and Barker, 1979).

The hepatopancreas secretes a wide range of digestive enzymes: proteases, including specific collagenases, lipolytic enzymes, chitinase, cellulase (e.g. endo-β-1,4-glucanase and β-1,4-glucosidase) to be able to utilise the cellulose from plant cell walls, laminarinase which hydrolyses laminarin (a polysaccharide of glucose found in brown algae) and amylase which hydrolyses starch (Allardyce & Linton, 2008; Figueiredo & Anderson, 2009; Johnston & Freeman, 2005; Xue *et al.*, 1999). Species with a high dietary intake of protein show high proteinase activities. Species that feed on crustaceans synthesise chitinase. Herbivorous species tend to produce high levels of the various carbohydrases to be able to disrupt cell walls and make use of the cellulose from plant cell walls, laminarin and other non-starch polysaccharides (Johnston & Freeman, 2005; Xue *et al.*, 1999). Omnivorous species have high activities of several of the above enzymes.

3.1 Proteases

Protein is a major component in the feed of crustaceans and consequently proteolytic enzymes play a significant role in food hydrolysis and assimilation (Dall, 1991; Gibson & Barker, 1979a).

Proteases are involved in digesting long protein chains into short fragments, splitting the peptide bonds that link amino acid residues by catalysing the reaction of hydrolysis. Hydrolysis is a chemical process in which a molecule of a target substance is split into two parts by the addition of a molecule of water. Some proteases can detach the terminal amino acids from the protein chain (exopeptidases, such as aminopeptidases, carboxypeptidase A); the others attack internal peptide bonds of a protein (endopeptidases, such as trypsin, chymotrypsin, pepsin, papain and elastase). Thus, when trypsin hydrolyses a protein, it splits peptides into amino acids which can then be absorbed across the cell membrane (Fig. 6).

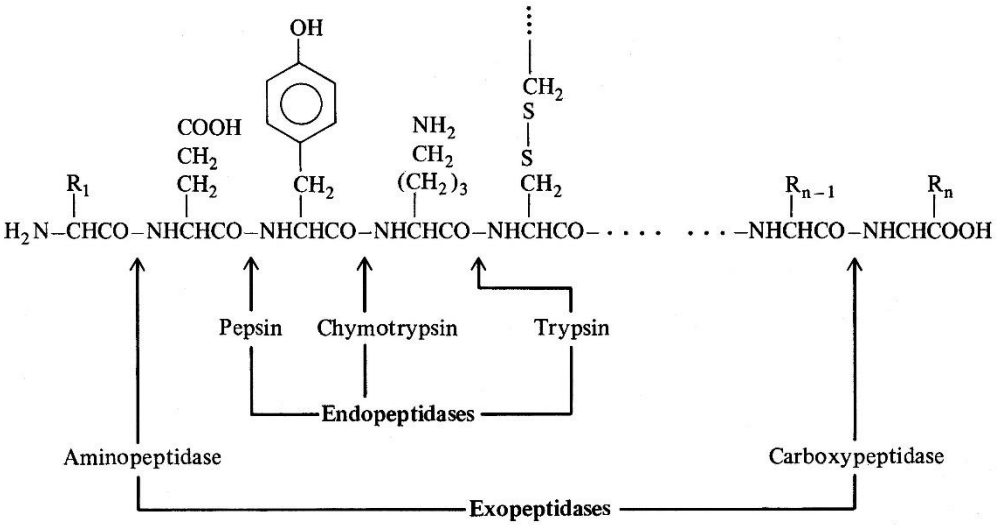

Fig. 6. Cleavage points for various protease enzymes - exopeptidases (aminopeptidase and carboxypeptidase) and endopeptidases (pepsin, chymotrypsin).

For many years it was thought that the vast majority of ingested proteins in all animals were completely hydrolysed to free amino acids before absorption. However, in mammals and teleost fish this view has been revised, and it is now believed that a significant proportion of amino acids are absorbed in the form of di- and tripeptides via a cotransport system driven by a hydrogen ion gradient (D'Mello, 2003). There is some evidence that the hepatopancreas of decapod crustaceans possess similar mechanisms (Thamotharan & Ahearn, 1996).

The vertebrate proteases (pepsin, chymotrypsin and carboxypeptidase) are secreted as inactive zymogens (pepsinogen, trypsinogen, chymotrypsinogen and procarboxypeptidase). This is in order to prevent digestion of intracellular proteins. These inactive precursors are activated by either inorganic ions (e.g., H+ activates pepsinogen), specific enzymes (e.g., enterokinase activates trypsinogen) other enzymes (e.g. trypsin activates chymotrypsinogen) or by the active enzyme itself (e.g. pepsin activates pepsinogen; trypsin activates trypsinogen).

Invertebrate endopeptidases generally are secreted in an activated form, although their activity is augmented by the presence of sulfhydryl (S-H) bonds in the gut. Various intracellular endopeptidases of both vertebrates and invertebrates, called cathepsins, are produced in the active form. It is likely that extracellular digestive endopeptidases are derived from intracellular cathepsins.

Proteases found in crustacean include trypsin, chymotrypsin, elastase, semi-collagenase, aminopeptidases and carboxypeptidases (**Table I**).

The chemistry of crustacean proteases has been the subject of a considerable research effort (e.g.Celis-Guerrero *et al.*, 2004; Dall *et al.*, 1990; DeVillez & Buschlen, 1967; Fernández Gimenez *et al.*, 2001; Muhlia-Almazán &

García- Carreño, 2003; Zwilling *et al.*, 1969). Several studies have shown that in crustaceans, especially penaeids, the synthesis of digestive proteases is sensitive to the level of protein in the food (Muhlia-Almazán & García- Carreño, 2003).

Table I. Proteases produced by the hepatopancreas

Enzyme	Substrate	Specificity or products
Trypsins	Proteins & polypeptides	Peptide bonds adjacent to arginine or lysine
Chymotrypsins	Proteins & polypeptides	Peptide bonds adjacent to aromatic amino acids
Cathepsin L	Collagen and elastin	Broad specificity for peptide bonds
Collagenase	Collagen	Hydrolyse the triple helix of native collagen

The main endopeptidases for most crustaceans are trypsin and chymotrypsin. Trypsin is considered one of the most important enzymes in decapod crustaceans. In *Marsupenaeus japonicus* and *Melicertus kerathurus*, this enzyme accounts for 40-50% of the total proteolysis (Galgani *et al.*, 1984), whereas it is reported to be about 50-60% in giant tiger prawn (*Penaeus monodon)*, kuruma shrimp (*Marsupenaeus japonicus)*, redtail prawn (*Fenneropenaeus penicillatus* formerly *Penaeus penicillatus*) speckled shrimp (*Metapenaeus monoceros)* and *Euphausia superba* (Tsai *et al.*, 1986) and 33% in the fiddler crab *Uca pugilator* (Eizen & Jeffrey, 1969) (Fig. 7).

Kuruma shrimp *Marsupenaeus japonicus*

Speckled shrimp (*Metapenaeus monoceros)*

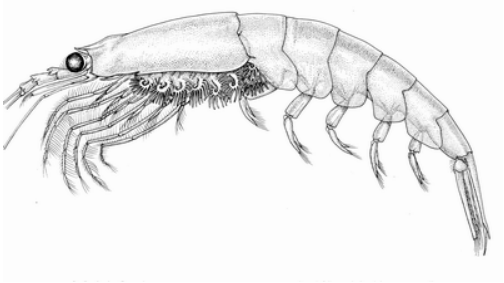

Fig. 7. *Euphausia superba*

Fiddler crab *Uca pugilator*

In giant freshwater prawn (*Macrobrachium rosenbergii*) trypsin and chymotrypsin account for between 55 and 82% of proteolytic activity (Chisty *et al.*, 2009). Some species produce cathepsin L as the main proteolytic enzyme e.g. the North Sea shrimp *Crangon crangon* and *Crangon allmani* most individuals of which lack trypsin and chymotrypsin (Teschke & Saborowski, 2005).

Trypsins have been described in decapod crustaceans that are capable of hydrolysing the triple helix of native collagen under physiological conditions. These enzymes, discovered in in the fiddler crab *Uca pugilator,* have been named "collagenolytic proteases" (Eizen & Jeffrey, 1969; Grant *et al.*, 1983). True collagenases are metalloproteases that degrade native collagen under physiological conditions, but are unable to hydrolyse denatured collagen or other native proteins (Woolley *et al.*, 1978). The main difference with the collagenolytic proteinases resides in the structure of the active sites (Sakharov *et al.*, 1994). Proteases able to degrade native collagen have also been found in *P. monodon* (Lu *et al.*, 1990; Tsai *et al.*, 1991).

In crustaceans, amino acids are absorbed by the R-cells and F-cells of the hepatopancreas and, to a lesser extent, by the midgut intestine. Amino acids are transported to the various tissues and are utilised as required, either being incorporated into proteins or used in a range of biochemical processes. Between 25% and 50% of dietary amino acids are incorporated into the body protein. The deposition of protein is consequently a major determinant of amino acid utilization and requirements.

3.2 Carbohydrases

The term carbohydrases applies to enzymes that digest carbohydrates. Carbohydrate digestion, like protein digestion, involves hydrolysis of polymeric bonds (the bonds between polymers - chemical compound or mixture of compounds consisting of repeating structural units) and proceeds in stages until the basic units (monosaccharides) are produced. There are two classes of enzymes that hydrolyze polysaccharides (polymers of saccharides): the polysaccharidases and the oligosaccharidases. Polysaccharides are long carbohydrate molecules of up to 3,000 monosaccharide units joined together. An oligosaccharide contains a relatively small number (typically 2-10) of monosaccharides.

The most common polysaccharidase enzymes are amylases, which hydrolyze plant starches (amylose and amylopectin) (Fig. 8) and animal glycogen; these all are polysaccharides with α-bonds between the subunits.

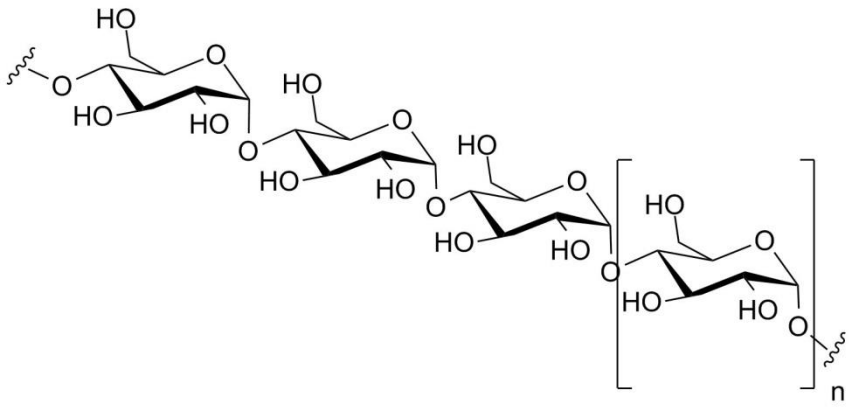

Fig. 8. Amylose is a linear polymer of glucose mainly linked with α(1→4) bonds. It can be made of several thousands of glucose units. It is one of the two components of starch, the other being amylopectin.

The oligosaccharidases hydrolyze trisaccharides such as raffinose, and disaccharides, such as maltose, sucrose, lactose (milk sugar) and trehalose (a common insect sugar). Maltose, sucrose and trehalose all contain a glucose bound by an α-bond to a second monosaccharide and are hydrolyzed by α-glucosidases (e.g., maltase and sucrase - also called invertase in invertebrates - and trehalase). Glucose linked to other monosaccharides by a β-bond (e.g. lactose, cellobiose) is hydrolyzed by β-glycosidases (e.g. lactase or β-galactosidase).

Decapod crustaceans are able to hydrolyze a great variety of polysaccharides and oligosaccharides, greatly surpassing the range of herbivorous fish. A wide range of enzymes have been identified in shrimp hepatopancreas and other sections of the digestive tract: α- and β-galactosidases, α-fucosidase, laminarinase, α-mannosidase, β-glucuronidase, β-glucosaminidase, xylanase, α-xylosidase, raffinase, β-fructofuranosidase, chitobiase and cellulase (Chuang *et al.*, 1992; Chuang *et al.*, 1991a; Chuang *et al.*, 1991b; Gibson & Barker, 1979a; Omondi & Stark, 1996; Trellu & Ceccaldi, 1977; Vega-Villasante *et al.*, 1995).

The activity of amylases is of great importance for herbivorous crustaceans and in the (omnivorous) common prawn (*Palaemon serratus)*, its activity is more important than proteolytic activity (van Wormhoudt, 1980). Sequencing of three α-amylases extracted from *Litopenaeus vannamei* has revealed close evolutionary relationships with mammals (59-63% identity) and with insects (52-62% identity) (van Wormhoudt & Sellos, 1996; Van Wormhoudt & Sellos, 2003).

The digestibility of starch is an important factor in determining the availability of glucose in feed formulations. The enzyme α-amylase hydrolyses starch into maltose. Additional enzymes, maltase and isomaltase, then hydrolyse maltose further into glucose. These enzymes are present in the gastrointestinal tract of fish and shrimp but their activity and effectiveness vary between species. Data are limited for crustacean species (**Table II**) but in fish, a reduction in digestibility is observed when the dietary level of starch increases, irrespective of the starch origin.

Table II. Apparent digestibility of starch from different sources by the Pacific white shrimp (*Litopenaeus vannamei*)

Source	Level in diet (g/kg)	Apparent digestibility coefficient
Raw maize starch	350	85
High amylose maize starch	350	63
Waxy maize starch	350	85
Gelatinized maize starch	350	94
Gelatinized waxy maize starch	350	96
Raw potato starch	350	72
Gelatinized potato starch	350	93
Raw wheat starch	350	92
Wheat flour	360	78
Raw cowpea	150	77
Cooked cowpea	150	83
Extruded cowpea	150	82

Data sources: Cousin et al. (1996) and Rivas-Vega et al. (2006).

According to Stone (2003) the reduction in the digestibility of carbohydrates when dietary levels increase may be the result of an overload on the digestive enzymes that become saturated with substrate. The threshold for enzyme saturation by substrate is unknown in crustaceans and it is not apparent in the data shown in Table II.

Herbivorous species of fish have high activity and efficacy of starch degrading enzymes. Omnivorous species have moderate activity whilst carnivores have only a limited ability to digest starch.

Since cellulase is quite rare in the animal kingdom, it is generally believed that the source of cellulase in decapod crustaceans is exogenous - symbiotic organisms living in the hepatopancreas. Even so, Yokoe and Yasumasu (1964) found more cellulase activity in hepatopancreas extracts from Procambarus clarkii than shown by Gram-negative bacteria living in the digestive tract.

3.3 Lipases and esterases

Lipids, like carbohydrates and proteins, are cleaved by hydrolysis reactions. Otherwise, lipid digestion and absorption are fundamentally different because lipid is not water soluble and coalesces into large droplets in water. The low surface-to-volume ratio of a large lipid droplet is not conducive to effective digestion by lipases, and so emulsification is extremely important for lipid digestion. Emulsification stabilizes small lipid droplets in

aqueous solution by coating them with molecules that are partly hydrophobic (to dissolve in the lipid droplet) and partly hydrophilic (to dissolve in water).

Vertebrates produce bile in their livers, which flows into the small intestine. Bile salts are moderately effective emulsifying agents and become much more effective in concert with polar lipids such as monoglycerides (the digestive products of triglycerides) and phospholipids (Kanazawa, 1985) (Fig. 9).

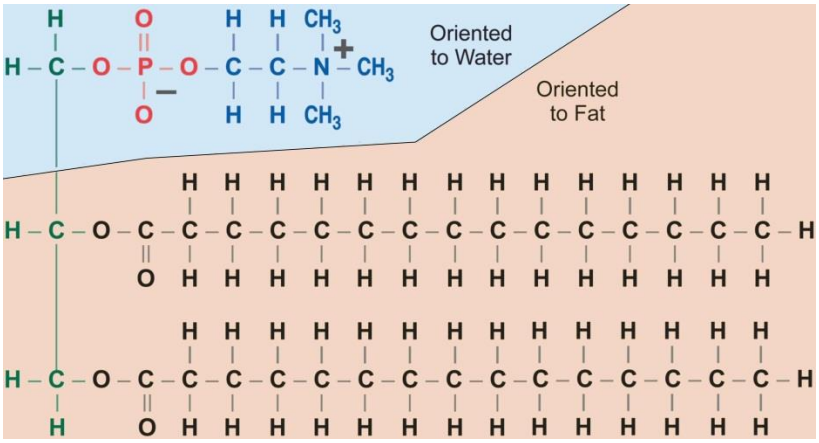

Fig. 9. Phosphatidylcholines, typical phospholipids, are made from glycerol (green), two fatty acids (black) and a phosphate group (red), with another molecule attached to its end (blue). The phosphate end of the molecule orients to water (blue background) due to its electric charge, while the non-polar fatty acid end orients to fat.

Invertebrates do not produce bile. Lipase enzymes attach to lipid droplets which form an emulsion and hydrolyse triglycerides to 2-monoglycerides, some glycerol and free fatty acids. Phospholipids play a significant role in lipid emulsification in crustaceans.

Lipases which are capable of hydrolysing triglycerides and phospholipids (**Table III**) have been observed in several species of shrimp (Deering *et al.*, 1996; González *et al.*, 1994; Moss *et al.*, 2001). Due to their function, lipases are commonly named esterases, although they almost exclusively hydrolyse carboxylic esters.

Table III. Lipases produced by the hepatopancreas

Enzyme	Substrate	Specificity or products
Triglyceride lipase	Triglycerides	Hydrolyse ester linkages producing monoglycerides and free fatty acids
Phospholipase	Phospholipids	Produce fatty acids and other lipophilic substances

The presence of real lipases in the shrimp hepatopancreas has been a point of disagreement among specialists working on the physiology and biochemistry of these species, since most of the techniques employed in their study cannot establish whether the lipolytic activity found was due to real lipases or to non-specific esterases.

Both triglyceride lipase and phospholipase have been observed in *Litopenaeus vannamei* (del Monte *et al.*, 2003; Forrellat *et al.*, 2004). A comparison of substrate specificity of lipases from *Litopenaeus schmitti* indicated a strong preference for *n*-3 (*ω*−3) and n-6 (*ω*−6) fatty acids (del Monte *et al.*, 2002). Studies of lipases from *Litopenaeus vannamei*, *Farfantepenaeus californiensis* and *Farfantepenaeus notialis*, showed similar preferences (Forrellat *et al.*, 2004). Note, with the exception of the land snail (*Cepaea nemoralis*) animals are incapable of *de novo* synthesis of fatty acids with double bonds in the *n-6* (linoleic series) and *n-3* (linolenic series) positions; only plants are able to synthesize these fatty acids *de novo*.

Lipases and esterases are associated with the microvilli of R-cells as well as vacuoles of F-cells, the lumen of the hepatopancreas tubules and in intertubular connective tissue (López-López *et al.*, 2005).

4. Changes in enzyme activity

Large changes in enzyme activity are observed during moulting (e.g. trypsin and chitinase) (Sainz Hernández & Cordova Murueta, 2009). Chitinase digests the old exoskeleton so it can be resorbed and replaced by newly synthesized chitin. The production of digestive enzymes also seems to vary throughout the year and even within a species, depending upon the available nutrient resources or stage of development (e.g. Biesiot & Capuzzo, 1990). The Caridean shrimp (*Crangon crangon*) has high trypsin activity during the summer and low activity during the winter (Pöhlmann, 2007; Sahlmann, 2008).

Adjustments to diet composition for proteases, lipolytic enzymes and amylase have been shown for many species. The responses vary among species. In some species, responses are seen in proteolytic and amylase activity but not in lipolytic activity, but for other species, amylase and/or protease activity seem unresponsive (Gamboa-Delgado *et al.*, 2003; López-López *et al.*, 2005; Moss *et al.*, 2001). A high dietary starch content was found to increase the specific activity of α-amylase and α-glucosidase in *Litopenaeus vannamei* (Gaxiola *et al.*, 2005; Le Moullac *et al.*, 1997). The same species has shown variation in trypsin and chymotrypsin activity with variation in protein level (Le Moullac *et al.*, 1997; Lemos *et al.*, 2000; Muhlia-Almazán & García- Carreño, 2003; Muhlia-Almazan *et al.*, 2008). The magnitude of the stimulation seems to depend on the species and the dietary protein source.

Digestive enzymes seem to be released from the hepatopancreas upon feeding (Ong & Johnston, 2006). Passage of food from the midgut to the stomach has been found to induce additional synthesis and secretion of enzymes (Vogt *et al.*, 1989). The release of digestive enzymes by the hepatopancreas is influenced by the endocrine system. For example, crustacean hyperglycaemic hormone (CHH) induces the release of proteases and amylases from the hepatopancreas (Dietrich, 1988); this organ is also responsive to a variety of vertebrate gastrointestinal hormones (Resch-Sedlmeier & Sedlmeier, 1999).

5. Energy storage

In mammals and birds, prolonged fasting induces a mobilization of fat stores whilst minimizing protein loss (Cherel *et al.*, 1992). Fish, such as the Atlantic cod (*Gadus morhua*) respond to starvation by mobilizing hepatic lipids first, then muscle and hepatic glycogen and finally muscle protein (Guderley *et al.*, 2003). Long-term flying insects utilize lipids as their main energy source (Van der Horst & Ryan, 2012) while other insect species can use carbohydrates or proline (Gäde & Auerswald, 2002). In crustaceans, it is thought that protein contributes significantly to energy reserves (New, 1976) a conclusion that is consistent with some reports showing a limited capacity of marine crustaceans to store lipids and carbohydrates (Dall & Smith, 1986; Rosas *et al.*, 2001).

The hepatopancreas is the main storage organ in crustaceans, mainly accumulating lipids (Adamczewska & Morris, 1994; Luvizotto-Santos *et al.*, 2003; Yepiz-Plascencia *et al.*, 2000) and to a lesser extent, glycogen (Verri *et al.*, 2001). It has been proposed that in crustaceans, neutral lipids (mainly triglycerides) are preferentially catabolized during starvation, while polar lipids (phospholipids and cholesterol) are conserved due to their role as structural components of cell membranes (Bourdier & Amblard, 1989; Heath & Barnes, 1970; Stuck *et al.*, 1996). A large reduction in total lipids in response to starvation has been reported in larvae, sub-adult and adult lobsters (Ritar *et al.*, 2003; Stuck *et al.*, 1996).

Glycogen is the most widely distributed animal polysaccharide and can be readily converted to glucose which, being soluble, can then be transported via the blood (Awapara & Simpson, 1967). Crustaceans utilize glycogen as an energy store but the proportion of all energy reserves depends upon the nutritional history of the individual. For example, in the estuarine grapsid crab *Chasmagnathus granulata*, 21 days of starvation reduced glycogen reserves by 51% in animals previously fed a high protein diet and by 64% in animals fed a high carbohydrate diet (Oliveira *et al.*, 2004). The difference between the utilization of reserves becomes more significant when one considers the initial glycogen concentrations in the hepatopancreas organs: 1.71% in high protein-fed individuals and 2.82% in high carbohydrate-fed individuals. In these experiments, haemolymph glucose was principally

maintained by effective gluconeogenesis from protein reserves in animals previously fed a high protein diet and from glycogen reserves in animals previously fed a high carbohydrate diet.

MOLLUSCS

Most bivalve molluscs are microphagous (feed on small particles) and feed on particles that are deposited on the substrate (deposit feeders) or in suspension (suspension feeders). Most of the species that are commercially exploited belong to this latter category and use their gills to filter particulate organic matter, usually phytoplankton, from the water.

Suspension feeding requires that organic, digestible, materials are trapped, sorted from indigestible mineral particles and passed to the mouth (LaBarbera, 1981). Bivalves achieve this by means of capture mechanisms of the gills and sorting mechanisms of the gills, labial palps and stomach. Intertidal animals possess a feeding rhythm linked to tides (Morton, 1977; 1983).

Dissolved organic substances (monosaccharides, amino acids) or lipid droplets may be absorbed directly by the epithelium of larvae or adults. Many studies (reviewed in Hily, 1991) have demonstrated the existence of active transfer in the mantle and the gills, while certain carbohydrates present in sea water stimulate the rate of pumping and particle retention. Péquignat (1973) found that *Mytilus edulis* removed dissolved amino acids and glucose, most of which were absorbed onto the gills before transfer to the mantle and digestive gland. Pasteels (1968; 1969) has suggested that the mucus secreted onto the gills of *M. edulis* has a digestive as well as a mechanical function. The absorptive mechanism is chemically selective, but small particles are removed non-selectively by pinocytosis.

Dissolved organic matter plays an important role in nutrition, possibly as a growth factor, particularly in littoral or estuarine zones. Héral *et al.* (1983) have established a correlation between the production of flesh in cupped oysters in the Marennes Oléron basin and the concentrations of dissolved organic matter, expressed as carbon or nitrogen.

1. Feeding

1.1 The role of the gills in feeding

The lateral cilia of the gills draw a water current into the mantle cavity (Fig. 10).

A typical bivalve filters 30 to 60 times its own volume of water in an hour (Famme *et al.*, 1986). Water must pass between the gill filaments in order to reach the exhalant chamber.

1.11 Food capture

As the water passes between the filaments (propelled by lateral cilia), food particles are held back by straining cirri (fused large cilia) and are carried forward to the ventral particle groove of the gill towards the mouth by other cilia in mucous strings (Silverman *et al.*, 1999) (Fig. 11).

Suspension feeding bivalves are capable of capturing suspended particles over a wide range of sizes, typically between 10^{-1} and 10^{2} µm (Møhlenberg & Riisgård, 1978).

In lamellibranchs, the frontal surface with its cilia is important in the capture of food. For this reason, the frontal surface is large relative to that of other molluscs and, and the filaments are slender. The combined frontal surface of the filaments forms large sheets known as lamellae (this is why bivalves are also known as lamellibranchs). The gill filaments are attached to the central axis, which is itself attached to the mantle (Fig. 12).

Each gill filament drops ventrally (the descending limb) from the central axis and then climbs dorsally (the ascending limb). The ascending lamellae attach either to the mantle skirt (lateral filaments) or the visceral mass (medial filaments).

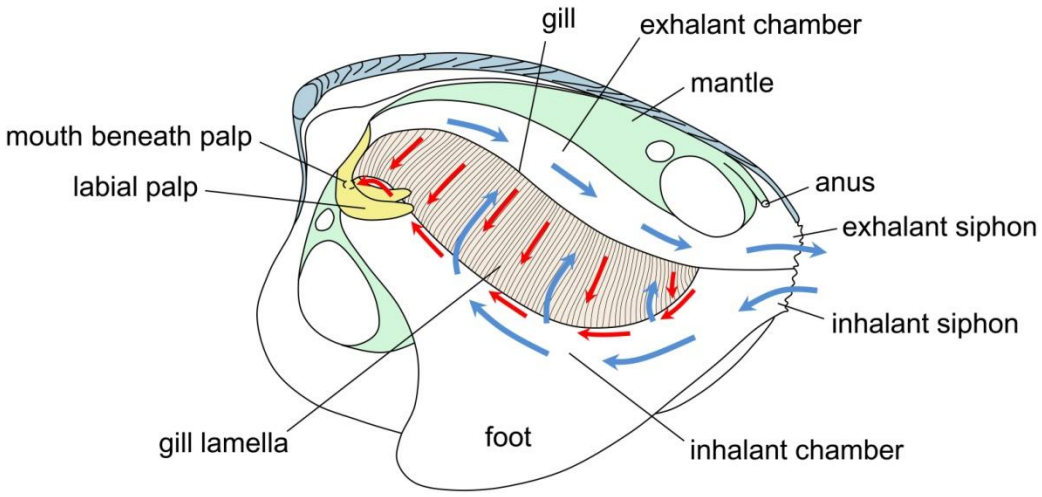

Fig. 10. Movement of water and suspended particles involved in the feeding mechanism of a bivalve mollusc, *Mercenaria mercenaria*. The left valve and left mantle skirt have been removed. Blue arrows - water current; red arrows - particle movement.

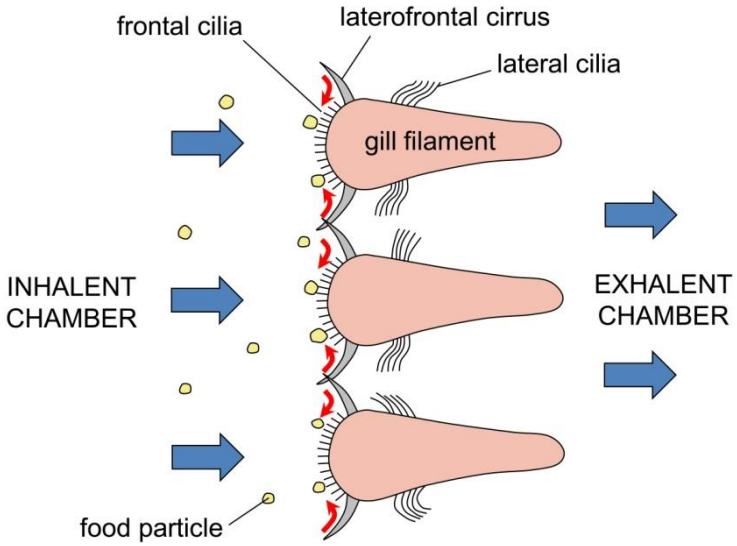

Fig. 11.

Section of three gill filaments of *Mytilus* showing capture of suspended food particles by the laterofrontal cirri.

Water (blue arrows) is propelled between the filaments by the beating lateral cilia.

Food particles are shifted to the frontal cilia (red arrows) which transport them to the labial palps. After Silvester & Sleigh (1984).

In the majority of bivalves, adjacent filaments are held together by junctions; this gill type is known as eulamellibranch. In more primitive bivalves (mussels and scallops), neighbouring gill filaments are attached to one another simply through interlocking clumps of cilia; this gill type is known as filibranch. Together, these filaments form a lamella. Therefore, each gill has four lamellae. The combined descending limbs of all the lateral filaments together form a descending lamella and the ascending filaments form an ascending lamella (Fig. 12). Captured food is passed into longitudinal ciliated food grooves formed along the dorsal and ventral edges of the lamella.

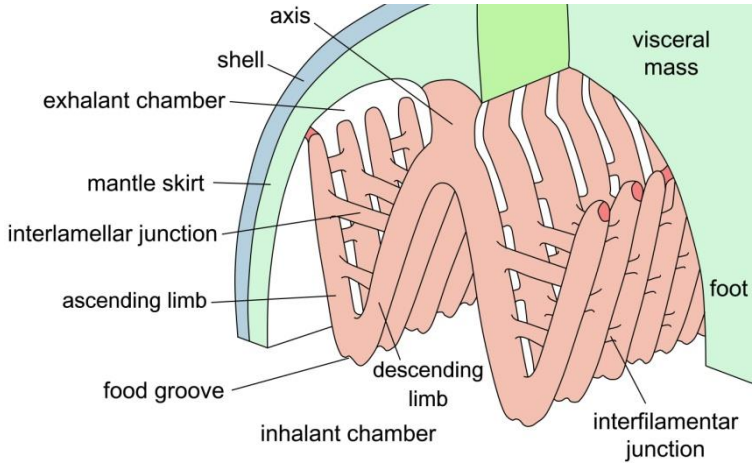

Fig. 12.

Structure of the bivalve (lamellibranch) gill.

Each filament is folded into a V shape. There are two Vs (demibranchs), one medial and one lateral to the central axis. Together these form a W shaped holobranch.

1.12 Food sorting

The food grooves of the gill lamellae are located on their dorsal and ventral edges. A maximum of five such grooves, two ventral and three dorsal, are associated with each holobranch (Fig. 13).

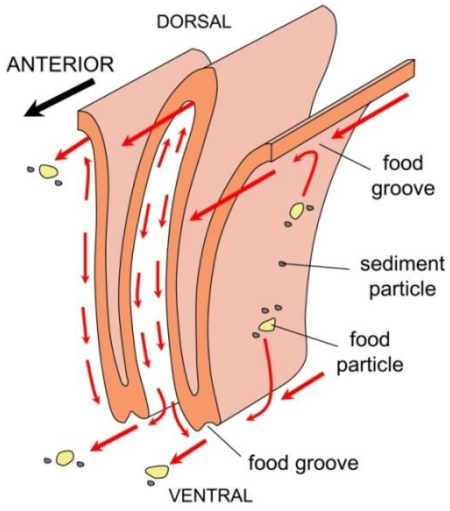

Fig. 13.

The movement of captured particles by the gill frontal cilia in *Mytilus*.

Food and sediment particles are transported by frontal cilia to the five anteriorly directed food grooves.

In mussels and pen clams, food and sediment particles are transported both ventrally and dorsally (mostly ventrally) by frontal cilia to the five anteriorly directed food grooves which carry them to the labial palps. Very little sorting occurs in these species. In less advanced lamellibranchs, such as the Arcidae (e.g., Ark clams) Anomiidae (saddle oysters) Ostreidae (true oysters) and Pectinidae (e.g., scallop) the gills sort food from sediment. In these groups, tracts of frontal cilia transport food particles ventrally to the three anteriorly directed food grooves, while sediment particles are transported dorsally to the two ventral tracts which are directed posteriorly. Sediment and mucus are then ejected as pseudofaeces.

1.2 The role of the labial palps in feeding

Particles are graded for size by the ciliated labial palps before being ingested (Fig. 14).

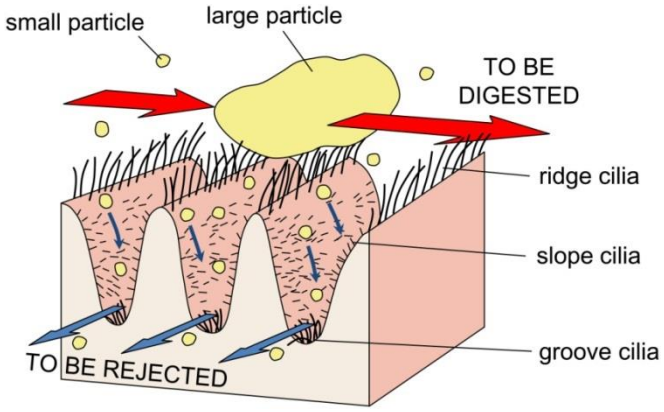

Fig. 14. Selection of food particles by the labial palps for transfer to the mouth.

Ridge cilia generate a current across the ridges, towards the mouth. Slope cilia beat downwards towards the groove. Groove cilia generate a current towards the edge of the palp.

Sediment particles which are rejected are transported to the edge of the lamellae in the grooves between the ridges and fall as pseudofaeces into the mantle cavity. This material, which consists of particles and mucus, is moved posteriorly in a ventral ciliary tract on the inner surface of the mantle skirt to a position close to the inhalant syphon. Occasional rapid contractions of the adductor muscles compress the mantle cavity and force water and pseudofaeces out of the inhalant syphon. Excess food material is removed by the labial palps in order to prevent gill saturation. In dense suspensions, the palps channel most of the filtered material away from the mouth and towards incorporation into pseudofaeces so that the animal can continue to filter and ingest at an optimum rate (Bayne *et al.*, 1976).

The mucus food string from the labial palps enters the mouth and is transported by cilia posteriorly through the oesophagus to the stomach.

2. Digestion

The digestive strategy of bivalve molluscs is unique among higher animals because both extracellular and intracellular digestion are important. The mouth of bivalves has foliar lips which may be folded and meshed (e.g., in scallops). The short oesophagus leads into the stomach which is irregular and complex in form with highly folded ciliated walls (Fig. 15 and Fig. 16). The stomach extends posteriorly, surrounded by digestive caeca (which form the digestive gland), into the intestine, rectum and anus.

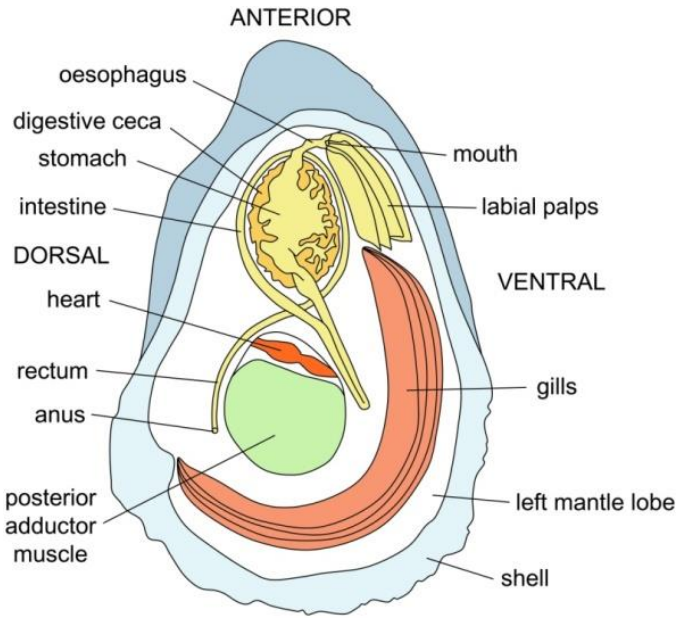

Fig. 15.

Internal anatomy of the American oyster *Crassostrea virginica* dissected to reveal the digestive system.

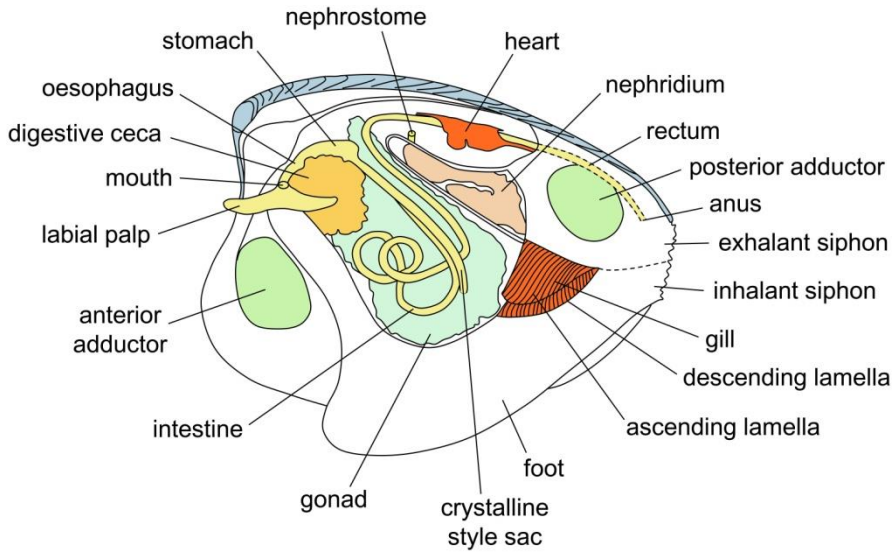

Fig. 16.

Internal anatomy of the hard clam *Mercenaria mercenaria* (dorsal uppermost) dissected to reveal the digestive system.

2.1 The stomach

Cilia and mucus are just as important in the gut as in the mantle cavity. The stomach is generally elaborate, with large ciliary sorting areas and a long rotating style (Fig. 17).

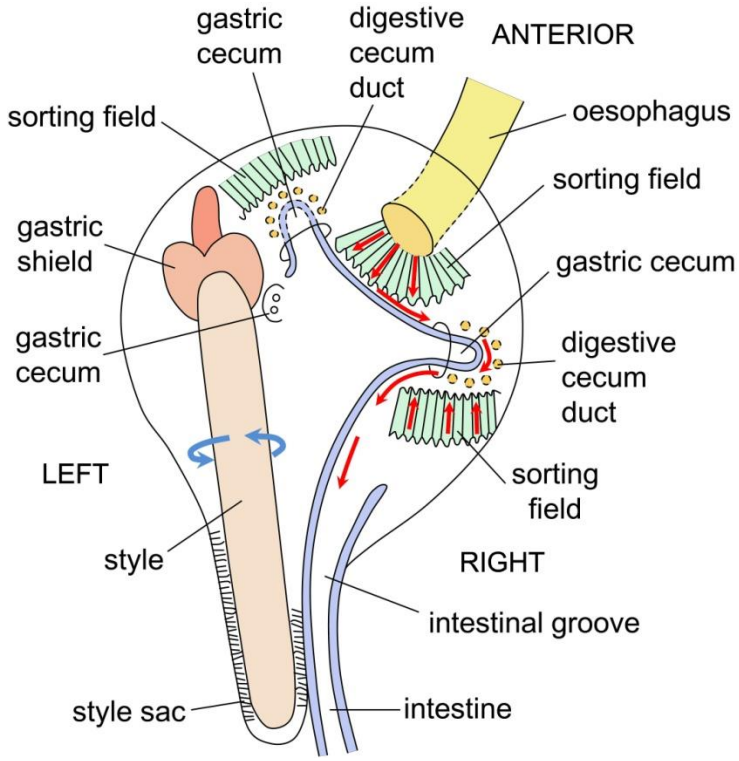

Fig. 17.

Section of a bivalve stomach (dorsal view).

The typhlosoles are shown in blue (left - major, right - minor).

The path of ingested materials is indicated by red arrows.

The oesophagus enters the stomach anteriorly and the intestine exits posteriorly. Evaginations in the stomach wall form the gastric ceca (diverticula). These give rise to the digestive ceca (diverticula) ducts which are associated with ciliary sorting fields, consisting of ciliated ridges and grooves, similar to those in the labial palps.

The sorting fields move large particles over the crests of the ridges; smaller mineral particles are collected in the grooves and moved to rejection tracts. The rejecting tracts move material to the intestine while food particles tend to be re-suspended in the fluid in the stomach.

A ciliated intestinal groove extends from the stomach into the intestine. The left side of the groove is bordered by a ciliated ridge, the major typhlosole, which arches over the groove and partially isolates it from the stomach. Another ridge, the minor typhlosole, lies on the right side of the intestinal groove. The major typhlosole and intestinal groove originate in the left gastric cecum and extend into the intestine, together with the minor typhlosole. The typhlosole and intestinal groove provide a continuous stream of mineral particles out of the stomach and into the intestine.

A style sac with a ciliated secretory epithelium extends posteriorly from the stomach. The epithelium secretes the crystalline style which consists of a protein matrix onto which are secreted a variety of digestive enzymes, including amylases, glycogenases, lipases and cellulases (Morton, 1983). There are no proteases in the stomach, since these would digest the proteinaceous enzymes of the style; protein digestion is intracellular in the digestive ceca.

The cilia of the style sac cause the style to rotate, resulting in its abrasion and release of digestive enzymes which perform extracellular digestion (Fig. 18). Rotation of the style stirs the contents of the stomach and drags the mucus food string through the oesophagus. Acid released from the style and digestive ceca reduces the pH of the stomach fluid and this reduces the viscosity of the mucus, releasing bound food particles onto the sorting fields.

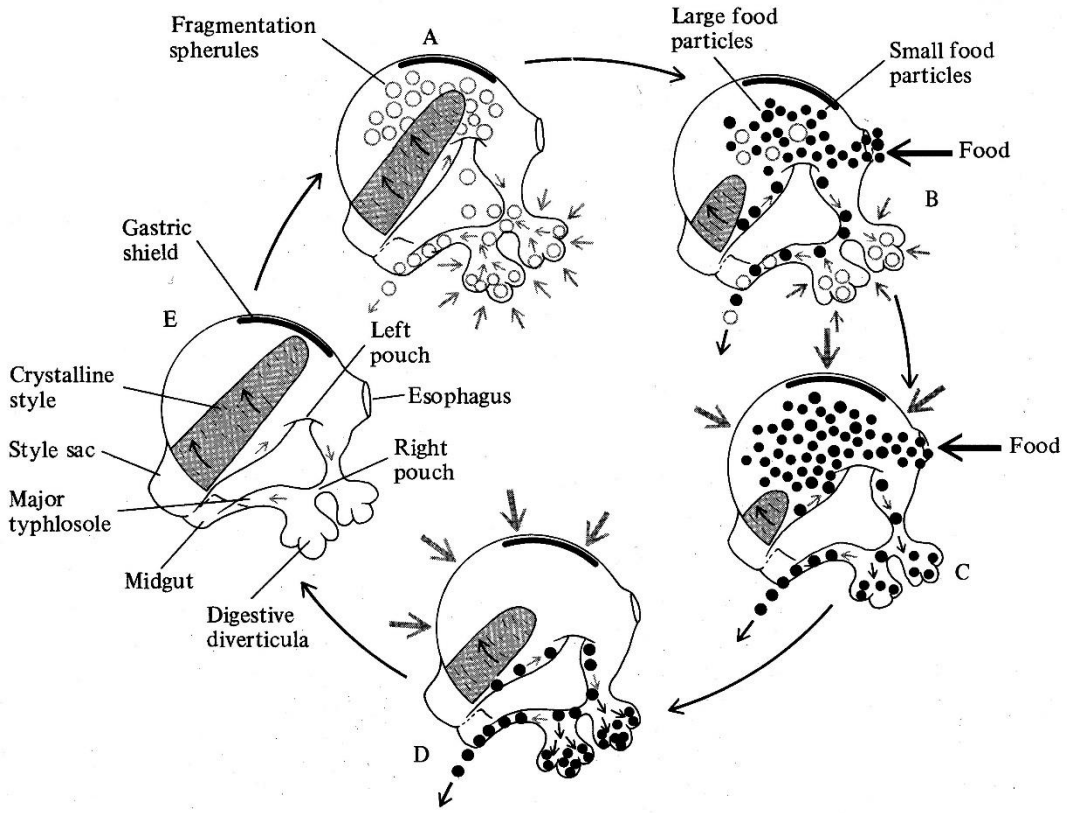

Fig. 18. Processes of extracellular and intracellular digestion in the bivalve mollusc stomach and digestive diverticulae. (A) The basic digestive cycle starts before food ingestion with dissolution of the crystalline style and presence of fragmentation spherules in the stomach. (B) Food arriving in the stomach is mechanically reduced by the rotating style and shield and extracellular digestion begins. (C) Particles are then passed to the digestive diverticulae for intracellular digestion. (D) Cessation of feeding is followed by progressive passage of food particles from the stomach to the digestive diverticulae. (E) During the quiescent interfeeding phase, the stomach empties and the crystalline style is re-formed, while intracellular

digestion in the diverticulae is completed and fragmentation spherules begin to form. (A) The movement of fragmentation spherules to the stomach starts the next cycle. From Morton (1983).

Partially digested food passes into the digestive ceca via the gastric ceca. The digestive ceca ramify into secondary ducts that terminate in blind-ending pouches (acini). The acinar epithelium is responsible for enzyme secretion, nutrient absorption and pinocytosis and intracellular digestion.

These tubules are of two cell types, digestive cells and basophil secretory cells. Digestive cells are the most abundant type. They are columnar and vacuolated and are responsible for intracellular digestion of food. The free surface of the digestive cell is extended into microvilli and the cytoplasm is characterised by the presence of numerous cytoplasmic vesicles types. Particulate matter is taken up at the base of the microvilli by pinocytosis and is initially stored within small vesicles called phagosomes. Phagosomes fuse with lysosomes in their maturation process. Digestion takes place within these lysosomes which contain hydrolytic enzymes. The end products of digestion are released directly into the haemolymph system and waste products are contained in residual bodies within the digestive cells. The cells eventually rupture and the waste material is swept along the ciliated secondary and primary ducts of the ceca towards the stomach, and ultimately to the intestine.

The ducts of the ceca are divided longitudinally into inhalant and exhalant channels. Cilia provide an exhalant current which carries undigested material to the typhlosoles and intestinal groove; this current also draws undigested material into the inhalant channel. It is possible (Morton, 1983) that the currents alternate with time. Mathers (1973) has shown the existence of an interval between the time when the food arrives in the ducts and the return to the intestine of residues from the digestive process.

2.2 The intestine

The function of the intestine is primarily to form faeces. The intestine often passes through the pericardial coelom and is surrounded by the heart ventricle. It then passes dorsally over the posterior adductor muscle and terminates at the anus where faeces are discharged into the exhalant chamber at the base of the exhalant siphon.

3. Digestive enzymes

Both stomach and digestive ceca contain a variety of digestive enzymes, particularly carbohydrases. In bivalves, digestive enzymes are produced by the style, by the cells of the digestive ceca (diverticula) and, in some genera examined, in the midgut (Purchon, 1971). Bivalve molluscs show an evolutionary trend towards extracellular digestion, which is believed to provide the most efficient utilisation of food (Reid, 1968).

Digestive enzymes include amylase, cellulase, alginase, lipase, phosphatase and several proteases (including chymotrypsin and cathepsins) (Mathers, 1973; Morton, 1983).

Kristensen (1972) examined carbohydrases from the crystalline styles of a number of bivalves, including the blue mussel (*Mytilus edulis*) and found less activity than in the digestive gland. However, Wojtowicz (1972) recorded a 40-times greater activity of α-amylase in the style than in the digestive gland of the scallop *Placopecten magellanicus*, but other carbohydrases (α-glycosidase, β-glucosidase, β-galactosidase, laminarase and chitobiase) were confined to the digestive gland. Cellulase has been recorded in the style and digestive glands of several filter feeding bivalves, including *M. edulis* (Crosby & Reid, 1971). The high activity of cellulase in the stomach contents suggests that this enzyme is produced by gut bacteria.

The localisation of enzymes in the digestive tract of the Pacific oyster (*Crassostrea gigas)* is shown in Table IV.

The activities of the carbohydrases (amylase, cellulase and laminarinase are located throughout the digestive tract in the epithelia, such as the lysosomal enzyme, N-acetyl glucosaminidase, which is particularly strong around the gastric shield and in the cells of the tubules of the digestive gland. Non-specific esterases are present in the canal of the digestive gland and the apical part of the stomach and intestinal epithelium. Acid phosphatase, a lysosomal enzyme, is very active in the digestive gland while alkaline phosphatase, which indicates active transport, is localized in the canals of the digestive gland and the base of the tubules.

Protease activity, in the tubules of the digestive gland and some parts of the intestine, is low at the start of digestion but builds up towards the end. These enzymes have an optimum pH of 5.5, as with lysosomal enzymes; this is associated with intracellular digestion. Given their location on the apical zones of microvilli, these enzymes appear to be membrane-bound (Boucaud-Camou *et al.*, 1985).

Table IV. Localization of enzymes in the digestive tract of the Pacific oyster (*Crassostrea gigas*)

Enzyme	Digestive gland		Stomach		Intestine	
	Canals	Tubules	Ciliated cells	Gastric shield	Ascending	Descending
Amylase	+++	+++	+	+	+	+
Cellulase	++	++	++		++	++
Laminarinase	+	+	+		+	+
Alginase						
β-N-acetylglucosaminidase	++	++	++	+++	++	++
β-glucuronidase						
Non-specific esterases	+++	++	+++		+	
Lipase						
Acid phosphatase	+	+++	++		++	
Alkaline phosphatase	++	+			weak +	weak +
Proteases		++				
Chymotrypsin	++	weak +	+		weak +	weak +
Cathepsin B	+		++		++	++
Cathepsin D						
Cathepsin C (DPP I)	+++		++		++	++
DPP II	+++		++		++	++
DPP III					++	++
DPP IV	+		++			
Aminopeptidase-M	++	++	++	+	+++	+++

Data from Lebesnerais (1985). Lysosomal proteases (cathepsins) are also known as dipeptidyl peptidases (DPPs); they are classified according to letter or number (Agarwal, 1990). Most of the cathepsins become activated at the low pH found in lysosomes. Thus, the activity of this family lies almost entirely within these organelles.

Similar distributions of enzymes within the digestive tract of other bivalve species have been identified: grooved carpet shell (*Ruditapes decussatus*) (Henry, 1987); blue mussel (*Mytilus edulis*) (Janssen, 1981) and king scallop (*Pecten maximus*) (Henry *et al.*, 1993; Henry *et al.*, 1991). The movement of food through the digestive tract of the Pacific oyster *Crassostrea gigas* and the sites of enzyme action and uptake of nutrients is shown in Fig. 19.

Experiments performed on *C. gigas* have shown that when the animal is starved or has been out of the water for a long period, all of the cavities are empty (Boucaud-Camou *et al.*, 1985; Lebesnerais, 1985). Whole algae have been observed in the stomach, the main ducts of the digestive gland, the intestine and the rectum. Without being subjected to enzyme action, this material begins to be expelled through the anus, surrounded by mucus. The duration of this stage is strongly influenced by temperature: 6 h at 10°C and 3 h at 20°C. The animal continuously compensates for this loss by taking in new food. From the beginning of their entry into the ducts of the digestive gland (1-2h), the nutrient particles are exposed to enzymes, although living cells (algae) have been observed for around 6 h after food has reached the stomach and 8-16 h in the intestine.

Due to the pronounced endocytotic nature of the digestive caeca, this gland is a major site for the uptake of metal and organic contaminants. A wide range of enzymes that degrade organic contaminants has been detected in the digestive gland of numerous bivalves (Livingstone *et al.*, 2000; Livingstone & Pipe, 1992). Metallothioneins, antioxidant enzymes and oxyradical scavengers, such as glutathione have also been found in these glands (Lowe & Pipe, 1994; Moore, 1991).

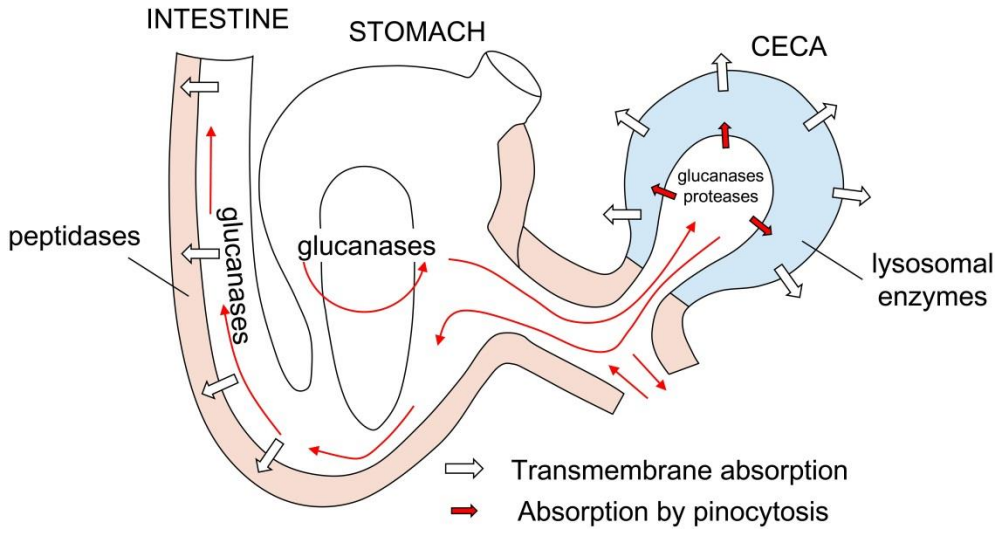

Fig. 19. Sites of digestion in the oyster *Crassostrea gigas*. After Boucaud-Camou *et al.* (1985).

In molluscs, proteins are taken up by pinocytosis by the acinar epithelium of the digestive ceca/diverticula, following which they are hydrolyzed to amino acids. Experiments on marine bivalves have provided evidence of significant uptake of amino acids via the gills (this is discussed in more detail in a later lecture).

4. Energy storage

A significant proportion of the energy coming from food is used to cover the metabolic requirements, particularly those associated with reproduction. The reserves are stored either in tissues which are specific to this function or in tissues which have a different physiological function (muscle, digestive gland, haemocytes). In addition to its role in the digestion of food, the digestive gland serves as a site for the storage of metabolic reserves and the control of the distribution of assimilated energy utilised during gametogenesis and during periods of physiological stress (Thompson *et al.*, 1974).

In the blue mussel (*Mytilus edulis*) storage tissues are present in the mantle and in the visceral mass and are made up of two types of cell in juxtaposition: adipogranulous cells (ADG); vesiculous cells with glycogen (vesicular connective tissue (VCT) (Houtteville, 1974; Labet, 1959; Pipe, 1987). The ADG cells (6–20 µm) are spherical and occupy the spaces between the VCT. Glycogen is very abundant and accumulates in a voluminous vacuole.

The flat oyster (*Ostrea edulis*) possesses only vesiculous cells with glycogen. The vesicular connective tissue forms a bed which may be highly developed, adhering to the mantle by the external surface and enveloping the visceral mass (gonad and digestive gland).

A correlation between the activity of the reserve tissue and that of the gonad was established several years ago in the blue mussel (*Mytilus edulis*). Gabbott *et al.* (1976) showed that glycogen disappeared progressively during the maturation of the gonad; at the same time both the volume and number of cells of the reserve tissue regressed (Peek *et al.*, 1989) (Fig. 20).

Studies of the ultrastructure and the cytochemistry revealed the existence of a complex processes of autophagy (a catabolic mechanism that involves degradation of unnecessary or dysfunctional cellular components by intracellular lysosomes) which destroy the ADG (Houtteville, 1974; Pipe, 1987). Metabolites are thus released and

these products of lysis become distributed around the tubules of the gonad and are absorbed by the young oocytes by pinocytosis. Most of the ADGs are destroyed. The VTCs also lose their glycogen through the action of lysosomes (cellular organelles that contain acid hydrolase enzymes that break down waste materials); this may also entail a total lysis of cells. The activity of glycogen phosphorylase is intense.

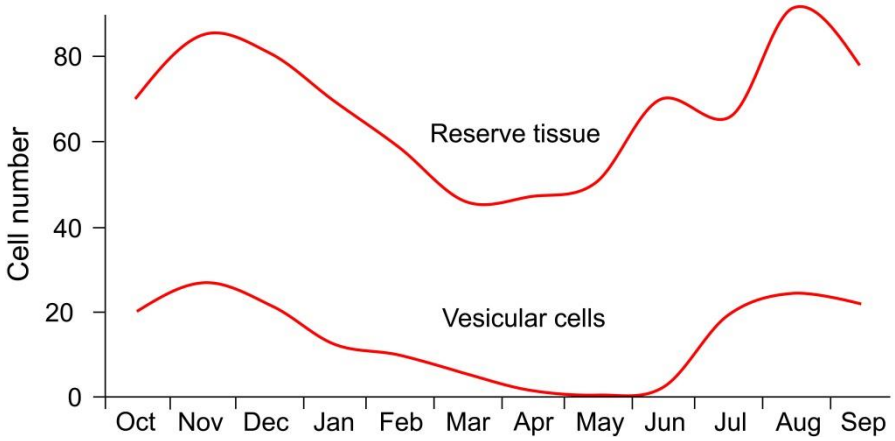

Fig. 20. Seasonal variations in the cells in reserve tissues of *Mytilus edulis*. After Peek *et al.* (1989).

Regeneration of reserve tissue begins at the end of the annual period of reproduction (Houtteville, 1974) and is brought about by haemocytes which accumulate between the gonad tubules and then form a loose mesh tissue. The synthesis begins from spring onwards and lasts to the autumn in mussels in northern latitudes, which corresponds both to the annual cessation of spawning and the period when food is most abundant. There is a significant increase in somatic growth following the drop due to the emission of gametes; this appears as a marked increase in the rate of linear growth.

The reproductive strategy of oysters is different from that of mussels. There is no relationship between the glycogen reserves and gonad maturation (Gabbott, 1976; Walne, 1970). In the European oyster (*Ostrea edulis*) gametogenesis and the accumulation of glycogen take place at the same time. The increase in the availability of food in spring meets the energy requirements of the oyster and the increase in temperature at this time accelerates gametogenesis. An identical pattern occurs in the Atlantic oyster (*Crassostrea virginica*) and the Pacific oyster (*C. gigas*). In all species, the highest level of glycogen occurs before the resumption of sexual activity, the period where growth in oysters is particularly fast. Glycogen levels drop dramatically after the emission of gametes in summer in species where fecundity is high, but this drop is less marked in the flat oyster (*Ostrea edulis*) which has a lower fecundity.

5. Recommended reading

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