MOLLUSCAN NUTRITION
ASPECTS OF BIVALVE FEEDING AND DIGESTION
RELEVANT TO AQUACULTURE NUTRITION

by

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ABSTRACT

Bivalves have been reported to be continuous, rhythmic, irregular or opportunistic in feeding behavior. Feeding stimuli may include tidal flow, the presence of food, and the condition of the digestive system. Mechanical stimuli appear to predominate over chemical ones. A variety of palial, ciliary organs with putative mechanosensory function are revealed by the scanning electron microscope. At present the feeding behavior of bivalves must be assessed species by species.

Within the category of commercially significant suspension feeders there is a range of discrimination of food particle size and density, Ostreidae, Mytilidae and Pectinidae being more selective than burrowing clams. Three functionally distinct stomach types occur in this category. Consequently all such suspension feeders under aquaculture may have specific requirements of food particle quality as well as feeding regime.

Studies of gastric digestive processes reveal in addition to amylase, enzymes such as trypsin, cathpsin B, exopeptidases, esterases and phosphatases. These are secreted in response to ingestion of food. Gastric enzyme levels, which are low in comparison with intracellular levels, are sufficient to lyse cell membranes, penetrate cell walls, and partially solubilise the dietary organisms, but digestion is largely completed intracellularly. The bivalve mid-gut possesses membrane-associated esterases, phosphatases and amino-peptidases, indicating a coupled digestive-absorptive process. Digestion may follow a rhythmic tidal or diurnal cycle, though not all digestive tissues are completely in phase. Some diverticular autophagy occurs during starvation; cathpsin D increases and lipid depots are mobilized.

Bacteria associated with bivalves may contribute to nutrition as food, as providers of soluble nutritive molecules for absorption by the gills or the gut, or as contributors to digestion. While the crystalline style has some antibiotic qualities, some bacteria, especially Cristispira, abound in the bivalve gut, and intracellular bacteria have been located.

Two bivalve genera prove the rules for the rest, Tridacna and
Solemya. Tridacna gigas is nutritionally dependent on its symbiotic zooxanthellae, but also feeds on suspended phytoplankton, reef detritus and coral exudates. Solemya reidi is a gutless protobranch. The clarification of its nutritive economy sheds some light on non-digestive nutritive processes in bivalves with normal alimentary tracts.

KEY WORDS: bivalve feeding; digestion; particle-retention; mantle cavity; stomach; crystalline style; digestive diverticula; bacteria; symbioses.

INTRODUCTION

In the aquaculture of bivalves it is necessary to know the food preference and feeding behavior of the organisms as well as knowing how the alimentary system utilizes the food. Since the beginning of the century our knowledge of bivalves in their natural environment has been growing. However, in order to meet the requirements of aquaculture in controlled conditions, more detailed knowledge of how bivalves function as individuals and as species is required. The fundamental causes and relationships within the alimentary system need to be understood, so that satisfaction of basic needs can be planned, and the unexpected dealt with before it becomes unmanageable. This paper will attempt to describe the fundamental elements of alimentation in the Bivalvia, and especially in those species which are commercially important.

FEEDING BEHAVIOR

The original portrayal of bivalves as continuous feeders set forth by C.M. Yonge in his seminal work on Mya arenaria and Ostrea edulis was based partly on observation, and partly inferred from the characteristics of the crystalline style (Yonge 1923, 1926). It was, of course, recognized that the continuity was interrupted in intertidal animals by the ebb and flow of the tide. One extreme case, Lasaea rubra, which is submerged in seawater for only brief periods, and possesses as a consequence a distinct digestive cycle, was originally thought to be exceptional (Ballantine and Morton 1956). Subsequent work by B.S. Morton (1970) on Cardium edule suggested that a discontinuous, rhythmic feeding behavior resulted in a distinctive four-phase digestive cycle, and that this cyclic activity might be more typical than exceptional, a view that was supported by Puchon (1971). Discontinuity in feeding behavior and variability in feeding rate has now been confirmed for many species of bivalve, with differences of opinion being expressed with regard to the periodicity of the activity, whether it be endogenously or exogenously rhythmic, or whether it be irregular and opportunistic (Morton 1973; Langton and Gabbott 1974; Mathers 1976; Mathers et al. 1979; Palmer 1980a; Robinson and Langton 1980). Palmer (1980b) has suggested that the function of discontinuity in feeding behavior is the provision of a continuity in the digestive process. Robinson and Langton (1980) suggest that efficiency of digestion is the goal of modifications of feeding behavior. The only safe conclusion is that feeding is not universally continuous, nor of constant rate. Therefore, in the design of feeding regimes for bivalves in aquaculture, each species must be individually assessed in order to maximize food utilization, providing food when the animals are in a receptive
condition and minimizing waste, particularly since the latter problem
can be compounded by contamination of the system with undesirable
microorganisms. Such regimes must also take the consequent digestive
processes into consideration. These consequences are discussed below.

There are a number of possible conditions for the initiation of
feeding behavior. The first and most obvious is a deprived condition,
due to tidal exposure or to the lack of food. Commencement of
respiratory activity after a period of valve closure brings suspended
material to the filtration organs. The material might provide a
mechanical or a chemical stimulus detected by the gills or labial palps,
resulting in more rapid feeding or rejection of the material as
pseudofoes. Despite isolated reports concerning the ability of palial
organs to discriminate on the basis of tastes (e.g., Loosanoff 1949),
there is little evidence to support the argument that palial
chemosensation is the primary factor in the acceptance or rejection of
filtered particles. The osphradium, an organ present in many bivalves,
and associated with chemoreception in Gastropoda, has never been shown
to possess this faculty in Bivalvia (Bayne et al. 1976). Kraemer (1981)
studying the osphradium of fresh water bivalves suggests the functions
of the organ may be related to exhalant current flow, adduction of the
valves, and it may also be light sensitive. A variety of palial
responses to mechanical stimuli have been demonstrated. Bernard (1974)
has suggested that distasteful chemicals, such as hydrogen sulphide
associated with the sulphur bacterium Chromatium warmingii, might result
in a generalized rejectory mucus secretion, but only if these are in
dense suspensions. The carnivorous Cuspudariidae possess a number of
siphonal mechanoreceptors, ciliated organs which are responsive to
low-frequency vibrations, including the turbulence created by the
passage of large particles in their vicinity (Reid and Reid 1974; Reid
and Crosby 1980). Similar, though smaller organs have been found on the
mantle edges and siphonal tips of all bivalve species investigated by
the author, using a scanning electron microscope. A variety of ciliated
organs are also found on the inner surfaces of the inhalant siphon of
many bivalves. These may have the function of detecting potential food
or general turbidity in the respiratory currents. The role of
particulate organic material in the diet and the relationship between
feeding rates and density of suspended material is discussed by Newell
in these proceedings.

PALLIAL ACTIVITY

Despite fifty years of study, the function of the palial organs in
bivalves is still not fully understood, especially with regard to the
mechanism and biochemistry of mucus secretion. Orton (1912) originally
suggested that mucus secretion was a local response to particles coming
in contact with the gill filaments. MacGinitie (1941) postulated that a
general mucus sheet secreted by the gills and hypobranchial gland was
moved down the gill surface to form food strings at the marginal food
grooves. Pasteels (1968) suggested that mucocytes in the ctenidia
respond with a thick, local secretion to particles of unsuitable size
and density resulting in their rejection as pseudofoes, while a thin
continuous mucus layer secreted by other epithelial cells of the
filament surfaces constituted the food collecting layer. In Solenya
reidii a copious mucus secretion from the hypobranchial gland contributes
to the formation of pseudofeces (Reid 1981). Bernard (1974), studying *Crassostrea gigas*, agreed with Pasteel's hypothesis, noting that physical contact between dense particle and single gill filament was not an effective stimulus, but contact with two or more filaments stimulated a heavy mucous secretion.

With regard to the size range of accepted particles, the upper limits can be determined by an examination of gastric contents. The largest dense particles, 300 - 400 \( \mu \text{m} \) in diameter, are found in class burrowing in sand, e.g., *Pivela stultorum* (Coe 1947) and *Macoma secta* (Reid and Reid 1969). I have found that other eulamellibranchiate clams (e.g., *Tresus capax* and *Tridacna gigas*) accept dense particles in the same upper size range. There is, however, a considerable range in the upper size limits of dense particles that will be ingested. *Macoma calcarea* rarely accepts dense particles larger than 10 \( \mu \text{m} \) (Reid and Reid 1969). Bernard (1974) calculated that in *Crassostrea gigas* the preliminary rejection of dense particles more than 14 \( \mu \text{m} \) in diameter is largely a consequence of their settlement in response to gill filament stimulation.

The efficiency with which smaller particles are retained by the gills differs from one species to another. Vahl (1972a) found that *Mytilus edulis* retained 60% of 2 \( \mu \text{m} \) particles but below that size there was a rapid decrease in efficiency or retention. In *Chlamys opercularis* 70% of 7 \( \mu \text{m} \) particles were retained, but smaller particles were largely lost (Vahl 1972b). Hughes (1969) found that *Scrobicularia* retained 100% of 4 \( \mu \text{m} \) particles. Haven and Morales-Alamo (1970) found that *Crassostrea virginica* retained 100% of 1 \( \mu \text{m} \) particles but that there was also significant retention in the 1-3 \( \mu \text{m} \) size range. Foster-Smith (1975a) found that *Cerastoderma edule*, *Venerapla pulastra*, and *Mytilus edulis* showed no size selection for graphite particles in the tested range of ca 5 \( \mu \text{m} \) - 120 \( \mu \text{m} \). He also found no discrimination between alumina particles (ca 7.5 \( \mu \text{m} \) - 31.5 \( \mu \text{m} \)) and the alga *Phaeodactylum tricornutum* (ca 29 \( \mu \text{m} \)) despite the fact that the alumina particles and *Phaeodactylum* cells were of different density and chemical composition. A more comprehensive bibliography on particle size retention and selection has been provided by Bayne et al. (1976).

Once the food strings have been formed in the marginal and dorsal food grooves of the gills they are passed anteriorly to the oral groove which leads to the mouth and is bounded dorsally and ventrally by the labial palps (Fig. 1). A sorting function is usually attributed to the labial palps of bivalves. This may be correct with respect to experimental conditions where the animal has been dissected, the labial palps separated, and particles dropped onto the inner palp surfaces. The relevance of such observations to natural conditions is debatable.

Some bivalves, especially the nuculid protobranchs, are able to use their palp lamellae as collecting and sorting organs under natural conditions (Stasek 1961). However, in most bivalves the bulk of material which comes into contact with the palps is in the form of mucous-bound food strings from the ctenidia, and a number of authors have concluded that the most significant function of the palps is the diversion of excess food strings from the mouth region to the mantle surface as pseudofeces (Gilmour 1964; Bernard 1974; Reid and Porteous 1980). Bernard (1974) observed, by means of a cystoscope inserted between the valves of an undissected *Crassostrea*, that the palps are always closely appressed, and has concluded that the major function is
the regulation of the amount of mucous-bound food entering the oral groove. Foster-Smith, who has revived MacGinitie's valve-window technique (MacGinitie 1941; Foster-Smith 1975b and 1978) has added another dimension to studies of the functional morphology of the bivalve pallium, by observing changes in activity related to changes in turbidity. In very turbid conditions, where thick mucous-bound food strings are formed, there is a high degree of indiscriminate rejection from ctenidia and palps. Alternatively, if turbidity is low all particles within the acceptable size range are accepted. The rejectory behavior of the labial palps can become acceptance behavior by muscular alteration of the disposition of rejection and acceptance tracts (Foster-Smith 1978).

Figure 1. The bivalve alimentary system. The dimensions of the digestive tract have been exaggerated, and the gill ciliary currents simplified, for the sake of clarity. AA = anterior adductor muscle; AN = anus; C = crystalline style; CM = marginal groove of ctenidium; CT = ctenidium; D = digestive tubule; DD = digestive diverticula; DH = dorsal hood; F = foot; G = gastric shield; I = intestinal groove; L = labial palp; M = mouth; MG = mid gut; O = oesophagus; PA = posterior adductor muscle; PF = movement of pseudofaeces on mantle; R = rectum.
Figure 2. The bivalve stomach. This generalized diagram displays a stomach as if the dorsal wall had been removed and the dorsal hood area spread out slightly. Arrows indicate direct ciliary currents; broken arrows indicate counter currents. A = appendix; C = crystalline style; D = digestive tubule; DD = digestive diverticula; DH = dorsal hood; FM = food-mass; G = gastric shield; H = hood groove; I = intestinal groove; LP = left pouch; MA = major typhlosole; MD = main duct; MT = minor typhlosole; O = oesophagus; P = posterior sorting area; S = style sac; SD = secondary duct.

GASTRIC FUNCTIONAL MORPHOLOGY

The gastric sorting functions of bivalves are the most complex in the animal kingdom. Purchon has described five basic stomach types of which three are found in commercially significant bivalves, namely types III, IV and V (Purchon 1957, 1958, 1960). The softening of the mucous food strings due to the pH of the gastric juice, and their disintegration caused by mechanical contact with the head of the crystalline style which rotates against the gastric shield, releases small particles. These are kept in suspension by a turbulent ciliary circulation of the gastric juice (Figure 2). Larger mucous masses and large dense particles come in contact with the stomach walls by gravity of centrifugal force and are subjected to a variety of sorting mechanisms (Reid 1965). The lightest masses are recycled back to the dorsal hood. In type III stomachs (e.g., Mytilidae, Ostreidae) a sorting caecum removes small dense particles. The sorting function of

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The caecum is therefore separate from the gastric digestive and absorptive functions. In type IV stomachs (e.g., Pectinidae) a recycling process retains all but the very small dense particles and therefore increases the effect of gastric digestion on ingested food, to the extent that large dense indigestible particles cannot be easily rejected, and are stored in a gastric appendix. From this sac they are possibly rejected periodically by convulsive muscular movements of the adjacent adductor muscle. In the type V stomach (e.g., Mactridae, Veneridae) there is a combined sorting and absorptive caecum, the duct caecum, which removes small dense particles and permits the uptake of gastric fluids by the ducts of the digestive diverticula by a ciliary counter-current (Owen 1955; Reid 1965). The large posterior sorting area of the type IV stomach has wide rejectory grooves and large dense particles released from incoming food strings are immediately rejected. In order that gastric function can continue the stomach must not become clogged with food. Excess particulate material may be indiscriminately eliminated by means of a muscular widening of the rejectory grooves and a peristaltic movement of the whole stomach. Several observers have noted that undigested and sometimes live algal cells may be found in the feces under such conditions (Bayne et al. 1976). Consequently gastric function must be considered in the planning of optimal feeding levels in maricultural systems.

The crystalline style has dominated most discussions of gastric digestion. The functional role of this unique structure is not altogether clear. Its role as a releaser of α-amylase is well known. The suggestion that the pH of the crystalline style buffers the gastric pH at a stable level (Yonge 1925) has been shown to be in error by Mathers (1974) who demonstrated that in Ostrea edulis gastric pH lies between pH 5.8 and 7.2, and in Crassostrea gigas between pH 5.9 and 6.9. Less well known are the antibiotic and emulsification qualities of the crystalline style (Kristensen 1972a). The digestive role of the crystalline style may have been exaggerated in some species (Kristensen 1972b). Low levels of phosphatase, esterase and protease activity have been found in some crystalline styles, probably originating from the gastric juice (Reid 1966). There is a correlation between style consistency and presumably ultrastructure, and the type of material found in the stomach. The crystalline styles of bivalves which ingest sand grains are stiffer and harder than those of other bivalves. This suggests that the physical integrity of the structure is important. Therefore, the mechanical role of mixing the stomach contents, or even triturination of incoming food strings as Yonge (1923) originally suggested may be more important than is generally appreciated. The crystalline style is susceptible to digestion by proteases, and the more flaccid crystalline styles found in oysters are more easily digested than the harder crystalline styles of some clams (Reid and Sweeney 1980). Judd (1979) has suggested that Cristispira bacteria may digest style carbohydrate. The process is also influenced by pH effects (Mathers 1974) and the rate of secretion of the crystalline style.

The full range of digestive enzymes found in the stomachs of bivalves is given in Table 1. Levels of enzyme activity differ according to the feeding type, but any generalization on this matter is fraught with exceptions. Amyloytic activity is lower in the stomach than in the digestive diverticula in some species (Kristensen 1972b) and higher in others (Wojtowicz 1972). Proteolytic activity is generally lower in the stomach than in the digestive diverticula with the
exception of the carnivorous septibranchs (Reid 1977, 1978). The
gastric enzymes, such as phosphatases, esterases, cathepsin B, trypsin,
and a number of exopeptidases, may emanate from the gastric epithelia,
the style sac and the digestive diverticula. Esterases, acid and
alkaline phosphatases are found in the A and B cells of the style sac.
In the C cells alkaline phosphatase is found. These tissues may be the
source of some style enzymes (Reid 1966). Gastric enzymes are secreted
in response to the ingestion of food (Mathers 1973a; Reid 1978). For
example, in Trisus capax the secretion of proteinases keeps pace with
the rapid increase in volume of the gastric juice, so that within an
hour of the commencement of feeding, after a hiatus caused by tidal
exposure, an eightfold increase in total enzyme activity occurs (Reid
1978). Such a direct response to the ingestion of food argues against
the view that gastric enzymes originate from mature digestive cells in
the digestive diverticula by "leakage" (Ballantine and Morton 1956), or
as "waste" enzymes (Morton 1977) or as exocytotic by-products of
digestive cells (Palmer 1979). Moreover, the complement of gastric
enzymes is different from the active enzymes of the digestive
diverticula both in terms of enzyme type and electrophoretic mobility.
Some diverticular enzymes, such as chymotrypsin, and a molecular form of
amylase, together with some esterases, are not found in the stomachs of
bivalves. The absence of trypsin from the digestive diverticular
extracts does not negate its possible origin there. There is some
evidence that trypsin originates in the digestive diverticula as
trysinogen (Reid and Rauchert 1976). I have also circumstantial
evidence that in Crassostrea gigas the diverticular cells in the
disintegrating phase (type III), which Palmer (1979) and Robinson and
Langton (1980) have proposed as the source of gastric enzymes, are
characterized by high levels of low pH (ca pH 3) proteolytic activity
which would be ineffective at gastric pH levels. These disintegrating
digestive cells are therefore not good candidates as the general source
of gastric enzymes. Owens (1970) suggested that digestive tubule cells
in their earlier stage of development, i.e., as basophilic cells were
actually secretory. Palmer (1979), noting that secretion from these
cells has never been positively demonstrated, concluded that these cells
are immature digestive cells which retain the primary lysosomes for
intracellular digestion. Frankner (pers. comm.), on the basis of
transmission electron microscope studies of Tridacna gigas considers the
diverticular duct epithelia to be the most likely source of gastric
enzymes. These are certainly histo-enzymologically active for
esterases, phosphatases, and leucine aminopeptidases (Reid 1966; Mathers
1973a; Palmer 1979). The overall picture is that the complement of
gastric enzymes is appropriate to the digestion of the carbohydrate and
proteinaceous components of the cell walls of diatoms, lysis of cell
membranes of food material and solubilization of cell contents. The
mechanical action of the crystalline style and inorganic particles aid
in these processes. Naked flagellates and bacteria are rendered
structurally unrecognizable within minutes of ingestion. Partially
digested food remains in suspension as gastric juice flows into the
ducts of the digestive diverticula, and is pinocytosed by the digestive
cells. While particulate matter can be phagocyctosed by the digestive
cells it is doubtful that this occurs with many large particles or
intact food organisms (Owen 1970; Palmer 1979).
### Table I. Digestive Enzymes Found in Bivalve Stomachs

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Activity reported in pH range</th>
<th>Origin</th>
<th>Selected references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypsin (endopeptidase)</td>
<td>5.5-8.0</td>
<td>Diverticular duct epithelia? Digestive cells?</td>
<td>Reid and Sauchert 1970, 1976</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gastric epithelia?</td>
<td>Reid 1977, 1978</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Kozlovskaia and Vaskovsky 1970</td>
</tr>
<tr>
<td>Cathepsin B (exopeptidase)</td>
<td>4.5-7.0</td>
<td>as for trypsin</td>
<td>as for trypsin</td>
</tr>
<tr>
<td>Cathepsin A (exopeptidase)</td>
<td>3.5</td>
<td>as for cathepsin A</td>
<td>as for trypsin</td>
</tr>
<tr>
<td>Cathepsin C (exopeptidase)</td>
<td>3.5-4.5</td>
<td>as for cathepsin A</td>
<td>as for trypsin</td>
</tr>
<tr>
<td>Carboxypeptidase A (exopeptidase)</td>
<td>5-7.5</td>
<td>as for trypsin</td>
<td>as for trypsin</td>
</tr>
<tr>
<td>Carboxypeptidase B (exopeptidase)</td>
<td>5-7.5</td>
<td>as for trypsin</td>
<td>as for trypsin</td>
</tr>
<tr>
<td>Leucine aminopeptidase (exopeptidase)</td>
<td>7-8</td>
<td>Ciliated epithelia</td>
<td>Mathers 1973a; Palmer 1979; Reid 1966; as for trypsin</td>
</tr>
<tr>
<td>&quot;True lipase&quot; (glycerol ester hydrolase)</td>
<td>6-7.5</td>
<td>Ciliated epithelia</td>
<td>Yonge 1925; Mansur-Rek 1944; George 1952; Reid 1966</td>
</tr>
<tr>
<td>Arylesterase</td>
<td>6-7.5</td>
<td>Ciliated epithelia</td>
<td>Mathers 1973a; Palmer 1979; Reid 1966</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>8-9</td>
<td>Ciliated epithelia, C cells of style sac</td>
<td>as for arylesterase</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>5-6</td>
<td>B cells of style sac; Crystalline style</td>
<td>as for arylesterase</td>
</tr>
<tr>
<td>G-Amylase</td>
<td>5.5-7.1</td>
<td>C cells of style sac; Crystalline style</td>
<td>Yonge 1923; Kristensen 1972b; Langton and Gabbot 1974; Wojtowicz 1972</td>
</tr>
<tr>
<td>True cellulase (C1)</td>
<td>5.5-6</td>
<td>Digestive diverticula; Crystalline style</td>
<td>Payne et al. 1972; Koudsman 1970; Stone and Morton 1958; Owen 1974 (review)</td>
</tr>
<tr>
<td>Poly-8-glucoamidase</td>
<td>5.5</td>
<td>Digestive diverticula; Crystalline style</td>
<td>Crosby and Reid 1971; and as for true cellulase</td>
</tr>
<tr>
<td>Chitinase</td>
<td>5.6</td>
<td>as for cellulase and poly-8-glucoamidase</td>
<td>As for cellulase and poly-8-glucoamidase</td>
</tr>
<tr>
<td>Chitinase</td>
<td>5.6-7.6</td>
<td>Digestive diverticula; Crystalline style</td>
<td>Kristensen 1972b; Jeuniaux 1963; Wojtowicz 1972</td>
</tr>
<tr>
<td>Chitobiase</td>
<td>5.6</td>
<td>as for chitinase</td>
<td>as for chitinase</td>
</tr>
<tr>
<td>Laminarinase</td>
<td>4.3-7.6</td>
<td>Digestive diverticula; Crystalline style</td>
<td>Kristensen 1972b; Wojtowicz 1972; Mathers 1973b</td>
</tr>
<tr>
<td>Alginate</td>
<td>4.2-7.3</td>
<td>Digestive diverticula; Crystalline style</td>
<td>Kristensen 1972b; Franssen and Jeuniaux 1965</td>
</tr>
<tr>
<td>α-glucosidase (maltaee)</td>
<td>4.3-7.6</td>
<td>Digestive diverticula; Crystalline style</td>
<td>Yonge 1926; Kristensen 1972b; Mathers 1973b</td>
</tr>
<tr>
<td>α-galactosidase</td>
<td>5.7</td>
<td>Digestive diverticula</td>
<td>Mathers 1973b; Wojtowicz 1972</td>
</tr>
</tbody>
</table>
Intracellular digestion is one of a number of important functions of the bivalve digestive diverticula, which also include food-storage, excretion and acid/base control. A complete list of diverticular digestive enzymes is given in Table II. Starch, disaccharides and other algal sugars can be digested. A true cellulase is rare in bivalves, but poly-β-glucosidases and cellobiases are widely distributed. This subject has been reviewed by Owen (1974). Proteins are digested by the typical range of lysosomal proteinases together with alkaline exopeptidases and a chymotryptic enzyme in some species.

Table II. Digestive Enzymes in the Digestive Diverticula

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Activity reported in pH range</th>
<th>Selected references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chymotrypsin (endopeptidase)</td>
<td>7-8</td>
<td>Reid 1966; Reid and Rauchert 1970, 1976</td>
</tr>
<tr>
<td>Leucine aminopeptidase</td>
<td>7-8</td>
<td>Reid and Rauchert 1976</td>
</tr>
<tr>
<td>Carboxypeptidases A, B</td>
<td>5-7.5</td>
<td>as for leucine aminopeptidase</td>
</tr>
<tr>
<td>Cathepsin B₁</td>
<td>4-5.7</td>
<td>Rosen 1949; Kamat 1957; Reid and Rauchert 1976</td>
</tr>
<tr>
<td>Cathepsin D (endopeptidase)</td>
<td>2-3</td>
<td>Reid and Rauchert 1976</td>
</tr>
<tr>
<td>Cathepsin A, C</td>
<td>3.5-4.5</td>
<td>Reid and Rauchert 1976</td>
</tr>
<tr>
<td>True lipase</td>
<td>6-7.5</td>
<td>Yonge 1926', Mansour-Bek 1946, 1948; George 1952</td>
</tr>
<tr>
<td>Arylesterase</td>
<td>6.5-7.5</td>
<td>Reid 1966; Mathers 1973a; Palmer 1979</td>
</tr>
<tr>
<td>Carboxylesterase</td>
<td>6.5-7.5</td>
<td>Reid 1966</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>8-9</td>
<td>Reid 1966; Mathers 1973a; Palmer 1979</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>5-6</td>
<td>as for alkaline phosphatase; Owen 1972</td>
</tr>
<tr>
<td>Carbohydrases</td>
<td>see Table I</td>
<td>see Table I</td>
</tr>
<tr>
<td>(all of those listed in Table I)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Studies of the putative digestive cycle in bivalves have generally focused more on changes in pH and the crystalline style, together with the histological condition of the digestive cells than on the more central question of enzyme activity, with the exception of Langton and Gabbott's (1974) and Langton's (1977) studies of amylases in Ostrea edulis and Mytilus edulis. Histochemical studies by Mathers (1973) and
Palmer (1979) are also relevant to this problem. I have made preliminary studies of protein digestion in Tresus capax and Crassostrea gigas in relation to the tidal cycle. In Tresus gastric proteolysis levels increased with the volume of the gastric juice after exposure by the ebb tide. Diverticular proteolysis began to rise within three hours of the commencement of feeding and within six hours reached a plateau of activity that remained constant whilst the animal was covered by the tide. This study was carried out under diurnal tidal conditions when there was a single ebb in daylight.

In Crassostrea gigas, studied under semi-diurnal tidal conditions, samples of ten specimens were taken at four hour intervals over 48 hours. Average gastric volumes were largest during the daylight high tide, and lowest during the ebb. At the nocturnal high tide there was considerable individual variation resulting in a low average volume. The range of gastric proteolytic activity at the gastric pH (6.0 - 6.5) was small, the maximum values being found at the high tide. Highest digestive diverticular proteolytic activity at pH 6.0 - 7.5 was found about 1 hour after the daylight high tide and there was no discernable peak associated with the nocturnal high tide. At pH 3 the highest activity in the digestive diverticula was found at night, developing 6 to 8 hours after the peak of pH 6.0 - 7.5 activity.

My interpretation of these observations is that under the conditions of this study the oysters were feeding during both light and darkness, and that the greater daylight feeding activity established the overall pattern of intracellular protein digestion, which commenced in the pH 6.0 - 7.5 range in the day and was completed at pH 3 at night. In addition, the individual variability at any sampling time indicates that not all of the digestive cells were in the same physiological phase at the same time.

Morton (1978) discovered that C. gigas had a diurnal 24-hour cycle, with feeding activity greatest in daylight. He related this to the inequality of the semi-diurnal tides during the time of his study, and suggested that equal semi-diurnal tides would establish a 12-hour cycle. Morton also found that "in general" all of the digestive tubules behaved synchronously. My observations indicate that a 24-hour cycle persists even when the semi-diurnal tides are of equal height. I would hypothesize that nocturnal feeding establishes a secondary physiological cycle which in most individuals is masked by the dominant daylight cycle; however in a few individuals the nocturnal cycle may be dominant. A study combining histological and enzymological observations is required to test this.

The tridacnids show a typical daylight filtering rhythmity since during the night they appear to go into a torpor (Morton 1978) which I can personally confirm for Tridacna gigas. I have also found that pH 3 proteolysis is at its peak during the later part of the torpid period in this species. However, my collaborator, P. Pankboner, has been unable to find any histological homogeneity in diverticular tissue, from the same specimens, that would support the hypothesis that all of the digestive cells were in phase with one another. McQuiston (1969) found that in Lasaea rubra two histological phases could be found at any time in the digestive diverticula. Mathers (1976) and Mathers et al. (1979) reported similar heterogeneity in Pecten maximus, Chlamys varia and Venerupis decussata. Robinson and Langton (1980) have reviewed the literature on this topic, and reported on a subtidal population of
Mercenaria mercenaria. They have found considerable histological variation within the digestive diverticula, regardless of the state of the tide. Although the cells were not in the same phase there were four peaks of absorptive activity that corresponded to variations of environmental levels of food organisms.

It is difficult to generalize on the topic of digestive cycles, significant though it may be for aquaculture. With regard to the histological condition, and by inference the digestive condition of the digestive diverticula, the weight of evidence suggests heterogeneity in subtidal populations at all states of the tide, with minor peaks of digestive activity correlated with food availability. In intertidal populations tidal movements impose a degree of digestive rhythmicity, though conflicting opinions have been expressed concerning the homogeneity of the digestive cell condition resulting from tidal rhythmicity. There is general agreement with Owen (1972) that prolonged exposure will bring the digestive cells into a 'holding phase'. Most authors admit to the possibility of circadian rhythmicity in some species, but the general opinion is veering back towards a modification of the traditional view of continuous feeding and digestion in bivalves, the modification being that feeding may be variable to take best advantage of food when available and to ensure a steady level of ingestion. It is difficult, however, to abstract anything of practical value from this generalization. Different species have been shown to differ in their behavior according to environmental conditions. Once more, each species in aquaculture must be characterized with these cautions in mind.

In addition to their other functions, the bivalves' digestive diverticula store energy in the form of lipid, and this lipid is mobilized during times of low food availability. The digestive diverticula cells may also be partly mobilized by autophagy during times of starvation. Cathepsin D levels remain high in Tresus when the food supply is poor in winter, suggesting autophagic lysosomal activity (Reid and Rauchert 1976).

In the mid-gut of bivalves a number of gastric enzymes are found, indicating that digestion continues in this region. Associated with the intestinal epithelia are esterases, phosphatases and leucine aminopeptidases (Reid 1966; Mathers 1973a). The distribution of the exopeptidase is similar to that found in vertebrates, and indicates a coupled digestion/absorption mechanism, i.e., as terminal amino acids are enzymatically released from the dietary protein they are transferred to an amino acid uptake site on the epithelial membrane. Digestion and absorption must therefore be added to the intestine's other function of fecal consolidation.

Thompson and Bayne (1972) identified in the feces of Mytilus edulis two distinct components, one consisting of rejecta from the gastric sorting processes, and the other consisting of excytotic material from the digestive diverticula. The proportions of the two components were assumed to be correlated with the digestive capacity of the digestive diverticula and the concentration of food in the stomach.
The role of bacteria in the nutritive economy of bivalves has come under closer scrutiny in the last decade. Previously bacteria had been recognized as important dietary constituents for detritus feeders (Braefield and Newell 1961), and possibly in suspension-feeders (Zobell and Feltham 1938). Braefield and Newell postulated that due to certain limits, the more finely divided detrital material provided a better physical substrate for associated bacteria. However, some bivalve species have modified their pallial behavior to make advantage of the epifauna and epifauna of large substrate particles such as sand grains. Braefield and Newell argued that the nutritive potential of bacteria associated with detritus was more important than that of the detrital material itself. Detritus-feeders have been regarded as the recipients of a continuously available diet of low nutrient level, in comparison to the discontinuous food availability for suspension feeders (Levinton 1972). It must also be remembered that some detritus feeders are facultative suspension feeders, and even those that cannot alter their feeding behavior to make advantage of suspended food must benefit from that food when it comes close to the substrate/water interface, or when it is deposited at that interface. Bacteria are known in the Galapagos Rift bivalves (Corliss et al. 1979). Furthermore, bacteria associated with the ctenidia have been found in two species of Solaena: S. velum (Cavanaugh et al. 1981) and S. panamensis (Felbeck et al., 1981). These bacteria possess enzymes of the Calvin-Benson cycle together with sulphide oxidation enzymes, indicating their ability to fix carbon dioxide using the energy of hydrogen sulphide. Masses of bacteria have been found to constitute the "gland" of Deshayes in the shipworm Bankia australis (Popham and Dickson 1973) and it was suggested that these ctenidial bacteria make up for the nutrient deficiencies of the shipworm's wood diet by fixing nitrogen (Trytek and Allen 1980). Another role for bacteria in the microenvironment of the burrow or mantle cavity might be the preliminary extracellular digestion of organic material, the hydrolysates of which could be used by the bivalve as food. A number of bacterial species of the spirochaete Cristispira from the bivalve gut have been described (Berkeley 1959; Tall and Nauman 1981). The contribution of bacteria to cellulose digestion in Scrobicularia plana has been assessed by Payne et al. (1972), and they concluded that none of the isolated bacteria had cellulolytic activity and therefore such an ability must be endogenous to the bivalve. There has been an active debate concerning the role of bacteria in shipworms, reviewed by Morton (1978) and by Mann at the Symposium on Marine Biodeterioration (1981). There is a consensus that in the wood-digesting caecum of teredinids, bacteria do not produce the cellulases (Mann 1981). However, Popham (pers. comm.) has detected intracellular bacteria in vesicles which arise from the digestive tubules in Bankia rochi. These bacteria have not yet been characterized.

EXCEPTIONS THAT PROVE THE RULE

The shipworms and the carnivorous bivalves are of no direct interest to aquaculturists, but they do demonstrate the adaptational plasticity of the Bivalvia, and indicate qualities that the more 'law-abiding' bivalves may possess to a lesser degree. Two other bivalve types of personal interest to the author prove the rules for the
rest. *Solemya reidi* (Bernard 1980) and possibly other members of this genus are completely gutless (Reid and Bernard 1980). Deprived of normal alimentation, they must depend on other sources of food. Members of the genus have already been shown to possess chemosynthetic symbionts (Pelbeck et al. 1981). I have detected a rapid uptake of dissolved organic molecules by the mantle and gill epithelia of *S. reidi*, and there remains the possibility of complex synergy between bacteria in the mantle cavity and burrow. Such studies may have relevance to the nutrition of better known bivalves.

**Tridacna gigas** is partially dependent on the symbiotic zooxanthellae held in the enlarged mantle edges. This bivalve now seems less exceptional in the light of recent findings concerning bivalves and symbiotic microorganisms. **Tridacna gigas** also feeds on phytoplankton, reef detritus and coral zooxanthellae released from heat-stressed corals, and possibly dissolved organic exudates from coral reefs (Pankboner and Reid 1981). Since this organism has potential for aquaculture or at least 'domestication' these environmental factors should be taken into account.

In conclusion, the exceptional may inform us about the 'normal' in bivalve biology. Indeed the 'normal' may be so exceptional that every species must be assessed individually for nutritive and digestive characteristics. The expansion of our knowledge of these matters in the recent years continues and each of the studies that I have discussed provides us with new directions of interest.

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**LITERATURE CITED**


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QUESTIONS AND ANSWERS

PRUDER (Delaware): Dr. Reid we've been growing bivalve molluscs particularly *Crassostrea virginica* in a controlled-environment system for almost ten years. During that time we've experimented with many different feeding regimes. The engineering considerations in a controlled-environment would seem to prefer a system which could be fed sequentially or pulse fed as compared to attempting to maintain a fairly low concentration of algal cells in water. We've had considerable experience and success supporting very rapid growth of the eastern oyster with pulse feeding techniques. The initial concentration of algae to which the animal is exposed can be as high as 2 million cells per ml of a combination of Tahitian Isochrysis and Thalassiosira pseudonana. If the animal is continually exposed to such high algal concentrations it will shut down and eventually perish. But if the animal biomass to seawater volume ratio is such that the animal exposed to the very high concentration can reduce the concentration down to low levels in a matter of 3, 4, or 5 hours by feeding, the process works. We have in a poster presented in this meeting showing results of experiments with silt and algae where we demonstrate once again that the pulse feeding technique works. Do you see how pulse feeding fits into a digestive pattern? There are other options, not just high algal concentration or low algal concentration. Could you give some explanation of why pulse feeding seems to be effective?

REID: For one thing in nature *Crassostrea virginica* will "pulse feed" by choice to some extent. It will show 2 to 3 peaks of intense feeding activity over 24 hours. This is on the basis of Robert Palmer's 1980 paper. Secondly, cultured bivalves may to some extent be like Clever Hans, the counting horse, in the sense that they're being trained to a certain feeding regime that in a way that the culturists don't consciously realize. I think it may be possible to bring all bivalves under culture into a feeding regime that suits both the bivalves and the culturists. That would be highly desirable if it's true, but it's only speculative.

NEWELL (Maryland): Would Dr. Reid like to comment on the recent work of Kiorboe et al. (1980)* which indicated that the mussel *Mytilus edulis* is capable of preferentially ingesting algae from a homogenous suspension of algae and silt particles? The pseudofeces were observed to have reduced algal content, on a dry weight basis, indicating that they may be used as a means of voiding the silt. My recent research also indicates that the oyster *Crassostrea virginica* can preferentially ingest the organic component of natural seston and reject inorganic or non-nutritive particles as pseudofeces.

REID: Were the particles you were feeding the same size?

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NEWELL: They appear from Coulter Counter analysis to be in the same size but we don't know if they are of the same density. We do not understand the mechanism of this selection but the labial palps have frequently been identified as an organ which may sort and select food particles.

REID: Obviously this subject is complex and requires further research. Bernard's observation that Crassostrea gigas will "reject" inorganic particles, because they settle out before reaching the sorting mechanism may be relevant here. During the last ten years we have moved out of the classical mold of studying molluscs. Instead of making a few observations for a few species and generalizing we have the same species being examined by two different sets of workers with contradictory results. To what extent the wishes of the researcher enter into this, I do not know.

McCORMICK (California): Dr. Reid over the recent years people have been rethinking bivalve nutrition. Early work in the 30s from Zobell looked at bacteria uptake, then in the 60s and 70s work from Winter looked at silt, and we see it at this symposium here, the importance of silt. Can you comment on what you feel the relative importance of diatoms vs. flagellates vs. bacteria vs. silt are?

REID: I can try. For one thing the digestive enzyme component of the stomach is fairly suitable for getting into diatom cells. It's not a matter of digesting the diatoms, it's a matter of lysing the cell walls sufficiently by digesting the carbohydrate and protein components. Naked flagellates are also lysed. Although the enzyme levels in the bivalve's stomach are generally quite low they are sufficient for lysis, to allow partial solubilization. The presence of mineral particles of the same sizes as the food may be significant in mechanical terms in helping break diatom frustules and in helping simply to reduce the particle size. I would guess from the silt experiments that have been described in the poster session there's no question of associated bacteria with that silt. There is no siliceous in bivalves; silt cannot be digested, so there must be some other role for it, if you take the causal view that is, and the mechanical role is the only one that's left. I find it very intriguing.
MOLLUSCAN BIOENERGETICS - A SYNOPSIS

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ABSTRACT

An understanding of the energy and nutrient flow through the individual, population, or community is one of the cornerstones of modern production ecology. Energy budgeting techniques are also being increasingly used by marine physiologists to quantify the degree to which various physiological adaptations and environmental perturbations alter an animal's growth rate and fecundity. Although these techniques have been extensively applied to terrestrial animal husbandry, they have not yet been widely adopted in mariculture.

This general review considers the application of bioenergetics to bivalve molluscs. Emphasis is placed on some adaptations that require further elucidation or are important for mariculturists to consider, such as optimum methods of providing the ration and methods for assessing growth.

INTRODUCTION

One of the major emphases in coastal marine zoology, until about ten years ago, was the explanation of animal distributional patterns in terms of a species tolerance to an array of environmental variables, e.g. temperature, desiccation, etc. Numerous such studies (reviewed by Newell 1979) demonstrated that the majority of littoral animals exist well within their zones of physiological tolerance. Recently, there has been increasing recognition that less tangible factors than had previously been studied, e.g. temporal and spatial variations in food availability, differential partitioning of energy to growth and reproduction, are of paramount importance in governing the distribution and abundance of species within its zone of physiological tolerance.
(Bayne et al. 1976b; Newell 1979). The complex interactions between such variables may be elucidated by measuring all factors in terms of energy units and combining the various energy gains and losses into an overall index of energy balance.

The primary components of an individual's energy budget are identified in the following "balanced energy equation" (Warren and Davis 1967) written using IBF terminology (Crisp 1971).

\[ P + G = C - (R + F + U) \]

where

- \( P \) = Somatic growth
- \( G \) = Germinal growth
- \( C \) = Energy value of food consumed
- \( R \) = Energy metabolically utilized
- \( F \) = Energy value of feces
- \( U \) = Energy value of material that is absorbed but later excreted as urine or other exudates

Units = Energy units (Joules)

The magnitude of a positive energy balance indicates the quantity of energy available for assimilation into either somatic or germinal tissue or to be sequestered in a nutrient reserve. Conversely, if there is a negative energy balance the organism can only survive under such conditions whilst it has a nutrient reserve to utilize. Thus, the index of energy balance, or "Scope for Growth" (Bayne et al. 1976c; 1979), is one sensitive method for assessing and quantifying the adaptability of an organism to a particular environment, including mariculture facilities.

In this paper I have not attempted to give an exhaustive review of the very extensive literature covering all aspects of molluscan bioenergetics as the field has already been adequately reviewed (Bayne et al. 1976a, b, c; Conover 1978; Widdows 1978a, b; Winter 1978; Newell 1979, 1980a, b). Instead, by concentrating on bivalve species which are both well studied and commercially important, I intend to give a broad overview of how a bioenergetic approach may be used to direct and focus research towards elucidating the key components of an organism's biology. I also hope to show that the efficiency of mariculture projects may be increased by the introduction of even a small number of the techniques currently available in bioenergetics.

FOOD CONSUMPTION AND ABSORPTION

The majority of bivalve species are sessile suspension feeders, and most can partially adjust their filtration rate in response to the quantity and "food quality" of the seston. In general, however, there is a rapid rise in pumping rate to a plateau level, with increases in particle concentration, from a certain minimum level necessary to supply oxygenated water to the gills. A point is reached, though, where any further increase in particle concentration causes the pumping rate to decline from this maximum plateau level (Winter 1978; Widdows et al. 1979). Superimposed on such short term variations in filtration rate are seasonal changes which may be a response to both changes in water...
temperature and food availability as well as cyclical changes in physiological condition, associated with the energy demands of gametogenesis (Bayne and Widdows 1978; Newell and Bayne 1980). The consumed ration is, therefore, dependent both on the food concentration and the animals' feeding behavior.

In bivalves, the digestive process is complex because as the rate of consumption increases the digestive gland becomes full and excess food then by-passes the gland and is voided as intestinal feces, together with feces from the digestive gland (Thompson and Bayne 1972; Widdows et al. 1979). When ingestion rates are below the level which results in intestinal fecal production, absorption efficiency, defined as the energy uptake from the food in the digestive system (Crisp 1971), is at a maximum. With increasing ingestion, and hence the ejection of undigested food as intestinal feces, absorption efficiency declines. A point is ultimately reached above which excess material is not ingested but is rejected as pseudofeces (Thompson and Bayne 1972; Winter 1978). Any further increase in consumption above this level does not further reduce the absorption efficiency of the material in the digestive system. However, such a consideration of absorption efficiency neglects the energy lost in pseudofecal production. Therefore, when constructing the energy budget of an animal, the energy absorbed should be expressed as a percentage of the total energy consumed, not just of the energy ingested. In practice this is achieved by pooling both pseudofeces and feces and analyzing them using the ratio method of Conover (1966). Widdows (1978a) found that for M. edulis, absorption efficiency declined with increasing food concentration and also with decreasing body size for animals feeding at the same food concentration. Such a result was to be expected as in both cases undigested food (both intestinal feces and pseudofeces) comprised an increasing proportion of the ejecta.

The production of pseudofeces assumes critical importance in closed or semi-closed mariculture systems because frequently one of the largest operating costs is the provision of a unicellular algal food. There have been numerous studies designed to determine the most efficient means of feeding algae to bivalves in order to minimize wastage. Many are based on the results of Morton (1969, 1970, 1973) who found that for intertidal bivalves there is a cyclical relationship between feeding and both extracellular and intracellular digestion. Epifanio and Ewart (1977) found that for continuously submerged oysters (Crassostrea virginica) there were also periods of feeding activity and quiescence, although there was no apparent tidal or diurnal rhythm (Palmer 1980). Epifanio and Ewart (1977) concluded that when rearing bivalves it would be advantageous to add food discontinuously. This would entrain the feeding and digestive activity of the animals to the feeding regime and hence prevent excess food being lost during periods of non-feeding.

Similarly, Langton and McKay (1974, 1976) found higher growth rates when the same total amount of algae was fed to the oyster Crassostrea gigas discontinuously, compared with a continuous feeding regime. This result is expected because the continuous addition of algae would have resulted in a low food concentration that may not have fully stimulated the oyster's feeding activity, whilst the discontinuous feeding regime would have resulted in much higher initial concentrations. This conclusion is supported by the results of Winter and Langton (1976) who found that for maximum growth in the mussel, M. edulis, food should be continuously replenished so that a constant optimum level is maintained.
However, before discontinuous feeding regimes are widely adopted, a complex series of economic and energy budget factors must be considered. In one scenario, if optimum ration levels were continuously maintained, animals would ingest a maximum ration with little or no pseudofecal production. Any synchrony between individual's feeding and digestive cycles in the continuously immersed animals would be lost within two weeks of being placed in such conditions (Langton and Gabbott 1974) and thus periods when all animals were not feeding would be unlikely to occur. In such conditions growth would be maximized. If it is necessary to reduce the algal ration, energy budgeting can be used to determine the least harmful way of achieving this. One alternative might be to adopt a discontinuous feeding regime where the daily feeding periods are balanced between economic restraints and achieving maximum growth. However, a more effective solution might be the maintenance of optimum ration levels during the seasonal periods of physiologically and genetically entrained maximum growth and a reduction in ration during the quiescent periods.

In addition to an animal's energy intake, it is also important to consider its requirement for specific nutrients, e.g., essential amino acids, lipids, etc. For example, Russell-Hunter (1970) has shown that the reproductive capacity of the marsh snail, Melampus bidentatus, is limited at certain times by lack of organic nitrogenous compounds. Unfortunately, as yet, very little research effort has been directed towards this type of study in bivalves, due to the technical complexities of measuring the various rate functions in terms of the selected nutrient. However, the development of artificial diets suitable for filter feeding bivalves (see Langdon - these proceedings), will allow experimental manipulation of the ration. This should yield the sort of detailed information concerning nutritional requirements that is already available for crustaceans (see Kanazawa - these proceedings).

**INFLUENCE OF INORGANIC PARTICLES ON FEEDING AND GROWTH**

The negative influence of high levels of particulate inorganic material (PIM) on the filtration rate of bivalves has been extensively studied (Foster-Smith 1975; Moore 1977; Widdows et al. 1979). However, there is some evidence that low levels of PIM added to an algal diet can enhance feeding and growth (Loosanoff 1962; Kiorboe et al. 1981). Winter (1976) found that mussels continuously fed a mixture of Dunaliella marina \(40 \times 10^6\) cells \(\text{L}^{-1}\) and 12.5 mg dry wt \(\text{L}^{-1}\) of PIM (produced by wet oxidizing natural sediment with \(\text{H}_2\text{O}_2\)) had a 32% greater dry weight after 26 days, compared with control mussels fed the same concentration of algae alone. Winter (1976) demonstrated that this enhanced growth was due to the stimulatory effect of low levels of PIM on filtration rate, and a reduction in pseudofecal production, which resulted in an increased rate of ingestion. Experiments by Ali (1980) confirmed the stimulatory effects of PIM added to the algal food, on the growth of the oyster, *C. virginica*.

Murken (1976) fed *M. edulis* a mixture PIM, the dissolved organic fraction (DOF) from fish waste, and algae and found that growth was improved compared with that of mussels fed on a mixture of just algae and DOF. Further experiments are required to see if the enhanced growth was mainly due to the stimulatory effect of increased particle
concentration on ingestion rate or if the bivalves were utilizing the DOF, or both. The active absorption, by aquatic molluscs, of dissolved amino acids (see Stephens - these proceedings) has clearly been demonstrated (Wright and Stephens 1977, 1978; Manahan et al. 1981). This may supply a proportion of the animal's energy requirements but perhaps more importantly specific nutrient requirements at certain critical periods in their life cycle.

The PIM filtered from suspension does not necessarily "dilute" the organic material ingested, as postulated by Widdows et al. (1979). Instead, recent research indicates that both the mussel M. edulis (Kiorboe et al. 1980) and the oyster, C. virginica (Newell, unpublished data) preferentially ingest algae and reject the PIM in the pseudofeces. C. virginica can also preferentially ingest the organic matter from natural seston which contains not only algae but bacteria, detritus, etc. (Newell, unpublished data).

There are many possible explanations for the effects that a PIM supplement to the algal diet can have on bivalve growth, including a) addition of bacteria or organic matter which enhances food levels; b) provision of a large surface area, for either the absorption of DOF which is thus made available to the digestive processes (Murken 1976), or the removal of inhibitory metabolites (Moore 1977); c) addition of an unknown growth factor(s) (Winter 1976); d) improvement of digestion efficiency by the mechanical grinding action of the inorganic particles in the gut (Murken 1976); and e) a stimulatory effect on filtration rate and hence an increase in the amount of material ingested (Winter 1976). Further investigation is required to determine which of these mechanisms is responsible for the enhanced growth. However, the last explanation seems perhaps the most plausible and is very similar to the effect found for crustaceans when a "bulking" fiber agent is added to their diet (Capuzzo - these proceedings).

RESPIRATION

Bivalves under normoxic conditions (i.e. active animal, freely ventilating its mantle cavity in fully aerated seawater) use approximately 65% of the energy of metabolic substrates to phosphorylate ADP to ATP by the classical tricarboxylic acid cycle (Calow 1978), with the remainder of the energy being lost as heat. In order to determine the amount of energy respired either the metabolic and mechanical heat output or the animal's oxygen demand must be measured. The latter measurement can then be converted to energy units using standard coefficients (Elliot and Davison 1975). Due to the relative ease and accuracy of measuring oxygen uptake, compared with heat output, it is almost exclusively used to quantify metabolic energy demands of aquatic molluscs (Crisp 1971).

In some circumstances, e.g. during periods of shell closure, a bivalve is deprived of oxygen. In such situations ATP is generated by perhaps as many as four anaerobic pathways, which may be differentially utilized, both temporally and spatially, within the animal's tissue (Kluymans et al. 1980; Zandee et al. 1980; Zurburg and Ebberink 1980). These pathways regenerate ATP by incomplete chemical oxidation of predominately glycogen, resulting in the accumulation of a succession of fermentation end-products, including succinate, amino acids and volatile fatty acids.
Anaerobic metabolic pathways have frequently been considered simply as useful adaptations that enable a "facultatively anaerobic" animal to supply its ATP requirements under hypoxic or anoxic conditions (Pamatmat 1979). Also, as the respiratory substrates are incompletely oxidized, anaerobiosis has been considered energetically inefficient, with ATP yields from glycogen being 6-8 times less than if an equisorb amount had been aerobically catabolized (de Zwaan 1977). However, the energy of the anaerobic end-products is not necessarily lost to the animal, as succinate and propionate can be converted to oxalacetate, which can be used during aerobic respiration in the tricarboxylic acid cycle (de Zwaan 1977). Recent research (Henry et al. 1980) also indicates that in some bivalves, anaerobic end-products are required during normoxic conditions for the production of intracellular osmolytes. There is also some evidence that indicates that even in the presence of high oxygen levels the metabolism of certain "deep seated" tissues, e.g. the adductor muscles, may be partially anaerobic (Chaplin and Loxton 1976; Hammen 1976; de Zwaan 1977; Booth and Manjun 1978). Few bioenergetic studies have considered the possibility that measuring oxygen demand under normoxic conditions may underestimate the total catabolism of energy reserves due to sustained anaerobic metabolism.

Hammen (1979) measured the oxygen demand and heat output under aerobic conditions of a group of mussels, Mytilus edulis, and found that the total metabolic heat output was 1.59 J hour⁻¹ g⁻¹ wet tissue, but their oxygen demand of 0.06 ml O₂ hour⁻¹ g⁻¹ was equivalent to a metabolic heat output of only 1.21 J hour⁻¹ g⁻¹ or, approximately 77% of that measured by calorimetry. A similar result was again reported for Mytilus edulis by Hammen (1980). Although such calorimetry experiments provide reliable estimates of heat output during anaerobiosis it was not until the work of Famme et al. (1981) that long term measurements of the heat output and oxygen consumption of M. edulis were made simultaneously, at a number of controlled oxygen tensions. Their results showed that under normoxic conditions anaerobic metabolism accounts for less than 5% of the total metabolism, which is probably associated with the energy demands of "deep seated" tissue. Although these experiments need to be performed on other species it does indicate, contrary to the work of Hammen (1979, 1980), that in M. edulis measuring oxygen consumption is an acceptably accurate technique for quantifying metabolic energy demand. However, at oxygen tensions as low as about 50% of saturation the oxygen consumption of M. edulis is reduced to 50% of its normoxic level but its total metabolism is not altered (Famme et al. 1981). This difference must, therefore, be sustained by anaerobic metabolic pathways. These results indicate that in maricultural facilities it is important that saturated oxygen tension levels are maintained either by aeration or by avoiding overcrowding so that the animals can sustain aerobic metabolism.

A bivalve's metabolism varies in response to short-term environmental perturbations such as temperature and food availability (reviewed by Bayne et al. 1979b; Newell 1979). The actual response elicited is dependent both on the species and its physiological condition. When an animal is subject to an abrupt increase in ambient temperature in conditions of high food availability, it may adopt an "exploitative" strategy. This involves an increase in feeding rate causing both the rate of consumption and metabolic costs to increase, with perhaps a reduction in absorption efficiency, but the net result is an increased scope for growth (Widdows and Bayne 1971; Newell 1979,
Conversely, in food limited situations a more "conservationist" strategy may be adopted in which activity is curtailed, thereby reducing metabolic energy demands to a "standard" level (Thompson and Bayne 1972), absorption efficiencies may be higher and hence the energy gain from the reduced ration is maximized.

Many commercial mariculture projects benefit from the faster "opportunistic" growth rates associated with artificially elevated temperatures. However, prior to investment in such projects it is vital to determine, using a regular sampling program, that the ambient food levels are high enough to maintain a positive annual scope for growth (Malouf and Breese 1977).

GENETIC INFLUENCES ON METABOLISM

It has frequently been found in breeding experiments that organisms which are heterozygous at a large number of gene loci grow faster than more homozygous individuals (Wilkins 1981). Zouros et al. (1980) found that for the oyster C. virginica, there was a positive relationship between the degree of heterozygosity at four to seven gene loci and the weight of a one year old animal. There was no clear explanation of this result until Koehn and Shumway (1982) demonstrated that the standard metabolism of more heterozygous oysters consumed less energy, and they suggested that this was possibly because metabolic pathways dependent on heterozygous enzymes are, on average, more efficient than homozygous ones. Koehn and Shumway (1982) postulated that reduced metabolic cost results in more energy being available for growth. This genetic component of an animal's metabolism and growth has frequently not been given sufficient consideration by physiologists and requires further study.

EXCRETA

Excreta, a term properly applied to the loss of energy in the form of nitrogenous waste compounds, is one of the least studied aspects of molluscan bioenergetics. I also include under this term exudates such as mucous (but not seminal products) (Crisp 1971).

The two main nitrogenous compounds excreted by bivalve molluscs are ammonia nitrogen (NH₄-N) and amino nitrogen (amino-N). Bayne et al. (1976b) concluded in a review of data from 8 bivalve species that NH₄-N accounted for about 65% and amino-N for about 28% of the total nitrogen excreted and the balance was made up by either urea or uric acid. Excretion of NH₄-N may be variable due to increased reliance on protein as a respiratory substrate during periods of stress, e.g. in M. edulis during and just after maximum reproductive condition (Bayne 1973; Widdow 1978a). Amino-N loss may partly be due to 1) the passive diffusion of amino acids from the body tissues (Hammen 1968); 2) an active excretory process associated with protein catabolism; and 3) osmotic adjustment to a decline in ambient salinity.

Amino-N excretion has been found to increase from a baseline value of approximately 11% of the energy of routine metabolism to a value as high as 63% when mussels are placed under environmental stress (Bayne 1973). This may perhaps be due to increased protein catabolism or to a
decreased stability of biological membranes. Amino acid excretion is an activity of great complexity considering the ability of bivalves to absorb amino acids actively from water (reviewed by Stephens - these proceedings). More research is required concerning the environmental and physiological conditions that affect the absorption or excretion of amino acids in cultured bivalves so that excretion of nitrogen is minimized whilst absorption is maximized.

The energy loss in the mucous voided with the feces and pseudofeces is not normally determined separately in energetic studies, but is included in the energy of the egesta. However, in species which produce copious pseudofeces under certain feeding conditions, e.g. *Crassostrea virginica*, it may be a significant loss.

**PHYSIOLOGICAL INTERGRATIONS**

The measurement of the physiological factors necessary to construct the complete energy budget for many molluscs may be made using standard techniques (Bayne et al. 1977, 1979). Once the appropriate interrelationships between the various functions, e.g. food concentration and feeding rate, variations in metabolic rate with season, etc. have been elucidated for a species, a computer based simulation model, can then be used to predict "scope for growth" values. A typical application could be the assessment of the maricultural potential for a species in a variety of localities. The only additional data required for each locality would be detailed information over an annual cycle of environmental factors, e.g. seston and total particulate organic matter levels, temperature, salinity, etc. The accuracy of using estimated "scope for growth" values based on simulation models to predict the actual growth of a species in the field has been tested by Newell (1977) and Bayne and Worrall (1980). The results obtained by Bayne and Worrall (1980) (Figure 1) indicate close agreement between the predicted and measured growth of *Mytilus edulis*.

Such techniques may be too sophisticated for maricultural facilities but at least frequent determinations of ambient food levels and absorption efficiencies should be made. The concentration of the added ration can thus be routinely regulated so that high absorption efficiencies are maintained. Widdows (1978a) found that the absorption efficiency of *M. edulis* was unaffected by temperatures between 5°C and 15°C in the laboratory. However, Bayne and Widdows 1978 and Newell (unpublished data) found that in field populations of *M. edulis*, the absorption efficiency ranged from about 70% in the summer to a winter minimum of about 5-10%, a result which may have been partly due to seasonal variation in food digestibility. However, it seems that absorption efficiency values between 60-70% can reasonably be expected when feeding bivalves on cultured algae (Winter 1978).

Information on the efficiency with which the cultured animals are utilizing the ration for growth is frequently required both to allow continuous adjustment of the ration to an optimum level and also to compare the performance of different groups of animals. Two useful, non-dimensional ratios are the "Gross Growth Efficiency" ($k_1$) and "Net Growth Efficiency" ($k_2$). These are defined as the efficiency with which an animal uses the energy in the consumed and assimilated ratio, respectively, for somatic and germinal growth (Klekowski and Duncan 1975).
Figure 1. Comparison of the growth of a population of *Mytilus edulis* as calculated from age-class analysis (broken line) and from physiological estimates of the scope for growth (solid line) from Bayne and Worrall (1980).

Gross Growth Efficiency ($K_1$) = $\frac{P+G}{C-F-U}$

Net Growth Efficiency ($K_1$) = $\frac{P+G}{(C-F-U)}$

Where $P$ = Somatic Growth; $G$ = Germinal Growth; $C$ = Ration Consumed; $F$ = Energy Lost in the Feces; $U$ = Energy Excreted.

Perhaps the most extensive use of $K_1$ for the description of the interrelationships between ration, metabolism and growth in bivalves has been by Thompson and Bayne (1974) and Widdows (1978b). Thompson and Bayne (1974) found for *M. edulis* (Figure 2) a rapid increase in $K_1$ from a negative value occurring at low food rations to a point ($C_m^*$) where $K$ is zero, which is when the absorbed ration is equal to the metabolic energy demands; thus $C_m^*$ is a measure of the maintenance ration. Growth efficiency increases further with ration size until a maximum point is attained at the optimum ration level ($C_{opt}$) for that sized animal. Any further increase in ration results in a decline in growth efficiency. Actual values for $K_1$ for *M. edulis* range from about 45% for animals between 0.1-0.2 g dry weight to below 10% for animals over 1 g dry weight (Jorgensen 1976; Bayne et al. 1975c; Widdows 1978b).

ENERGY PARTITIONING

The discussion so far has been mainly concerned with the energy available for somatic and germinal growth and not how this energy is partitioned between these two types of growth. The majority of bioenergetic studies have not been able to quantify the differential allocation of energy because this varies not only between species but is also dependent on the individual's age, size, history and environmental
conditions. This is further complicated by the use of a nutrient reserve in many species to supply energy for gametogenesis and maintenance metabolism during periods of reduced food ingestion.

Figure 2. Generalized curve for growth efficiency ($K_1$) of *Mytilus edulis* as related to ingested ration. The solid line represents the control condition and the dashed line a decrease in growth efficiency due to increased energy demands of respiration. Where $C_m$ and $C_{m1}$ are the maintenance rations and $C_{opt}$ is the optimum ration (from Thompson and Bayne 1974).

The somatic growth of a bivalve, above a certain species specific minimum size (e.g., approximately 10 mm for *Mytilus edulis* (Thielsen 1973) can be described by a logarithmic curve, that is, a continuously decreasing weight specific growth rate. This is due, in part, to a decreasing efficiency of feeding, and an increasing metabolic maintenance cost, as the animal increases in size. This was demonstrated by Vahl (1973) for the cockle, *Cardium edule*, where the exponent ($b$) relating metabolism ($R$) to body weight ($W$) in the allometric equation

$$ R = aW^b $$

was 0.77 whereas that for feeding rate was only 0.58. Also, an
increasing proportion of the scope for growth of larger animals is channeled into reproduction such that at some maximum size somatic growth virtually ceases. This is clearly illustrated (Figure 3) in the energy budget of the oyster Ostrea edulis (Rodhouse 1978). The amount of energy allocated to somatic growth in oysters under 5 years (equivalent to an animal of about 50 mm shell height) is higher than that allocated to germinal production but in older animals somatic growth virtually ceases.

![Figure 3](image)

Figure 3. Age dependent metabolic costs and annual production of germinal tissue, somatic tissue, and shell organics in terms of the energy expended for the oyster Ostrea edulis (after Rodhouse 1978).

A significant proportion of the absorbed energy is not used for either somatic or germinal growth but is incorporated into the matrix of the shell. The mean shell energy content, expressed as a percentage of the total tissue energy count, is 35.3% for a number of different bivalve species (Table 1). There is wide variation in these percentages because of different shell thicknesses e.g. for the thin-shelled Scrobicularia plana the dry tissue weight is equivalent to 28.1% of the shell weight whereas for the heavy-shelled Crassostrea virginica the tissue is only 2.6% of the shell weight. Also, as the tissue weight varies with the seasonal changes in reproductive condition the relationship between the energy content of the tissue and the shell is not constant. These values do not indicate that a third of the absorbed energy is incorporated into the shell matrix each year, as the shell has been gradually built up over the animal’s entire life, whereas of the body tissues, only the somatic portion accretes and the energy allocated to germinal production is largely lost as gametes each year. Little is known of the energetic cost to the animal of producing the shell as it is simply considered part of the metabolic demand. However, there are differences of up to 45% in the shell weight of similar sized mussels originating from different habitats (Meyers 1980). It would be of
interest to know if a reduction in shell weight significantly improves an animal's scope for growth. Furthermore, for certain bivalves, e.g. the mytilids, the production of byssus threads is also a drain on the absorbed ratio which can represent up to 8-15% of the total tissue energy content (Griffiths and King 1979),

Table I. The Energy Content of Various Bivalve Shells, Expressed as a Percentage of Their Tissue Energy Content, Calculated Using the Information Supplied in Each Reference

<table>
<thead>
<tr>
<th>Species (Reference)</th>
<th>Shell Length (mm)</th>
<th>Dry wt (g)</th>
<th>Dry Energy content (KJ)</th>
<th>Tissue Dry wt (g)</th>
<th>Dry Energy content (KJ)</th>
<th>Energy content of shell as % of tissue energy content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scrobicularia plana</td>
<td>50</td>
<td>3.2</td>
<td>0.27</td>
<td>0.9</td>
<td>18.8</td>
<td>1.4</td>
</tr>
<tr>
<td>(Hughes 1970)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crassostrea virginica</td>
<td>8.0 a</td>
<td>5.76</td>
<td>1.19</td>
<td>0.15</td>
<td>3.18</td>
<td>37.4</td>
</tr>
<tr>
<td>(Dane 1972)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mytilus edulis</td>
<td>35-40</td>
<td>1.3</td>
<td>4.3% b</td>
<td>0.28</td>
<td>NG</td>
<td>20.0</td>
</tr>
<tr>
<td>(Jørgensen 1976)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geukensia demissa</td>
<td>60</td>
<td>9.3</td>
<td>5.4% b</td>
<td>1.15</td>
<td>NG</td>
<td>43.6</td>
</tr>
<tr>
<td>(Jørgensen 1976)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Ostrea edulis</td>
<td>100</td>
<td>NG</td>
<td>46.4</td>
<td>3.63</td>
<td>78.9</td>
<td>58.8</td>
</tr>
<tr>
<td>(Rodhouse 1978)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>35.3</td>
</tr>
</tbody>
</table>

*a Whole wet weight of an oyster.

b Values are % weight loss on ignition at 450°C.

NG = not given.

The majority of energy converted to germinal growth is liberated as gametes and is therefore not reflected as an increase in marketable meat, unless the animal is harvested when gravid. Currently attempts are being made to produce sterile triploid oysters (Hidu et al. 1981) which would possibly grow faster than normal diploid animals. More of such research is required on the breeding of specialized strains of bivalves, especially suited to mariculture, which incorporate both the genetic advantages of heterozygosity (Koehn and Shumway 1982) and triploidy.

In certain situations, e.g. with low pollution levels or environmental changes leading to reduced food availability, bivalves may experience stress which is not severe enough to cause adult mortality. Instead, it may have a subtle but deleterious effect on the animals' energy budget (Hayne et al. 1979). Bivalves fed below the maintenance ration seem to produce fewer eggs, which weigh less than normal eggs because of a reduction in their nutrient content (Sastry 1975; Bayne et al. 1978). This means that when the eggs are spawned and fertilized the resulting larvae with lowered nutrient reserves may have a reduced probability of successfully attaining metamorphosis (Bayne et al. 1975). In some species, starvation may also result in the absorption and
utilization of the gonads for metabolic maintenance (Bayne et al. 1978). These findings indicate that endogenous mechanisms that regulate energy partitioning between metabolic processes and somatic or germinal growth are extremely complex and incorporate feedback loops which are dependent on the animal's physiological and reproductive condition. It is perhaps only by studying such control mechanisms that we can fully interpret the mollusc's responses to changing environmental conditions.

CONCLUSIONS

1) Energy budgets are useful techniques for integrating a number of measurements so as to provide a single index (the Scope for Growth) of an animal's ability to survive, grow and reproduce in a particular environmental regime.

2) Although the concept of energy flow is useful, there is a need to trace individual nutrients through the animal both in the laboratory and in the environment.

3) Bioenergetic techniques should be applied to mariculture so as to provide information on optimum feeding levels and growth potentials.

4) Although a large number of bioenergetic techniques have been developed and applied effectively there are still areas of ignorance. These mainly concern the loss of energy in the excreta and the mechanisms, presumably hormonal or neurosecretory, that govern the differential allocation of energy between growth and reproduction in bivalves.

ACKNOWLEDGEMENTS

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LITERATURE CITED


QUESTIONS AND ANSWERS

WEINER (U. of Maryland): Available energy is also a very important consideration. Has anyone looked at adenylate energy charge, that is E. C. = (ATP + 1/2ADP)/(ATP + ADP + AMP), under, for example, various dietary regimes, oxygen levels or stages in the life cycle of the animal?

NEWELL: Yes, a group of scientists (Rainer et al. 1979) have used the energy charge as a means of assessing the severity of an environmental stress on molluscs. Although they obtained good measurements of all of the adenine nucleotides they concluded that the use of adenylate energy charge does not provide an absolute measure of stress in molluscs. I am not aware of any extensive monitoring of energy charge on a seasonal basis but I agree that it could provide a useful index of the animal's metabolism.