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HISTOLOGY, HISTOCHEMISTRY AND ENZYME BIOCHEMISTRY OF THE DIGESTIVE GLANDS IN THE TROPICAL SURF BARNACLE *TETRACLITA SQUAMOSA*

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The tropical surf barnacle *Tetraclita squamosa* (Bruguière) (Crustacea: Cirripedia) has a pair of lobulate digestive glands each connected to the dorsal surface of the foregut by a single duct. These glands have a central lumen surrounded by columnar epithelial cells and are the primary site of enzyme synthesis and secretion within the alimentary tract. A number of carbohydrases and proteases were detected, all of which exhibited optimal activity at an acidic pH. Gland cells discharge secretory products into the foregut ventriculus via apocrine secretion and this process is continuous; it is not correlated with tidal and subsequent feeding cycles. Possible reasons for the absence of feeding and secretory co-ordination are discussed.

INTRODUCTION

Barnacles have attracted much interest on account of their successful colonization of intertidal regions and their economic importance as fouling organisms. Research has focused on their ecology (Foster, 1987; Hui & Moyse, 1987; Otway & Underwood, 1987) and larval settlement (Nott, 1969; Crisp, 1976; Achituv, 1981), the latter for commercial interest in effective antifoulants. Despite numerous studies on cirripede feeding mechanisms (Southward & Crisp, 1965; Anderson & Buckle, 1983; Anderson & Southward, 1987), knowledge of their digestive physiology is poor, with only two studies on temperate species such as *Balanus improvisus* Darwin (Törnävä, 1948), *Semibalanus balanoides* (Linnaeus) and *Chirona hameri* (Ascanius) (Rainbow & Walker, 1977). Digestive physiology of tropical surf barnacles has not yet been examined.

The alimentary canal of temperate cirripedes is U-shaped, with an ectodermal cuticle-lined foregut and hindgut and an endodermal midgut with associated caeca (Rainbow & Walker, 1977). All barnacles have a pair of digestive glands of midgut origin, each connected to the anterior midgut by a single duct. *Tetraclita squamosa* (Bruguière) is a large surf barnacle (basal diameter up to 50 mm) common on exposed shores of tropical Queensland. It is an extension-feeding planktivore which extracts a broad range of zooplankton and phytoplankton from the water column (Hunt & Alexander, 1991).

Knowledge of the cirripede digestive gland is limited. Its structure was briefly described in temperate species by Törnävä (1948) and Rainbow & Walker (1977); however, its function was not investigated. Cirripede digestive glands are branched

tubular organs composed of large columnar epithelial cells with an apical brush border and large basal nuclei. Cells rest on a basal membrane, beneath which are thin layers of longitudinal and circular muscle; they are of one type and are histologically distinct from those of the midgut epithelium (Rainbow & Walker, 1977). In contrast, the decapod digestive gland is recognized to contain five distinct cell types: E-, F-, B-, R- (Gibson & Barker, 1979; Caceci *et al.*, 1988) and M-cells (Al-Mohanna *et al.*, 1985a), all of which have specialized functions.

Biochemical studies of digestive enzymes in only two temperate cirripede species, *Balanus nubilus* (Darwin) (Harnden, 1968; DeVillez, 1975) and *Balanus crenatus* (Bruguière) (Kristensen, 1972) have been published. These were limited to qualitative estimates of enzyme concentration as amounts of protein extracted were negligible. From *T. squamosa* sufficient quantities of protein can be extracted for quantitative determination of enzyme concentration.

Histology, cytology and histochemistry of the digestive gland of *T. squamosa* are described and biochemical methods have been used to identify enzyme types and their primary sites of synthesis. Enzyme activity is discussed in relation to diet and feeding cycle and the principal role of cirripede digestive glands is postulated.

MATERIAL AND METHODS

Specimen collection

Tetraclita squamosa adults (basal diameter >30 mm) were removed from the seaward side of the eastern breakwater of Townsville Harbour, Australia (19°15'S 146°30'E). Specimens were immediately preserved in formaldehyde calcium acetate (Lillie & Fullmer, 1976), or transferred to an aquarium of circulating sea-water which was drained periodically to simulate tidal conditions. Barnacles were maintained on particulate food within the aquaria and supplemented at intervals with *Artemia salina* (L.) nauplii.

Histological, histochemical and cytological methods

For histological examination, excised tracts were fixed for at least 48 h in formaldehyde acetic acid - calcium chloride (formaldehyde 4%, calcium chloride 1·3%, acetic acid 5%). For histochemistry, specimens were fixed in formaldehyde calcium acetate (formaldehyde 4%, calcium acetate 2%) or Gendre's fluid (after Cook, 1974). Following dehydration in graded ethanol solutions, tissues were embedded in paraffin wax and longitudinal sections (4-7 µm) were then cut and stained with Mallory-Heidenhain or haematoxylin and eosin (after Winsor, 1984).

The presence of carbohydrates, proteins and lipids within the digestive gland was determined using histochemical techniques (Table 1). Fixation for carbohydrates and proteins used the methods outlined above. For lipids, specimens were transferred to gum sucrose for 12 h after formaldehyde calcium acetate (see above) fixation, embedded in OCT compound (Ames Tissue Tek, Bayer Diagnostics, Brisbane, Queensland) and 10-µm frozen sections cut on an IEC Minitome cryostat.

For ultrastructural examination, digestive glands were dissected in sea-water and

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Table 1. *Histochemical tests applied to the alimentary tract and digestive glands of* Tetraclita squamosa

Compound	Stain	Control	Fixative	Reference	Results
Carbohydrate					
Glycogen	Diastase controlled PAS*	*Bufo marinus* liver	Gendre's fluid	Cook 1974	
Acid/neutral/ basic mucins	Alcian blue/ PAS'	Cat pyloro-duodenal junction	formal Calcium acetate	Cook 1974	+
Protein					
General NH_2 gps	Mercuric bromo-phenol blue	*Bipalium kewense*	formal Calcium acetate	Winsor 1984	+++
Basophilia	Buffered azure A	-	formal Calcium acetate	Lillie & Fullmer 1976	+
Acidophilia	Biebrich scarlet	-	formal Calcium acetate	Lillie & Fullmer 1976	
Lipid					
General	Bromine Sudan black	Delipidized section	formal Calcium acetate/ gum sucrose	Winsor 1984	

PAS', Periodic acid-Schiff reagent. blank, negative reaction. +, positive reaction. +++, strongly positive reaction

fixed in 2·5% glutaraldehyde in sea-water (pH 7·1) for 1 h, washed in 0·1 M cacodylate buffer and post-fixed in 1% osmium tetroxide in 0·1 M cacodylate buffer for 20 min at 20°C. Following washing in cacodylate buffer they were dehydrated in graded ethanols and embedded in resin (Spurr, 1969). Optical sections of 1·5 µm were stained with 1% toluidine blue in 2% borax and 60-nm sections were stained with acidified, saturated uranyl acetate in 50% ethanol for 10 min, and with lead citrate for 1 min (Reynolds, 1963) and examined in a JEOL 2000 FX transmission electron microscope at 80 kV.

To determine whether gland secretion is dependent on tidal feeding activity, ten barnacles were randomly collected from the eastern breakwater at 1, 3 and 5 h after feeding on the receding tide. As Townsville has a semi-diurnal tidal pattern (Easton, 1970) specimens were collected at three successive times during their approximate 5-7 h feeding period. Five barnacles were fixed in 2·5% glutaraldehyde in sea-water (pH 7·1) and digestive glands processed for resin embedding. Optical sections were examined for structural changes associated with secretion and thin sections examined for cytological changes in rough endoplasmic reticulum (RER), Golgi apparatus and mitochondria. The remaining five barnacles were fixed in buffered formalin and glands processed for paraffin wax embedding. Sections of 4 µm were stained with mercuric bromophenol blue and the glands' staining intensity (protein concentration) categorized to indicate the degree of secretory activity.

Enzyme extraction

The alimentary tracts of 30 adult barnacles were placed in chilled sea-water (4°C). Fifteen whole tracts were pooled and used to detect enzyme presence. The remaining 15 were divided into glands, fore, mid and hindguts. Each region was pooled to determine primary sites of enzyme synthesis. Digestive fluid in the tracts was removed to prevent

bias. Samples of whole tracts and tract regions were homogenised in chilled buffer pH 7·2 (7·5 ml; 10 mM sodium phosphate, 50 mM NaCl), centrifuged for 5 min at 1500*g* and the supernatants assayed.

Total protein was determined using the Bio-Rad micro protein assay (Bradford, 1976). Digestive tract fluid from five recently fed adults was pooled, diluted (1:10) in distilled water and the pH determined at 25°C using a micro-electrode.

Enzyme assays

Two replicate assays were carried out in duplicate to ensure the rate of hydrolysis was representative for each tract region. Controls lacked enzyme extract to account for residual substrate hydrolysis occurring over the long incubation periods. Assays were conducted at pH 5·0 and 7·2 to estimate optimal activity.

(A) Carbohydrases

α-amylase was measured using starch remazolbrilliant blue (5 mg) as substrate (Rinderknecht, 1967). Assays were conducted at both pH 5·0 (0·1 M phosphate-citrate buffer) and pH 7·2 (20 mM HEPES buffer). α-glucosidase and β-glucosidase activity were determined using the acid and alkaline buffers and p-nitrophenol α-D-glucopyranoside and β-D-glucopyranoside (20 mM) as substrate respectively. To stop the reaction, 1 M Na_2CO_3 was added prior to measuring the absorbance at 400 nm. The ability to hydrolyse a range of carbohydrates was investigated by cellulose thin-layer chromatography (TLC). Assays consisted of either the acid or alkaline buffer (250 μl), tract extract (5 μl) and substrate (5 mg): amylopectin, carboxymethyl cellulose (Sigma), laminarin (Pfansteil Laboratories Inc.), fucoidin (Koch-Light Laboratories Inc.), chitin (Calbiochem), sodium alginate (BDH) maltotriose (Hayashibara Biochemical Laboratories Inc.) and cellobiose (BDH). Samples were incubated under a cover of toluene for 24 h at 37°C and then 10-μl aliquots spotted on TLC plates which were run at 25°C for 5 h in the solvent n-butanol, glacial acetic acid and distilled water (4:1:1). Chromatograms were developed according to Trevelyan *et al.* (1950).

(B) Proteases

Protease activity was measured using hide powder azure (0·5 mg) as substrate with both acid and alkaline buffers (as above). Tryptic activity was determined according to Hummel (1959) using the above buffers and 2 mM p-toluenesulphonyl-L-arginine methyl ester (TAME). Chymotrypsin was determined using 1 mM N-benzoyl-L-tyrosine ethyl ester (BTEE) (Hummel, 1959). The activity of carboxypeptidase A and B was measured according to the method of Gates & Travis (1973).

RESULTS

The alimentary tract of *Tetraclita squamosa* can be divided into foregut, midgut and hindgut as shown in Figure 1A. The foregut is divided into a narrow pharynx and

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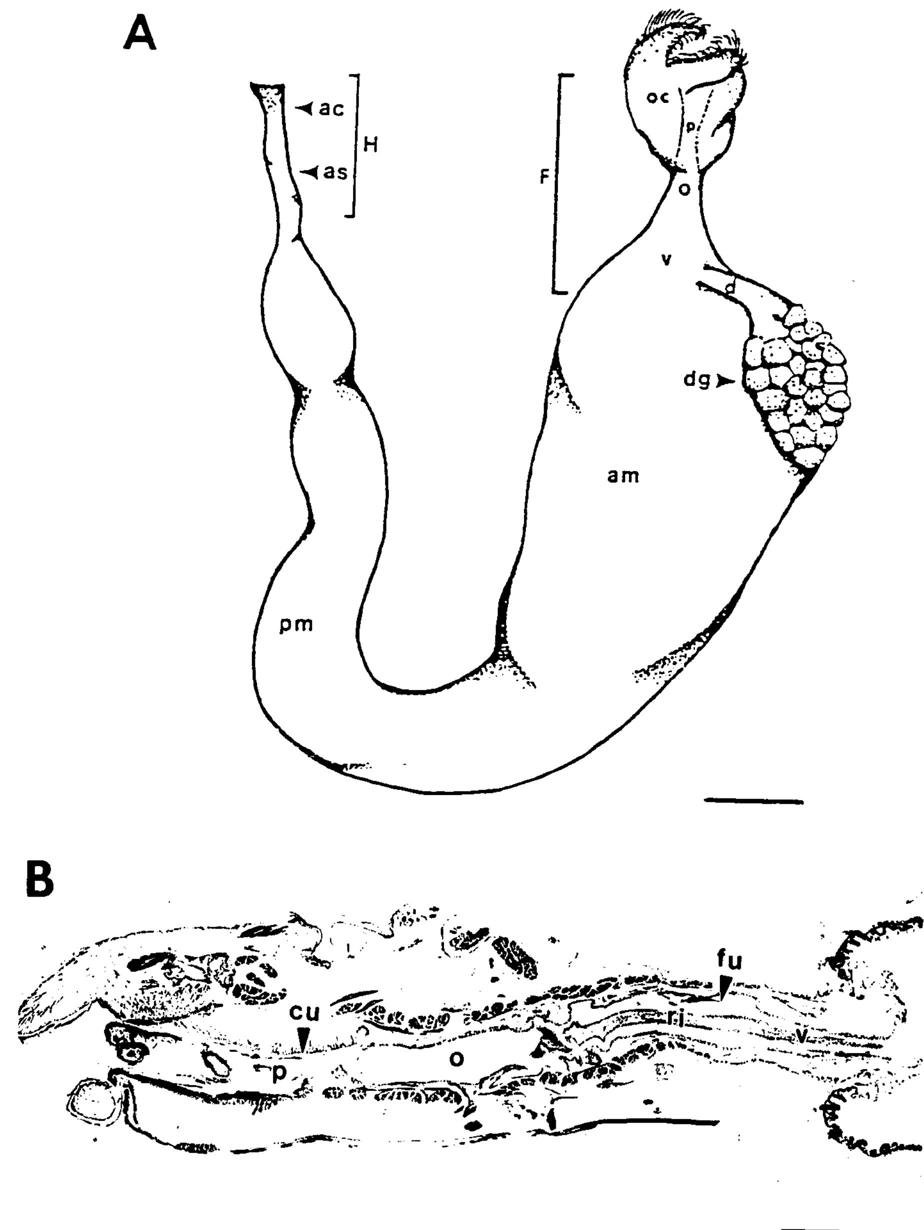

Figure 1. (A) External lateral view of the alimentary tract of *Tetraclita squamosa*. Scale bar: 2 mm. (B) Longitudinal section (wax, 6 μm) through the foregut region of the alimentary tract of *Tetraclita squamosa* showing the position of pharynx, oesophagus and ventriculus. Scale bar: 0·45 mm. ac, anal chamber; am, anterior midgut; as, anal sphincter; cu, cuticle; d, gland duct; dg, digestive gland; F, foregut; fu, furrow; H, hind gut; o, oesophagus; oc, oral cone; p, pharynx; pm, posterior midgut; ri, ridge; v, ventriculus.

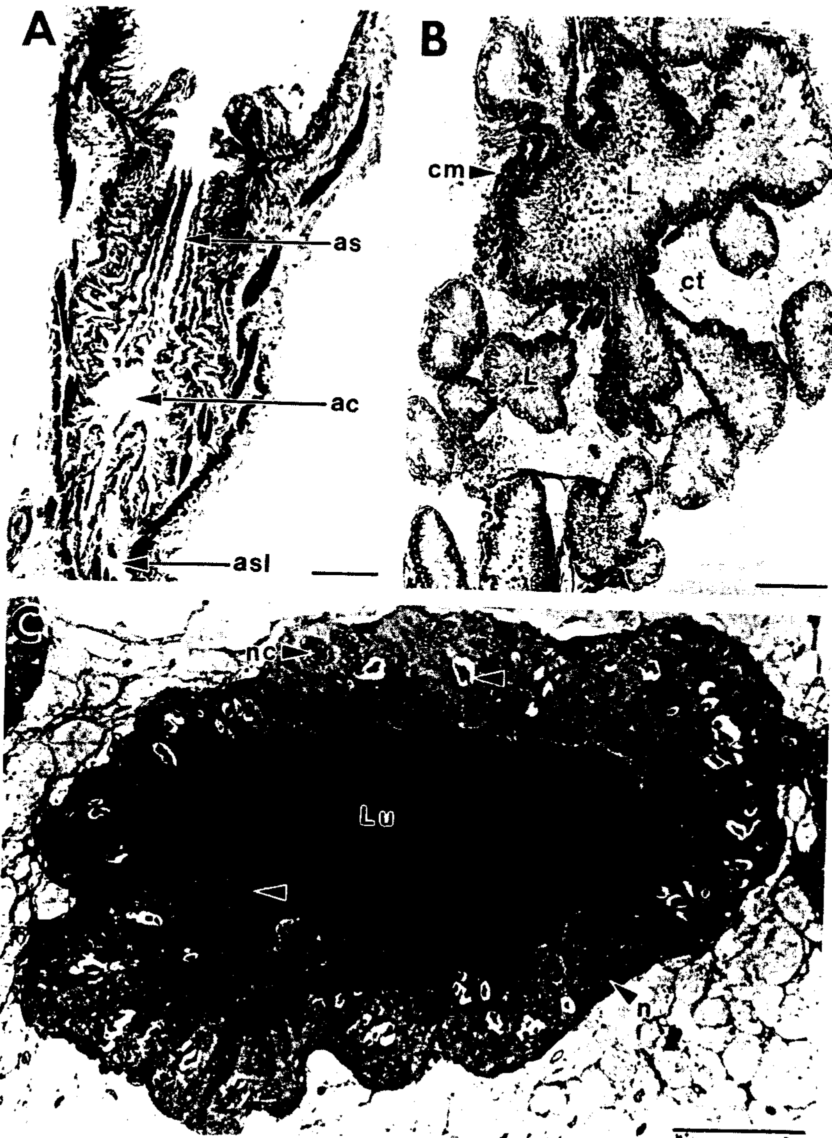

Figure 2. *Tetraclita squamosa.* (A) Longitudinal section (wax, 6 µm) through the hindgut showing the position and structure of the anal sphincter, chamber and anal slit. Scale bar: 0·25 mm. (B) Longitudinal section (wax, 4 µm) through the digestive gland showing lobule structures and the musculature. Scale bar: 0·25 mm. (C) Longitudinal section (resin, 1·5 µm) through a digestive gland lobule showing structural characteristics of the epithelial cells. Note pigmented granules scattered throughout the cell cytoplasm (arrowheads). Scale bar: 40 µm. ac, anal chamber; as, anal sphincter; asl, anal slit; cm, circular muscle; ct, connective tissue; L, lobule; Lu, lumen; n, nucleus; nc, nucleolus.

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oesophagus and a wide posterior chamber, the ventriculus, characterized by a system of ridges and furrows (Figure 1B). Paired digestive glands are connected dorsally to the ventriculus by single ducts (Figure 1A). The U-shaped midgut comprises the major portion of the tract. Midgut caeca, as described in temperate cirripede species by Rainbow & Walker (1977), are absent. The hindgut is a short structure, the lumen of which is much narrower than that of the midgut. It is divided into an anterior anal sphincter and posterior anal chamber from which a slit-like anus opens between the sixth pair of cirri (Figure 2A). Tract histology is similar to that reported in temperate cirripedes by Törnävä (1948) and Rainbow & Walker (1977).

In an adult barnacle (basal diameter >30 mm), each digestive gland is approximately 1·5 mm wide and 1·7 mm long. Their colour varies from translucent to white although most are cream. They are lobulate structures connected to the tract by connective tissue. Each lobule is surrounded by a layer of circular muscle (Figure 2B) and has a central lumen lined by large columnar epithelial cells 40-60 µm long and 15 µm wide (Figure 2C). However, cell size and shape vary depending on their secretory stage (see Figure 4B). Cells have a large spherical basal nucleus within which are characteristic dense aggregations of chromatin and a prominent nucleolus. Numerous small vesicles and irregular-shaped granules are scattered throughout the cell cytoplasm (Figure 2C). Transmission electron microscopy elucidated the cytological features of gland cells. Large quantities of RER are present, particularly adjacent to the basal nucleus, often

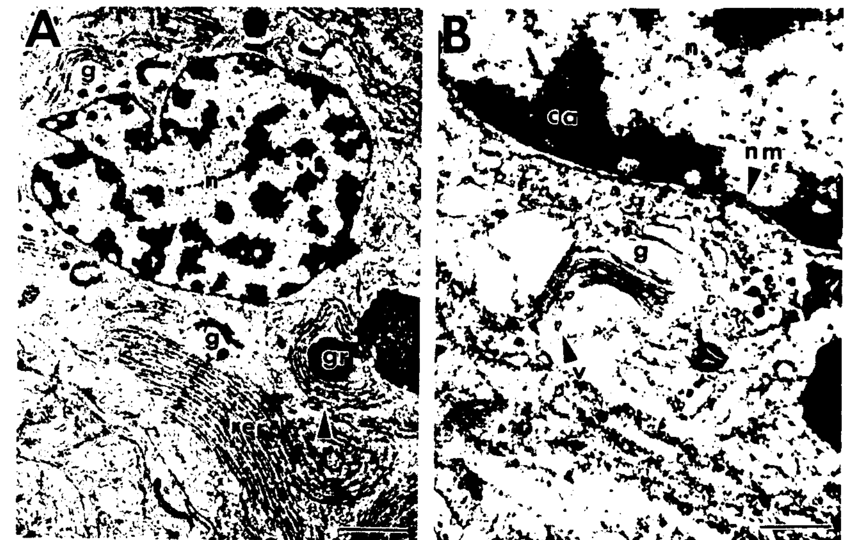

Figure 3. Transmission electron micrographs of *Tetraclita squamosa* digestive glands. (A) Rough endoplasmic reticulum (RER) and Golgi apparatus arrangement within the gland cell cytoplasm. Note whorl-like formation of RER (arrowhead). Scale bar: 2 µm. (B) Golgi apparatus and its associated secretory vesicles positioned adjacent to the gland cell nucleus. Scale bar: 0·6 µm. ca, chromatin aggregation; g, Golgi apparatus; gr, granule; n, nucleus; nm, nuclear membrane; rer, rough endoplasmic reticulum; v, vesicle.

forming complex whorl-like arrangements up to 5 μm in diameter (Figure 3A). Golgi bodies are common, the majority being situated close to the nucleus (Figure 3B). There are also large numbers of mitochondria dispersed throughout the cytoplasm.

Histochemical tests (Table 1) revealed a strong positive reaction of gland cells to mercuric bromophenol blue and buffered azure A indicating the presence of basophilic proteins, but these did not appear to be concentrated in any specific region of the cytoplasm. Cells also stained positive for neutral mucopolysaccharides, although glycogen and lipid reserves were not found.

Although gland cells are of one type, each exhibits structural changes associated with apocrine secretion. Four distinct stages have been identified (Figure 4B). In stage 1, cells are columnar with small, densely-stained granules surrounding the basal nucleus. In stage 2, cells elongate and widen apically, the granules enlarge and migrate towards the apex, whilst the nucleus remains unchanged. Stage 3 is characterized by distension of the cell apex into a balloon-like shape and accumulation of granules apically. Stage 4 involves the formation of secretory bundles (within which are the accumulated granules) and their subsequent budding from the cell into the gland lumen.

Mercuric bromophenol blue was too insensitive to detect changes in concentration of proteins (digestive enzymes) that may be produced by glands in response to tidal and associated feeding cycles. Structural analysis was therefore used to determine whether

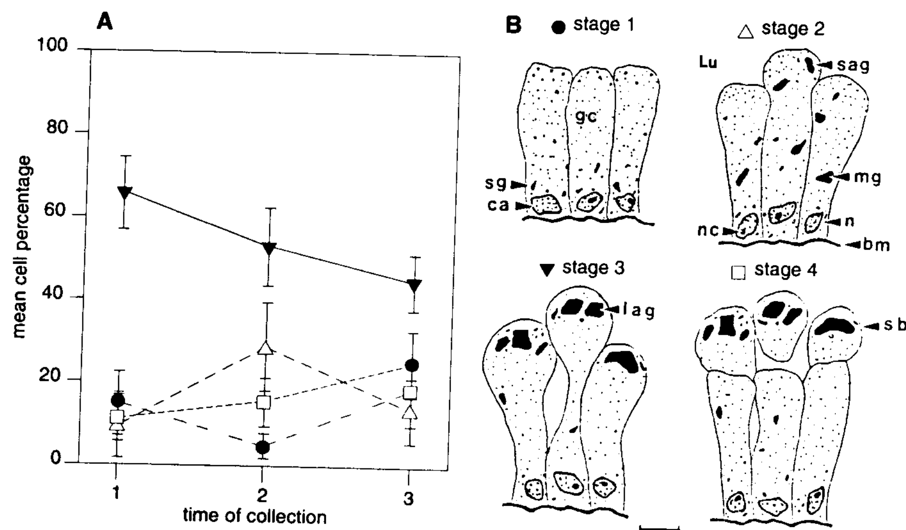

Figure 4. (A) Relationship between the percentage of secretory cell stages within the digestive gland and time of collection during a tidal feeding cycle. Standard errors around the mean percentage for each time interval are shown. Note that cell stages (Figure 4B) represent the key applicable to this graph. (B) Characteristic cell stages of the apocrine secretion cycle within the digestive glands of *Tetraclita squamosa*. Stage 1: production of small basal vesicles/granules. Stage 2: apical movement and enlargement of secretory granules. Stage 3: apical accumulation of secretory granules. Stage 4: apocrine secretion of granules and movement of secretory bundles into the cell cytoplasm. Scale bar: 10 μm. bm, basement membrane; ca, chromatin aggregation; gc, granular cytoplasm; Lu, lumen; lag, large apical granule; mg, median granule; n, nucleus; nc, nucleolus; sag, small apical granule; sb, secretory bundle; sg, small granule.

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gland cell secretion (measured as percentage of stages 1-4 within glands) was correlated with feeding activity (time of tidal collection). Figure 4A suggests there was no significant change in the mean percentage of each cell stage with time after feeding as illustrated by the large standard errors. This is substantiated by the high probability values ($P>0.05$) of the Kruskal-Wallis one-way analysis of variance summarized in Table 2. Thus, the null hypothesis that secretion was not significantly correlated with time of collection after feeding was accepted. This poor correlation between the apocrine secretion cycle and tidal feeding activity was further supported by the absence of structural change in gland cell organelles involved in protein secretion and the presence of digestive fluid in the

Table 2. *Kruskal-Wallis one-way analysis of variance on the significance of changes in percentage of secretory cell stages within digestive glands of* Tetraclita squamosa *during the tidal (feeding) cycle* ($\alpha=0.05$)

Source of variation	df	F	P	H_o
Cell stage 1	29	0.83	0.447	accept
Cell stage 2	29	1.64	0.212	accept
Cell stage 3	29	1.37	0.270	accept
Cell stage 4	29	1.07	0.356	accept

Table 3. *Specific activities of digestive enzymes in the alimentary tract regions of* Tetraclita squamosa

Enzyme	Digestive glands pH5	Digestive glands pH7.2	Foregut pH5	Foregut pH7.2	Midgut pH5	Midgut pH7.2	Hindgut pH5	Hindgut pH7.2
'General proteases'	7.6	2.55	0.88	0.54	4.54	2.68	2.77	2.48
Trypsin	0.9		0.22		0.26		0.21	
α-amylase	0.01	0.047	0.07	0.024	0.04	0.037	0.036	0.033
α-D-glucosidase	11.4	8.75	3.4		5.1	2.67	2.75	4.9
β-D-glucosidase	100	8.33	10.23	7.6	14.85	5.2	10	0.7

Results are the mean of replicate assays measured in units per mg barnacle protein, where units = nmoles glucose produced per hour (glucosidase assays) or change in spectrophotometric absorbance per hour (rate of hydrolysis). Blank cells = no activity.

Table 4. *Qualitative determination of carbohydrate hydrolysis by enzymes within the alimentary tract regions of* Tetraclita squamosa

Carbohydrate	Alimentary tract region: Digestive glands	Foregut	Midgut	Hindgut
Maltotriose	+++	+++	+	+
Amylopectin	++	++	++	++
Laminarin	++	++	+	+
Carboxymethyl cellulose	+++	+++	++	++
Cellobiose	+	+	+	+
Fucoidin	-	-	-	-
Chitin	-	-	-	-
Sodium alginate	-	-	-	-

Activity is expressed on a scale of +++ to -, where the former represents significant substrate hydrolysis and the latter no substrate hydrolysis

alimentary tract at all stages of the secretion cycle, suggesting digestive processes are continuous in *T. squamosa*.

A range of digestive enzymes was detected in the alimentary tract and digestive glands of *T. squamosa*. Quantitative and qualitative measurements of carbohydrase and protease activities are summarized in Tables 3 and 4 respectively. As shown in Table 3 specific activities were low in comparison to those reported in decapods by Kristensen (1972) and DeVillez (1975). Greatest enzymic activity was located in the digestive glands. All enzymes exhibited considerably higher activity at the acidic pH, particularly trypsin (no neutral activity) and β-D-glucosidase. Digestive tract fluid was slightly acidic at pH 6·2.

DISCUSSION

The alimentary tract and digestive glands of *Tetraclita squamosa* are morphologically similar to those of temperate cirripede species (Törnävä, 1948; Rainbow & Walker, 1977). Differences in histological complexity of digestive glands between cirripedes and malacostracans reflect their functional status. Gland cells of malacostracans, in particular decapods, are highly differentiated, each cell type performing a specialized role in digestion. These include synthesis and secretion of enzymes, nutrient absorption and intracellular digestion, storage of metabolic reserves (lipid/glycogen) and excretion of wastes (Gibson & Barker, 1979). In contrast, there appears to be only one cell type within the digestive gland of *T. squamosa*, with histochemical, cytological and biochemical evidence suggesting the glands are primarily involved in the synthesis and secretion of both mucus (mucopolysaccharide) and digestive enzymes.

Histochemical detection of neutral mucopolysaccharide and basophilic protein within the gland epithelia and lumen implied cells synthesize mucus and possibly digestive enzymes. Cytological features of the cells, namely abundant RER, Golgi bodies and mitochondria, provided evidence for this latter role. Rough endoplasmic reticulum is involved in the synthesis of proteins (translation), which are subsequently modified and packaged into secretory vesicles for cytoplasmic transport by the Golgi bodies (Figure 3B). Mitochondria are responsible for the production of adenosine triphosphate (ATP), a crucial energetic requirement of protein synthesis (Carr & Toner, 1982). Quantitative biochemical analysis detected the highest concentration of enzymes within the digestive glands, demonstrating that these glands are the primary site of enzyme synthesis in *T. squamosa*. In contrast, only the F-cells of decapod digestive glands are involved in the synthesis and secretion of enzymes (Vogt *et al.*, 1989).

The absence of glycogen and lipid reserves within the digestive glands suggests they do not contain significant energy stores. These reserves were found deposited within the connective tissue surrounding the midgut, as was the case in temperate cirripede species studied by Rainbow & Walker (1977).

It is likely that digestive enzymes are released into the foregut by apocrine secretion. Rainbow & Walker (1977) postulated a similar mechanism in temperate cirripedes, although individual stages were not described. Although vesicles associated with Golgi bodies have been observed in *T. squamosa* (Figure 3B), it was not possible to follow a

sequence of formation to prove conclusively that apocrine secretion occurs. However, when substantiated, this secretory mechanism would be unique in the Crustacea, as merocrine secretion has only been reported thus far in decapods (Al-Mohanna *et al.*, 1985b; Vogt *et al.*, 1989) and cladocerans (Elendt & Storch, 1990).

Examination of cell structure revealed that four cellular stages characterize the apocrine secretion cycle. The absence of significant changes in the proportion of each cell stage during the feeding cycle (Figure 4A) suggests that digestive glands in *T. squamosa* secrete enzymes continuously, irrespective of feeding. This is supported by the constant presence of digestive fluid in the alimentary tract which indicates digestive processes are also probably continuous.

This secretory and digestive continuity reflects the biochemical processes occurring within *T. squamosa*. This species ingests a wide range of planktonic organisms, many of which are composed of complex carbohydrates and proteins. For complete hydrolysis of these dietary components a number of digestive enzymes is required, each with appropriate catalytic periods. As enzyme activities are low in *T. squamosa* (Table 2), continuous glandular production ensures effective hydrolysis is achieved within the short 5-7 h tidal feeding period of *T. squamosa*. Comparable studies have not been reported for crustaceans, although Merdsoy & Farley (1973) found a similar secretory strategy in the digestive glands of the gastropod *Littorina littorea* (L).

In agreement with studies on proteases in *Balanus nubilus* (DeVillez & Buschlen, 1967; DeVillez, 1975), the trypsin-like enzyme in *T. squamosa* was active at acidic pH and had no activity at neutral pH. Indeed, all other enzymes were more active at acid pH and only marginally active at pH 7, which supports the statement made by Van Weel (1970) that the majority of crustaceans secrete digestive enzymes with acid pH optima. The acidity of the digestive fluid of *T. squamosa* ensures that enzymes are optimally active *in situ*. Although chymotrypsin and carboxypeptidases A and B could not be detected here, or in crayfish (Zwilling & Neurath, 1981) and shrimp (Galgani *et al.*, 1984), it is likely that enzyme equivalents are produced for complete protein degradation to occur. Further studies using a range of substrates are needed to investigate these negative results.

Flagellates and diatoms represent the largest proportion (52%) of total plankton ingested by *T. squamosa* (Hunt & Alexander, 1991). Thus the broad range of carbohydrases produced, including polysaccharases and glucosidases, suggests many of their constituent carbohydrates can be hydrolysed, at least to some extent, by the barnacle. Algae make up 12% of ingested material (Hunt & Alexander, 1991) so the production of cellulase and laminarinase indicates that the cell wall can be degraded, and laminarin, the main storage polysaccharide of some brown algae and diatoms, can also be hydrolysed (Bull & Chesters, 1966; Percival, 1970). Cellulase has been reported in many decapods and amphipods (Elyakova, 1972; Monk, 1976) but has not been previously detected in cirripedes. This may be because early assay techniques using insoluble substrates were too insensitive, whereas this study used a water soluble derivative, carboxymethyl cellulose. Cellulase production in *T. squamosa* is most likely endogenous as gut contents and any associated cellulolytic microflora were removed prior to protein extraction.

Negligible hydrolysis of cellobiose into its constituent glucose residues suggests only

small quantities of β-(1-4) glucosidase are produced by *T. squamosa*. However, as cellulose was hydrolysed into glucose and cellobiose it was assumed that a sufficient amount of this enzyme must be secreted in order to utilize the cellulose. Poor hydrolysis of sodium alginate and fucoidin indicates that their respective enzymes may not be produced by *T. squamosa*. Although chitinase has been reported in temperate cirripedes (Harnden, 1968; Kristensen, 1972), no hydrolysis of this structural polymer was detected in *T. squamosa* digestive tract extracts. This is unusual as this species ingests crustaceans, but appears to lack enzymes to digest the exoskeletons which contain chitin.

Detection of the polysaccharases α-amylase (α-(1,4) glucanase) and maltase (α-(1,4) glucosidase) indicates that *T. squamosa* can hydrolyse starch grains which form the major storage polysaccharide in dinoflagellates and green flagellates (Clayton & King, 1981). α-amylase activity has been demonstrated in the majority of crustaceans investigated (Dall & Moriarty, 1983).

The broad range of carbohydrases and proteases detected reflects the diversity of zooplankton and microplankton ingested by *T. squamosa*. This relationship may contribute to the successful colonization of intertidal regions by this and other barnacles, as not only can *T. squamosa* efficiently extract and ingest a wide range of food from the water, it is also capable of digesting and utilizing many of their dietary components for metabolic, reproductive and somatic energy requirements.

These investigations have highlighted major weaknesses in the knowledge of cirripede enzyme biochemistry, stemming from use of insufficient amounts of protein extract which has resulted in poor characterization of many digestive enzymes and a subsequent lack of quantitative data. Future research must focus on rectifying these problems before a comprehensive understanding of digestive physiology in both temperate and tropical cirripede species is assured.

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