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CHARACTERIZATION OF THE DIGESTIVE TRACT OF GREENLIP ABALONE, *HALIOTIS LAEVIGATA* DONOVAN. II. MICROENVIRONMENT AND BACTERIAL FLORA

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ABSTRACT Microelectrodes were used to measure pH and dissolved oxygen within the gut environment of adult greenlip abalone (145 to 160 mm), *Haliotis laevigata* Donovan. Oxygen levels were found to be below the limit of detection for the oxygen microelectrode (0.38 mg $DO.L^{-1}$), suggesting either microaerophilic or anaerobic conditions. The pH profile of the gut revealed a decrease from the external environment (pH = 8.20) to pH 5.31 within the crop, increasing through the intestine to 6.64 in the rectum. Enrichment cultures of bacteria from within the abalone gut revealed mostly isolates from the family Enterobacteriaceae. These isolates occurred throughout all regions of the abalone gut, and almost all showed hydrolytic ability for one or more carbohydrates. *Cytophaga* spp. isolates appeared from esophageal and intestinal enrichments of the digestive tract and were all capable of both carboxymethylcellulose and agar hydrolysis. A decrease in diversity of bacterial types in the stomach, crop, and style sac corresponded with reduced pH

KEY WORDS: Abalone, *Haliotis laevigata*, digestive tract

INTRODUCTION

The gastrointestinal tract is a microenvironment that has been examined in marine invertebrates in terms of pH (Mathers 1974) and dissolved oxygen or redox potential (Plante and Jumars 1992). As well as having a significant effect on enzyme activity, high or low pH can favor maintenance of symbiotic microbes (Plante and Jumars 1992). The selective nature of the microenvironment directly influences microbial composition and activity. In turn, it is possible for the microorganisms, through their metabolism, to modify the microenvironment.

The most commonly reported association between microbes and invertebrates involves the ingestion of bacteria (e.g., Garland et al. 1985, Vitalis et al. 1988). Bacterial associations with digestive tracts of marine animals have revealed a restricted range of microorganisms, suggesting the existence of strong selective pressures within the gut that result in characteristic gut microflora (Unkles 1977, Sochard et al. 1979, Tall and Nauman 1981). Studies on the bacterial flora of digestive tracts require an understanding of the microenvironment in order to mimic these conditions in culture. Microelectrodes are ideally suited for studying, *in situ*, some aspects of the physiology of undisturbed microbial communities, such as microbial respiration (Revsbech and Jørgensen 1986).

Nearly all terrestrial herbivorous animals have one or more parts of the digestive tract expanded into an organ that accommodates a microbial population valuable in the digestion of food, for which the host animals do not necessarily produce the correct complement of enzymes (McBee 1971). The activity of such bacteria often benefits the host through cellulose breakdown, nitrogen fixation, increased host resistance to toxins, or preconditioning of food (Harris 1993). In some herbivorous animals, there exists a specific fermentation organ, in which food (cellulose) is subjected to highly reduced conditions arising as a result of microbial metabolism. Abalone are aquatic herbivores. If an analogy can be drawn between herbivorous animals that ferment and abalone in terms of microbial cellulase activity, then the conditions prevalent in these fermentation chambers should also be repeated. These conditions would include a highly reduced environment in which oxygen has been removed. The most effective means of detecting these conditions is with microelectrodes because of their small size, accuracy, and sensitivity. Oxygen depletion as seen within the digestive tract of vertebrates does occur in some aquatic deposit feeders (Plante and Jumars 1992).

Less is understood about the relationships that microbes have with invertebrate hosts than with vertebrates, with the exception of the cellulose-degrading bacteria found within Teredinidae (Bivalvia) (Morton 1978). Associations between microbes and aquatic invertebrates have been reviewed by Harris (1993). If not digested, microbes can travel the length of the gut and pass out unaffected, or may proliferate in a favorable region of the gut. Attachment often leads to the development of residential populations. The importance of the role these microbes play is unclear. Vitalis et al. (1988) found that bacteria that degraded algae contributed significantly to the nutrition of the sea-hare, *Aplysia* spp. However, Galli and Giese (1959) described several isolates from within the gut of the herbivorous aquatic snail, *Tegula funebralis*, few of which could degrade either agar, alginic acid, or carrageenan.

Algal carbohydrates (Table 1) have been used as substrates to examine the role of microbes in aquatic herbivore digestive physiology. Alginate lyase, amylase, cellulase, agarase, laminarinase, carrageenanase, and b-1,4-glucanase activities have all been evaluated (Galli and Giese 1959, Vitalis et al. 1988, Harris 1993, Sawabe et al. 1995, Erasmus et al. 1997). Bivalves that exhibit cellulase activity have been shown to possess a cellulolytic microflora (Crosby and Reid 1971). Other bacterial strains isolated from aquatic invertebrate guts have shown agarase, protease, lipase, laminarinase, amylase, alginase, and chitinase activities (Harris 1993).

Most commonly, facultatively aerobic bacteria have been identified from the digestive tracts of invertebrates (Galli and Giese 1959, Prim and Lawrence 1975, Musgrove 1988). However, few attempts to isolate strict anaerobes have been made (Harris 1993, Sawabe et al. 1995). In vertebrates, facultative aerobes are present, but in lower numbers than other types. The activities of these facultative aerobes quickly deplete the oxygen within the digestive tract, thus providing favourable conditions for growth of obligate anaerobes.

TABLE 1.

Common polysaccharides found in some marine algae (Kreger 1962, McCandless 1981, Craigie 1990).

Algal Division	Polysaccharides	
	Storage	Structural
Rhodophyceae	Floridean starch	Cellulose, agar, carrageenan, mannans
Chlorophyceae	Starch	Cellulose, xylans
Phaeophyceae	Laminarin	Cellulose, alginic acid, fucoidan

In aquatic invertebrate guts, bacteria can occur within the esophagus, the stomach, intestine, midgut, style sac, cecum, and hindgut (Harris 1993). In bivalves, the hindgut is the most heavily colonized region (Harris 1993). Accumulation of bacteria in the hindgut of bivalves occurs because of the extended passage time of food (Prieur et al. 1990). Bacteria have doubling times that range from 15 minutes up to several days, so passage times of up to 3 days through bivalve guts are sufficient for adapted bacteria to survive and grow (Prieur et al. 1990). Observations of blacklip abalone, *H. rubra*, indicate that feces are produced up to 7 days after feeding (Wee et al. 1992), suggesting ample time for bacterial colonization. Similarly, the relatively long intestine in abalone, with numerous folds and grooves (Campbell 1965), provides ample surface for bacterial colonization (Harris 1993). However, Harris et al. (submitted) found relatively few bacteria within the gut of *H. laevigata*.

The abalone digestive tract contains several functional regions through which there is a continuous, one-way flow of ingested material (Harris et al. submitted). Characterization of the microenvironment of these areas would enhance understanding of the microbial and physiological processes occurring within the digestive system of abalone. The nature of the microbes from the greenlip abalone, *H. laevigata*, also requires characterization to determine their potential importance to the digestive physiology of the host. The purpose of this study is to determine the physical conditions within the gut of the greenlip abalone and to examine the ability of microbes isolated from the gut to digest algal carbohydrates. This information complements another study on the gut structure of this species (Harris et al., submitted).

MATERIALS AND METHODS

Maintenance System

Adult greenlip abalone (135 to 185-mm length) were collected from several locations in northern Tasmania, 40° to 41°50′S, 146°50′ to 148°50′E (Petal Point, Foster Islands, Port Sorell, and Flinders Island) and maintained in recirculating systems. Macroalgae were collected in southern Tasmania, 42°50′ to 43°50′S, 147°50′ to 148°E (Blackman Bay and Port Arthur). Macroalgae of the genera *Polysiphonia* sp., *Ulva* sp., and other epiphytic algae associated with the macroalgae *Amphibolus* spp. collected by divers were used as food for the abalone (Harris et al. 1998). The algae were added to the maintenance tank and left for 10 to 14 days. To remove algal debris, tanks were siphoned every second day. A diatom film, which was grazed by the abalone, developed within the tank during the study period.

Preparation of Abalone for Experiments

Abalone were removed from the maintenance tank with either a commercial abalone iron, a warm water siphon, or a flat plastic spatula with grease. Abalone were anesthetized in 1 mL/L of ethyl p-aminobenzioc acid (benzocaine) solution for 15 minutes to prevent any movement. The stock benzocaine solution was made up from 100 g ethyl p-aminobenzoic acid dissolved in 1 L of 95% alcohol (Hahn 1989).

Microprobe Analysis of the Abalone Digestive Tract Microenvironment

Seven adult abalone, 145 to 160-mm, were used for pH analysis. Two of these were also used for determining dissolved oxygen levels in the gut. Once anesthetized, the abalone were removed from their shells, and the integument was removed to expose the gut (see Harris et al. 1998). All measurements were recorded after the electrodes were fully inserted into the lumen and the electrode response stabilized, which took less than 4 seconds.

The pH microelectrode was a MI-413 microcombination pH electrode in an 18-gauge needle (Microelectrodes Inc., New Hampshire, USA). It was connected to a Hanna Instruments (HI 9017) microprocessor pH meter, accurate to ±0.0001 mA. The pH electrode was calibrated with buffered solutions of pH 7.00 and pH 4.00, at 20°C. pH measurements were performed at 18 to 20°C and 35.5 ppt.

The dissolved oxygen probe was a Clark-type oxygen microelectrode (OME) with guard cathode (Diamond General Corp., MI, USA). It was used with a Keithley 485 autoranging picoammeter. The dissolved oxygen electrode was prepared for calibration by immersion in air-saturated water for 30 min. The current output was measured for 1 hour after saturating aquarium water of 35.5 ppt and held at 16.1 ± 0.05°C with N_2, air or O_2. The electrode had good linearity in its response and a low stirring effect of 1.2%, which was considered negligible (Revsbech and Jørgensen 1986). Before testing each animal, water samples were taken for Winkler analysis to check electrode calibration. Electrodes were held in position using a micromanipulator (Maerzhauser, Germany), attached to a heavy stand (Fig. 1). Any lateral stress on an OME may cause it to crack, so the abalone were held in position by pinning them to a soft polystyrene base. The base was fixed to a wire rack and positioned within a small (30 × 30 × 20 cm) aquarium con-

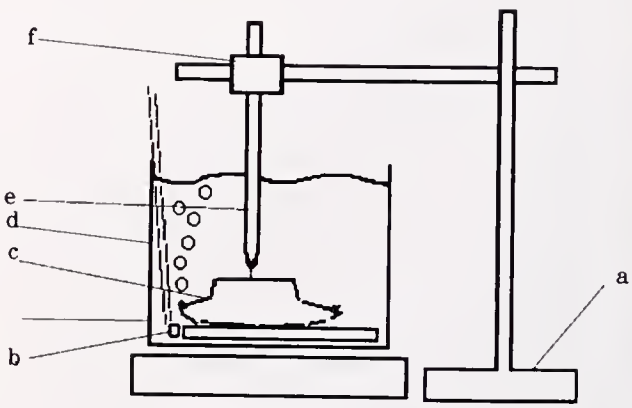

Figure 1. Experimental setup (a = 15 kg stand base; b = airline; c = abalone; d = seawater aquarium; e = dissolved oxygen OME; f = micromanipulator).

taining seawater. The gut wall was punctured with the electrode, which was then inserted into the lumen. Positioning the OME in the lumen was achieved once the gut wall slid up the probe.

OME's give an output in pA which is directly related to the partial pressure of oxygen. Because temperature and salinity were constant, the dissolved oxygen concentration was calculated as:

$$\text{DO sample} = \frac{\text{I sample}}{\text{I standard}} \times \text{DO standard}$$

(I sample = current output in pA; I standard = current output from aquarium water; DO standard = DO of aquarium water as measured by Winkler titration; DO sample = DO of sample in mg/L.)

The limit for detection by the OME was 15 pA, or 0.38 mg $DO.L^{-1}$. The oxygen saturation can be calculated by dividing the calculated concentration in mg/L by the saturated concentration of oxygen at 16.1°C and 35.5 ppt, which is 7.93 mg/L.

Bacterial Flora of the Abalone Digestive Tract

To obtain bacterial samples from the abalone, sections of the digestive tract (postesophagus region 1, crop, stomach, style sac, and intestines) were excised with scissors, scalpel, and tweezers (Harris et al. 1998) and placed into each of three enrichment broths (CarboxyMethylCellulose (CMC), starch, or agar). Smaller sections, such as the esophagus, were sampled as whole gut sections; whereas, larger areas, such as the stomach, required the removal of one wall. Before use, these broths were boiled for up to 30 minutes to remove dissolved oxygen, then placed in an ice-bath to cool rapidly. Enrichments were performed in anaerobic and microaerophilic conditions according to methods modified from Lewis et al. (1992). Carbohydrate enrichment broths were incubated at 21°C and examined on the third day.

Loops of enrichment broth were transferred to solid media containing either starch, agar, or CMC. CMC and starch plates were made using modified seawater, agar, and vitamins (SWAV) medium with agar at 10 g/L and CMC or starch at 10 g/L. pH concentrations of enrichments broths and solid media were adjusted to 6.5 for esophagus and intestines II and IV, and 5.5 for crop, stomach, and style sac samples. Plates were incubated in anaerobic and microaerophilic conditions at 21°C and examined after 4 days. Visible colonies were subcultured onto the same media to purify the isolates. Bacteriological peptone and yeast extract were omitted from the (SWAV) medium of Lewis et al. (1992) to enable single carbon sources (starch, agar, or CMC) to be added.

CMC was dissolved in distilled water before adding to seawater. CMC degradation was detected visually by observing clearance zones around colonies. Localized clearance of the medium was taken as hydrolysis. Hydrolysis on agar plates was seen by a lowering of colonies into the agar. Combined ingredients for solid media were autoclaved at 121°C and 15 psi for 15 minutes.

Tests performed on pure isolates were: gram reaction and cellular morphology, colonial morphology, OF test, Craigie tube motility test, catalase, oxidase, and susceptibility to the antibiotic 0/129 (150 μg) (Oxoid). Organisms found to be fermentative were tested against this antibiotic for presumptive *Vibrio* spp. identification. All three media types were tested for their effect on the catalase and oxidase reactions and were found to have no effect.

Statistical Analysis

Data were subjected to single, fixed factor analysis of variance (ANOVA) after meeting assumptions of normality using the Shapiro–Wilk test (Zar 1996) and homogeneity of variance using Cochran's test (Underwood 1981). Multiple comparison of means (Tukey–Kramer HSD) was performed on data that showed a significant ANOVA result (Sokal and Rohlf 1995). All analyses were conducted using JMP 3.0 software (SAS Institute).

RESULTS

Microenvironment of the Abalone Digestive Tract

Readings below the limit of detection for the OME (0.38 mg $DO.L^{-1}$) indicated that conditions were, at least, microaerophilic. This was consistently found throughout the length of the digestive tract, with little variation evident. All the dissolved oxygen concentrations were calculated to be ≤5.7% oxygen saturation at 16.1°C and 35.5 ppt. The crop was significantly ($p < .05$) more acidic than the esophagus and the intestines (Table 2).

Bacterial Flora of the Abalone Digestive Tract

The attempt at isolating anaerobic bacteria produced few organisms. The microaerophilically incubated plates showed growth after 4 days incubation and were subcultured after 7 days. Approximately three bacterial types were evident on each plate for a total of 51 isolates.

Subculturing revealed both pigmented and nonpigmented colonies, mostly as Gram-negative rods. Strains showed both negative and positive catalase reactions, and all strains examined were oxidase negative. Physiological types included mostly fermentative reactions (36 isolates), although no reaction (13 isolates), and oxidative reactions (two isolates) were also observed. Only two isolates were obligate microaerophiles, both being members of the family Enterobacteriaceae; the remainder were facultatively aerobic. Most fermentative strains were resistant to 0/129, although six isolates showed inhibition zones ranging up to 20 mm.

Most of the microbial isolates showing positive hydrolytic activity occurred within the oesophagus (11 isolates) and intestines (17 isolates). Fewer isolates were recovered from the crop, stomach, and style sac. Hydrolytic activity varied among the isolates (Table 3), being prevalent among those isolates identified as *Cytophaga* spp. and Enterobacteriaceae. Degradative ability on agar and CMC was common among the isolates, although no isolates were observed that could degrade both starch and agar.

TABLE 2.

pH profile of the digestive tract of greenlip abalone, *H. laevigata*.

Sampling Site	Mean ± SE	*n*
Esophagus	6.20 ± 0.16^{a}	8
Crop	5.28 ± 0.08^{b}	8
Stomach	$5.53 \pm 0.10^{a,b}$	8
Style sac	$5.49 \pm 0.12^{a,b}$	7
Intestine II	5.80 ± 0.12^{a}	8
Intestine III	6.34 ± 0.04^{a}	7
Intestine IV	6.65 ± 0.06^{a}	8
Intestine V	6.64 ± 0.04^{a}	3

Means sharing a common superscript are not significantly different ($p > .05$).

TABLE 3.

Genera, location, and hydrolytic activity of bacterial isolates from the digestive tract of *H. laevigata*.

Site	Bacterial Groups	No. Isolates	Polymer Degrading Activity[a]		
			CMC	Agar	Starch
Esophagus	Enterobacteriaceae	6	4	3	2
	Cytophaga	4	4	4	
	Alteromonas	1	1	1	
Crop	Enterobacteriaceae	6	4	3	
	Aerococcus	2	2		
Stomach	Enterobacteriaceae	6	3	1	3
	Neisseriaceae	1	1		1
Style sac	Enterobacteriaceae	6	4	1	2
	Neisseriaceae	1			
	Alteromonas	1			
Intestine II	Enterobacteriaceae	6	5	1	3
	Alteromonas	1		1	
	Listeria	1			1
Intestine IV	Enterobacteriaceae	4	2	1	
	Cytophaga	3	3	3	
	Acinetobacter	1	1		1
	Aerococcus	1			
Total		51	34	19	13

[a] Number of isolates showing positive hydrolytic activity.

DISCUSSION

Within the abalone gut, dissolved oxygen levels were below the detection level of this OME and the gut should, therefore, be regarded as anoxic, or at least microaerophilic. Low dissolved oxygen levels, similar to those found within the abalone digestive tract, have been reported in other invertebrates. Plante and Jumars (1992) found that even within the digestive tracts of deposit-feeders known to have consumed oxygenated sediment, oxygen levels were similar to animals known to have consumed anoxic sediments. From this, Plante and Jumars (1992) proposed that the low dissolved oxygen levels were attributable to biological or chemical processes in the foregut that quickly consumed added oxygen, with the gut contents effectively acting as an oxygen sink.

The low oxygen tension and weakly acidic conditions within the digestive tract of *H. laevigata* provide a selective environment. Prieur et al. (1990) reviewed the microbiology of bivalve digestive tracts and noted a higher proportion of fermentative bacteria than in the surrounding seawater. Most of the bacteria isolated from the guts of aquatic invertebrates have been facultative aerobes, although obligate aerobes and anaerobes have been reported (Harris 1993). The metabolism of facultative aerobes quickly depletes the available oxygen, thereby creating conditions favorable for anaerobic fermentation. However, anoxia is an insufficient variable with which to define microbial activity, and fermentation in particular, in an environment such as the abalone gut, because an anoxic environment may still have oxidizing conditions. Combined Eh and dissolved oxygen measurements provide a better understanding of the microbial environment (Plante and Jumars 1992).

The pH profile along the greenlip abalone digestive tract is similar to other gastropod and bivalve mollusks. The lowest values recorded are in the stomach of *Patella* sp. (5.55) (Hyman 1967), *Crepidula* sp. (6.00) (Hyman 1967), *Buccinum* sp. (5.6) (Hyman 1967), *Ostrea edulis* sp. (6.02) (Mathers 1974), and in the style sac of *Mya* sp. (4.4) (Owen 1966), although few authors have reported pH levels in the crop of mollusks. The lower pH in the crop and stomach reduces the viscosity of mucus, allowing the gut contents to mix readily. Raising pH increases the viscosity of the mucus in the intestine, helping to consolidate the loosely bound mucus string into cohesive pellets (Morton 1968). Crop contents in *H. cracherodii* are considerably less viscid than in other regions of the gut (Campbell 1965). The only direct measurement of pH within the digestive tract of abalone was described by Gómez-Pinchetti and García-Reina (1993). They measured the pH of digestive gland homogenates from *Haliotis coccinea canariensis*, and recorded values between 5.5–6.0. This suggests that the crop is the most acidic region in haliotids in general and specifically in *H. laevigata*. This organ is believed to act as a food storage and digestion organ, because both recognizable food pieces up to 3-cm long and unrecognizable food have been found (Campbell 1965). Esophageal valves restrict the movement of larger food particles from the crop into either the stomach or the stomach cecum (Crofts 1929).

Natural seawater has pH values varying between 7.5 to 8.5 (Austin 1988). The decrease in pH within the abalone gut portrays an environment that differs from relatively stable, alkaline seawater. The acidic abalone gut provides an environment that would select against organisms unable to tolerate acid pH. The genus *Vibrio*, for example, is tolerant of mildly alkaline conditions and is generally grown on media of pH 8.6 (Baumann and Schubert 1984); whereas, other marine bacteria such as *Alcaligenes* are commonly isolated in neutral pH (Kersters and De Ley 1984).

The enzyme activity peaks found in other abalone also illustrate the pH changes found throughout the gut of *H. laevigata*. Digestive activity in the esophagus of *H. rufescens* is highest at pH levels between that of seawater and 6.8 (McLean 1970). Peak alginase activity in *H. rufescens* and *H. corrugata* occurs from pH 7.4 to 7.6 (Nakada and Sweeney 1967). However, the lower pH levels found in the crop of *H. laevigata* are still within the range of pH that enables efficient amylase and protease activity in *H. rufescens* (McLean 1970), even though different enzymes with different pH optima are likely to be present in *H. laevigata*.

The stomach functions to collect food and secretions from the salivary glands, cecum, and the digestive glands (Crofts 1929), so the pH within the stomach should also be a mixture of these influences and the secretions of the stomach. Observations from *H. laevigata* indicate that the stomach has similar pH to that of the crop and style sac. In the digestive diverticula of *Haliotus* sp. (= *Haliotis*), the maximum activity of enzymes such as fucoidanase occurs at pH = 5.4 (Thanassi and Nakada 1967), a value of pH similar to that found in the crop, stomach, and style sac of *H. laevigata*.

In some invertebrates, pH and redox conditions are sometimes at unusual levels that favor association between the host and specific microbial communities (Harris 1993). The selective nature of these changes in pH imparted on the microbial communities will favor those microbes best adapted to the conditions. The different pH readings and the microaerophilic environment found in the abalone digestive tract, therefore, provide several niches for microbes to exploit and grow.

Most of the bacteria found within the abalone gut were able to degrade starch, CMC, or agar. The ability of several different isolates to degrade both agar and CMC indicates that these bacteria were capable of growth on two of the more common substrates available in this environment. Although the isolation media were

as close as possible to the conditions within the gut environment, the use of selective media can sometimes fail to detect some bacteria capable of hydrolytic activity (Harris 1993). Therefore, it is likely that there are some bacterial types present that were not isolated through the enrichment process. The larger diversity of bacteria isolated from the esophagus of the abalone, with a decrease in species in the crop and stomach, is directly related to the selective conditions of the gut environment. Dissolved oxygen and pH are two variables likely to influence microbial growth strongly in the abalone.

Bacterial genera found within the digestive tracts of bivalve molluscs include: *Achromobacter, Flavobacterium/Cytophaga, Pseudomonas, Vibrio, Corynebacterium, Arthrobacter, Escherichia, Neisseria, Streptococcus, Micrococcus, Moraxella, Acinetobacter,* and *Aeromonas* spp. (Prieur et al. 1990). Juvenile blacklip abalone, *Haliotis rubra,* have been shown to consume bacteria with coralline algae (Garland et al. 1985). These bacteria were predominantly *Moraxella,* although *Pseudomonas, Vibrio, Alteromonas,* and smaller numbers of *Flavobacterium/Cytophaga* and *Aeromonas* spp. were also present. Bacterial isolates obtained from the South African abalone, *H. midae,* showed an ability to use a range of complex polysaccharides (Erasmus et al. 1997). In terms of hydrolytic capabilities, the types of bacteria found within the abalone gut are similar to those found in the sea hare (Gastropoda), *Aplysia juliana,* (Vitalis et al. 1988). However, not all the bacteria ingested by abalone may be able to exploit the gut environment. From our study, it seems that the marine bacteria capable of growth at reduced pH are different in composition to those isolated by other authors at higher pH (Sawabe et al. 1995, Erasmus et al. 1997). Consequently, the reports of other authors may have revealed bacterial populations that are present, but not necessarily capable of contributing to the digestive ability of the host in a typical gut pH regime. It may be that the bacteria reported in this study differ from those reported elsewhere by being capable of digesting algae within the gut environment, from the wider variety of bacteria ingested by the abalone.

Vibrio spp. have been recorded as predominant microorganisms in several marine invertebrate digestive tracts (Unkles 1977, Sochard et al. 1979, Harris 1993), including abalone (Erasmus et al. 1997, Sawabe et al. 1995). It may seem surprising that so few isolates of *Vibrio* spp. were obtained from the greenlip abalone. However, *Vibrio* spp. are usually isolated on alkaline media (Baumann and Schubert 1984), suggesting growth is reduced or prevented in acidic conditions. The microbial isolates from the most acidic region, the crop, were almost entirely from the family Enterobacteriaceae, suggesting that these bacteria are well adapted to the acidic gut environment. The Enterobacteriaceae are rarely recorded from the marine environment or from the guts of invertebrates (Harris 1993). The occurrence of the Enterobacteriaceae in *H. laevigata* may represent a normal bacterial flora that specifically developed within the gut and adapted to the reduced pH and microaerophilic environment. Their presence throughout the digestive tract suggests that these bacteria may be indigenous.

Wild greenlip abalone are obligate drift algae consumers, able to consume many different types of algae (Shepherd 1973). Ingestion of diatoms, detritus, bacteria, and sand also occurs as a result of the mode of feeding (Campbell 1965). The diet fed to the abalone during this study was limited in diversity as compared to that of abalone in the wild. The restrictions this would place on microbial growth may be subtle, although some decrease in normal microbial species diversity could be expected. By restricting available food types, this may also reduce bacterial diversity. Digestive tract analysis of bacterial biota in other animals maintained in laboratory systems supports this theory, because the selective pressures imposed by the artificial environment influence the normal bacterial flora occurring in the gut of aquatic invertebrates (Sochard et al. 1979).

Seaweeds are known to have epiphytic colonies of diatoms, yeasts, and bacteria (Austin 1988), some of which have known algal cell-degrading abilities, such as *Cytophaga* spp. Mechanical breakdown of algae by the radula would release cellular contents previously unavailable to epiphytic or free-living bacteria, and this would be expected to stimulate microbial growth. However, few bacteria were seen to be associated with the gut surface of the greenlip abalone. This may be caused by the action of cilia and the presence of mucous and secretory cells (Harris et al. 1998), and the results from this study that suggest that variation in pH from the external environment may also be a factor. We obtained 44 isolates of bacteria from the gut of *H. laevigata.* These bacteria were capable of degrading algal polysaccharides at levels of pH and dissolved oxygen similar to gut values. Therefore, bacteria may contribute to *H. laevigata* nutrition. Because the bacteria do not seem to be strongly associated with the gut epithelium, then bacterial digestive activity is likely to be restricted to the lumen (Harris et al. 1998). Some output of feces still occurs several days after feeding has ceased (Wee et al. 1992), allowing the possibility for sustained bacterial activity within the intestines. The less intimate association of bacteria with bivalves as compared to terrestrial animals (Kueh and Chan 1985) also seems apparent in the greenlip abalone. Kueh and Chan (1985) suggested that, for oysters, the gut flora are mainly derived from the external environment and a more indigenous population of bacteria dominate the lower digestive tract because of selective pressures and multiplication. This situation seems analogous to that of the greenlip abalone.

CONCLUSION

The presence of bacteria within the digestive tract of the greenlip abalone, and their ability to break down algal carbohydrates at pH levels found within the gut, suggests that bacteria are capable of contributing to the nutrition of their host, although the amount remains in question. The lack of physical association of these bacteria with gut epithelium suggests a different digestive strategy to terrestrial herbivores. If bacteria contribute significantly to host nutrition, they are more likely to contribute through activity within the gut lumen. The selective pressures of the gut environment give rise to bacterial populations that are different in composition to those reported from the external marine environment.

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