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The digestion of microbial and detrital resources by an omnivorous shrimp, *Penaeus vannamei* Boone

Burgett, Jeff Montgomery, Ph.D.

University of Hawai‘i, 1994
THE DIGESTION OF MICROBIAL AND DETRITAL RESOURCES
BY AN OMNIVOROUS SHRIMP, *PENAEUS VANNAMEI* BOONE

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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ZOOLOGY

DECEMBER 1994

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DEDICATION

This work is dedicated to my parents,

M. Frances Burgett

and

Laurence M. Burgett

whose unwavering love and support have made it all possible.
ACKNOWLEDGEMENTS

Financial support for various portions of this research project were provided by The East-West Center, the Edmondson Trust, the ARCS Foundation, and Sigma Xi. The staff and management of The Oceanic Institute and Amorient Aquafarms generously allowed access to their ponds and provided Penaeus vannamei for experimentation.

This project could not have been done without the lab space, equipment and material support provided by David Karl. Fred Dobbs provided advice, expertise and lab facilities for the lipid extractions. The fatty acid analyses were performed by David Hedrick and David Ringelberg, University of Tennessee. Comments by Robert Findlay helped in interpretation of the lipid results. The HPLC used in the pigment analyses was provided by Bob Bidigare. Michael Ondrusek performed the pigment analysis and identified the peaks.

I thank Georgia Tien for her friendship, practical advice and excellent lab management. Shaun Moss, Karen Selph, Fred Dobbs, and Terri Rust gave me innumerable boosts when things looked dark.

My deepest thanks go to Rose Schilt, whose understanding, love, and faith in me helped transform struggle into success.
Amorphous detritus, composed of organic precipitates and microbial extracellular polysaccharide (EPS), is suspected to be a more important food source for marine animals than is the familiar morphous detritus formed by fragmentation of plant remains. Omnivorous juvenile stages of many marine species occupy shallow habitats where amorphous detritus is abundant. Although these animals ingest aggregates containing amorphous detritus and microbes, only the latter have been investigated as potential foods for omnivorous consumers. I tested whether a representative omnivorous shrimp, *Penaeus vannamei*, could use an abundant type of amorphous detritus, bacterial EPS, as a food source. For comparison, I measured the ability of *P. vannamei* to digest bacteria and microalgae contained in natural aggregates of amorphous detritus and sediment. Detrital aggregates and juvenile *P. vannamei* used in experiments were obtained from earthen aquaculture ponds.

Fluorescent microspheres were coated with [$^{14}$C] EPS produced by *Alteromonas atlantica* and then attached to detrital aggregates fed to shrimp. Two independent methods showed that 16% of this EPS was digested and assimilated. Amorphous detritus, unlike morphous detritus, is therefore a potentially valuable food of penaeids and other omnivores. Ingestion of this material by abundant consumers may constitute an important microbial-macrofaunal trophic link in coastal ecosystems.
Phospholipid fatty acid analysis showed that all groups of bacteria present in aggregates were digested with approximately 60% efficiency. Previous reports of 90-99% digestion of bacteria by penaeids may be based on methodological artifacts. Photosynthetic pigments, analyzed by HPLC, indicated that 63% of the diatoms in detrital aggregates were digested. When abundant in aggregates, diatoms could support growth of juvenile penaeids.

Microbes contain high proportions of protein, but EPS is mostly carbohydrate. Given the relative digestion efficiencies and the probable abundances of EPS and microbes in detrital aggregates, penaeids ingesting aggregates exclusively could encounter food energy limitation but not protein malnutrition. Detrital aggregates are nutritionally equivalent to dilute prey, and would supplement, rather than complement, prey ingested as part of an omnivorous diet. While foraging for benthic prey, penaeids consuming encountered detrital aggregates would increase their rate of food intake with little or no additional energetic expenditure for search or capture.
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CHAPTER 1. INTRODUCTION

Detritus in the benthic marine environment

Detritus is ubiquitous in the biosphere, but its complex role in food webs is poorly understood at both theoretical and empirical levels (Rich 1984, Mann 1988). Detritus can be conceived of as essentially all organic matter of biological origin that is not part of a living organism. Wetzel et al. (1972) provide a dynamic, system-oriented definition of detritus as "non-predatory losses of organic carbon from any trophic level (includes egestion, excretion, secretion, etc.) or inputs from sources external to the ecosystem that enter and cycle in the system (allochthonous organic carbon)." This definition explicitly includes dissolved material, which in some environments (e.g., the open ocean) can represent >90% of the detrital pool. Because dissolved detritus is generally unavailable to macroorganisms, zoological research has concentrated on particulate detritus. The diversity of detrital organic materials is mirrored by the variable utility of detritus to consumer organisms. In the benthic marine environment, the perceived role of detritus in animal nutrition has evolved as the responses of consumers to the wide spectrum of detrital types has been explored (review by Levinton et al. 1984).

The fragmented remains of higher plants were initially assumed to be the food source for a wide variety of obligate and facultative consumers. This type of
Particulate detritus was later found to be nearly indigestible compared to the associated heterotrophic microbes, which were therefore presumed to be supporting detritivores (Newell 1965, Hargrave 1970). Microorganisms such as bacteria and diatoms are vulnerable to digestion, but it has now become apparent that microbial biomass alone is insufficient to support the biomass of detritivores in most systems, and that considerable assimilation of detritus must occur (Cammen 1980a). A key result of numerous studies has been the realization that there is a wide spectrum of digestibility and nutritional value in detritus, depending largely on the source of the particles (Tenore 1981) as reflected in the content of indigestible fiber (Tenore 1981), interfering compounds (Valiela and Retsma 1984), and C:N ratio. For example, many invertebrates can assimilate algal detritus much more efficiently than they can the remains of higher plants (Tenore 1981).

Most research on detritivory have focussed on particulate detritus produced by fragmentation of macroalgae and higher plants. This *morphous detritus* (Bowen 1984) retains cellular structure and is readily identified in gut contents and field samples. Another type of detritus, termed *amorphous detritus* (Bowen 1984), lacks such structure and is less easily identified and characterized. A growing body of research suggests, however, that amorphous detritus could be a major component of food webs, especially in shallow coastal environments (see Mann 1988, Decho 1990 for reviews). Because this type of detritus lacks the structural carbohydrates that make morphous detritus physically and biochemically resistant to digestion by
animals, amorphous detritus is potentially more labile to consumers than is morphous detritus (Mann 1988).

Amorphous detritus is formed by two main processes: abiotic condensation of dissolved organic matter (DOM) and microbial excretion of extracellular polysaccharides (EPS). In the former process, a wide range of dissolved compounds precipitate onto inorganic particles (Bowen 1984) or air-water interfaces (Baylor and Sutcliffe 1963, Camilleri and Ribi 1986). When the precursor DOM is leachate from recently dead plant cells, the condensate may be more valuable to animals than that derived from more modified DOM such as humic acids (Camilleri and Ribi 1986). If widely ingested by consumer animals, this type of amorphous detritus would represent more efficient energy transfer to higher trophic levels than would detritus that required microbial decomposition. Despite the potential abundance of this type of amorphous detritus in certain environments (Sholkovitz 1976), studies of its use by consumers are difficult. This is partly because most DOM is difficult or impossible to characterize chemically at this time (Benner et al. 1992) and so the dissolved precursor compounds are mostly undefined. Much of the organic material in these condensates is similarly enigmatic (Bowen 1981).

The second broad category of amorphous detritus is EPS, produced by bacteria, diatoms, and some groups of macroalgae. Formed primarily of polymerized pentose and hexose sugars (Decho 1990), EPS is more clearly defined chemically than is precipitated DOM. Nevertheless, EPS displays a wide variety of physical properties, ranging from dense bacterial capsules and sheaths to loose
slimes. Because nearly all bacteria in nature produce EPS (Decho 1990), this type of amorphous detritus is ubiquitous, and in sediments and flocculent aggregates it can exceed the biomass of microorganisms (Uhlinger and White 1983). Although this extracellular material is not usually considered in bacterial productivity estimates (Paerl 1980), the proportion of bacterial metabolic energy expended in EPS production may be large (62%, Jarman and Pace 1984). If EPS is widely assimilated by consumer animals it could shunt more microbial production to higher trophic levels than is presently thought.

The two pathways leading to amorphous detritus can interact in at least four ways. First, heterotrophic bacteria which produce EPS often use free DOM for metabolic energy. Second, abiotically-produced particles can be colonized by bacteria (Camilleri and Ribi 1986), which could convert some of the precipitated DOM into biomass and bacterial EPS. Third, DOM is readily adsorbed onto EPS and may greatly increase its potential nutritional value to consumers (Rees 1976). Fourth, some of the DOM condensing on surfaces could be EPS, because EPS is suspected to be a component of the dissolved detritus pool (Paterson 1989). These interactions potentially blur the distinction between abiotically- and microbially-generated types of amorphous detritus.
Use of amorphous detritus by marine macrofauna

In shallow marine and estuarine environments, EPS may be the type of amorphous detritus most available to animals because it occurs in sediments and detrital aggregates in close association with bacteria and microalgae. In sediments EPS appears to play an important structural role (Grant et al. 1986, Dade et al. 1990, Watling 1988) and may represent the "missing carbon" in diets of deposit-feeding infauna (Hobbie and Lee 1980). The formation of detrital aggregates probably requires EPS (Biddanda 1986). These macroscopic aggregates contain microbial cells, EPS, inorganic particles, and often morphous detritus (Bowen 1987), and are found on vegetation and sediment surfaces and suspended in the water column. Although they are undersampled by most collecting techniques, detrital aggregates are known to be ingested by bivalves (Cranford and Grant 1990), fish (Lewis and Peters 1984) and benthic crustaceans (Robertson 1988).

Evidence for digestion of EPS by marine macrofauna is mixed. Deposit-feeding clams showed no uptake of labelled EPS (Harvey and Luoma 1984), but significant assimilation of EPS into tissues has been reported for a deposit-feeding holothurian (Baird and Thistle 1986) and a bacterivorous copepod (Decho and Moriarty 1990). The animals investigated in these experiments are all trophically specialized to process a single food type. Although omnivorous animals in freshwater systems have been shown to use amorphous detritus (Wallace et al. 195
the ability of non-specialist marine animals to assimilate EPS or other types of amorphous detritus has not been investigated.

If the EPS available in detrital aggregates is assimilated by abundant, unspecialized consumers, a potentially important food web linkage has been overlooked. Omnivorous juvenile fish and shrimp that inhabit inshore nursery areas can be extremely abundant. These animals tend to share a generalized feeding strategy characterized (Miller and Dunn 1980) by: 1) flexible feeding habits in time and space, 2) omnivory, 3) sharing a common pool of food resources, 4) multi-level exploitation of food chains, and 5) ontogenetic changes in diet with rapid growth. These features are assumed to be advantageous in nursery habitats where physical stresses and food abundance are spatio-temporally unpredictable (Miller and Dunn 1980). Although gut contents of these species can be dominated by detrital aggregates (Lewis and Peters 1984), these aggregates contain variable amounts of microbial biomass in addition to amorphous detritus. Thus the food resource being exploited is ambiguous. Whether amorphous detritus such as EPS is of equal or greater importance than microbial biomass to consumers of detrital aggregates depends to a large extent on the relative digestibilities of these resources.

**Focus of study**

This study was designed to determine the extent to which each of the dominant organic components of detrital aggregates (amorphous detritus and micro-
organisms) are potential nutritional resources for omnivorous consumers. To answer this question required an experimental animal that was representative of the opportunist omnivores found in shallow, vegetated habitats. The penaeid shrimp *Penaeus vannamei* was chosen because it spends its juvenile stages in mangrove estuaries and lagoons (Menz and Bowers 1980), is known to ingest detrital aggregates in nature and in culture (Hunter *et al.* 1987), and because small juveniles were easily procured. Like estuarine fishes, penaeids display omnivory and large shifts in diet during ontogeny (Dall *et al.* 1990), eventually becoming predominantly carnivorous. Penaeids are abundant in tropical and warm temperate coastal areas worldwide, and as prey for larger animals act as important food web links (Salini *et al.* 1990). In addition, much is known of penaeid nutrition and digestive physiology because of their use in aquaculture.

Using juvenile *Penaeus vannamei* as a model, I tested two hypotheses regarding the nutrition of opportunist omnivores:

1) Bacterial EPS is digested and assimilated.

2) Bacteria and microalgae in aggregates are efficiently (>80%) digested.

**Hypothesis 1: Bacterial EPS is digested and assimilated**

*by juvenile Penaeus vannamei.*

The study of digestion of amorphous detritus has been hampered by its transparent, inconspicuous nature and by lack of an easy method of measurement.
Quantification is made difficult by the heterogenous nature of amorphous detritus and by the lack of a chemical constituent common to all types that could be assayed for or labelled. Bacterial EPS was chosen as the representative type of amorphous detritus because of its relative chemical simplicity, its prevalence in detrital aggregates, and the existence of techniques to trace its digestion and assimilation (Decho and Moriarty 1990).

The null hypothesis, namely that there is no digestion or assimilation, is actually more plausible for several reasons. First, although penaeid carbohydrases are little known, digestive enzymes of the caridean shrimp *Crangon crangon* showed no activity against many polysaccharides tested, including pectin, gum arabic, carrageenan, and hyaluronic acid (Kristensen 1972), all of which have close similarities to bacterial and microalgal EPS. Second, penaeid guts do not generate the extreme pH conditions associated with digestion of amorphous detritus in fish (Bowen 1979a) and insects (Martin *et al.* 1980). Third, digestion of EPS is not universal even in invertebrates that exclusively ingest sediments and detrital aggregates (Harvey and Luoma 1984).

The first hypothesis would be falsified if EPS were fed to shrimp and no significant digestion or assimilation into tissue were found. Although EPS can be roughly quantified using chemical assays for uronic acid residues (Fazio *et al.* 1982), these are expensive, tedious and impractical for experimental use. An alternative way to estimate digestion of EPS, utilized in this study, was to attach 14C-labelled EPS to natural detrital aggregates as a tracer of digestion and assimilation. The
labelled EPS was cultured from a common marine bacterium and was apparently typical of EPS found in sediments (Dade et al. 1990, Uhlinger and White 1983).

**Hypothesis 2: Bacteria and microalgae in aggregates are efficiently (>80%) digested by juvenile Penaeus vannamei.**

Animals which eat detrital aggregates ingest microorganisms along with the EPS they produce. For animals that are able to digest amorphous detritus, growth on a diet of aggregates is dependent on high levels of microbial biomass (Bowen 1979a). If the detrital component of aggregates is indigestible by an organism, these microbes will be the only nutritional resource in the aggregates. Because bacteria and eukaryotic microalgae differ greatly in their content of specific, essential nutrients (Phillips 1984), a consumer's ability to digest these cells may affect its growth or survival.

Penaeid postlarvae (the initial benthic life-stage) feed at least partly on planktonic and epiphytic diatoms (Gleason and Wellington 1988), but the ability to ingest and digest these cells may change as growth progresses. Use of bacteria as a food resource by some species is suggested by the finding that bacteria can make up at least 14% of the organic gut contents of juvenile Penaeus merguensis (Moriarty and Barclay 1981). Experiments in which concentrated microalgae and bacteria are fed to juvenile penaeids have shown that these cells can be digested with high (>85%) efficiency (Moriarty 1976, Condrey et al. 1972). The second hypothesis is
based on these previous findings. However, the efficiency of digestion in nature could be lower, because microbial cells in detrital aggregates would be ingested under much different conditions than those present in such tests. For example, bacteria in sediments and aggregates are embedded in EPS capsules and slime layers (Moriarty and Hayward 1982, Paerl 1974), which are reduced or absent in most laboratory-cultured bacteria (Decho 1990).

To test the second hypothesis, I measured the abundance of two suites of biochemical components of microbes in natural detrital aggregates that were then fed to *P. vannamei*. By quantifying the fate of these biochemical markers, I estimated the extent to which this animal actually digests the microbes present *in situ*.

**Organization of the dissertation**

The experiment testing the first hypothesis is presented in Chapter 2. To test for digestion of EPS, I modified a technique pioneered by Decho and Moriarty (1990) for use with penaeid shrimp. I used a commercially-available bacterial clone for EPS production to facilitate comparative studies. I also developed a protocol for chemical conversion and binding of fluorescent microspheres to the EPS, without which this technique could not have been used with detrital aggregates. The results of this experiment corroborated the first hypothesis by showing significant digestion and absorption of EPS.
Chapter 3 presents the first of two experiments which measured the digestion of microbes using biochemical markers. In this study the membrane lipids of aggregate-inhabiting microorganisms were analyzed qualitatively and quantitatively. These lipids have proven to be sensitive and discriminating tools for monitoring microbial communities (Findlay and Dobbs 1993). Phospholipid fatty acids known to be constituents of defined microbial groups were used to estimate absolute and relative responses of these groups to digestion (Dobbs and Guckert 1988a). This type of analysis has not previously been used to examine digestion by crustaceans.

In the experiment described in Chapter 4 I used concentrations of pigments associated with photosynthesis to follow the fate of photoautotrophic microbes in aggregates ingested by P. vannamei. By separating and quantifying these pigments using high-performance liquid chromatography, the transformations associated with digestion could be observed and used to infer the fate of the microalgae. The results of both of these experiments indicated that efficiency of digestion of all microbial groups was much less than 80%, falsifying the second hypothesis.

In Chapter 5 I use the results of these three related studies to reinterpret the role of detrital aggregates in the diets of juvenile shrimp and other omnivores in vegetated coastal habitats. The potential value of amorphous detritus and microbes to omnivores is explored in the context of major and minor nutrient balances. Observations and tests are proposed that will help clarify the value of detrital aggregates in natural field situations and in the special environments of aquaculture ponds.
CHAPTER 2. POLYSACCHARIDE DIGESTION

Introduction

In shallow-water sediments, bacteria and benthic microalgae secrete extracellular polysaccharide (EPS), which forms part of the ubiquitous sediment matrix (Watling 1988) and is a key component of detrital aggregates (Biddanda 1986, Eisma 1986). These flocculent aggregates, which occur in suspension (Kranck 1984), attached to vegetation (Bowen 1981) and at the sediment surface (Bowen 1979a), typically consist of microbial cells, small inorganic particles, and detritus (non-living organic matter) (Bowen 1981). An extracellular organic material, EPS is a type of amorphous detritus (Bowen 1979a), a term which also includes abiotically-formed organic condensates (Mann 1988). Amorphous detritus contrasts with the more easily recognized morphous detritus (Bowen 1987) such as fragmented plant remains. It has been suggested that EPS and other types of amorphous detritus could be digestively labile sources of both organic carbon (Paerl 1974) and amino nitrogen (Decho 1990). In sediments (Uhlinger and White 1983) and flocculent aggregates (Biddanda 1985), EPS can be at least as abundant as microbial biomass. For animals that process sediments (Lopez and Levinton 1987) or facultatively ingest sediments and detrital aggregates (Ahlgren 1990a, Robertson 1988, Bowen 1979a), EPS may be an important but largely overlooked food resource (Hobbie and Lee 1980).
Some obligate marine deposit feeders can digest and assimilate EPS to some degree (Decho and Moriarty 1990, Baird and Thistle 1986), but others cannot (Harvey and Luoma 1984). No researcher has examined the resource value of EPS to less specialized consumers, even though juveniles of marine fish (Lewis and Peters 1984) and crustaceans (Robertson 1988) are known to ingest detrital aggregates in their nursery grounds. For example, juvenile penaeid shrimp forage in shallow tropical and subtropical sediments (Dall et al. 1990, p. 342) rich in EPS (Uhlinger and White 1983). These opportunistic omnivores are known to ingest detrital aggregates in the wild (Robertson 1988, Qasim and Easterson 1974) and in culture (Hunter et al. 1987) in addition to microalgae and invertebrate prey. Because penaeids grasp, taste and manipulate individual food particles (Alexander and Hindley 1985), it is unlikely that ingestion of aggregates is incidental to prey capture. What food value the various components of detrital aggregates have to shrimp is presently unknown.

In this study I investigated the ability of a commercially important shrimp, *Penaeus vannamei*, to digest EPS produced by a marine bacterium. Extracellular polysaccharide in natural systems is heterogenous and difficult to quantify (Decho 1990). No method currently exists to uniquely label EPS *in situ*. I used laboratory bio-synthesized EPS, uniformly labelled with $^{14}$C and added in tracer amounts to natural detrital aggregates, as a model for the digestion of the polysaccharide matrix present in surficial sediments and benthic detrital aggregates.
Digestion efficiency, the percentage of an ingested foodstuff that is removed during gut transit, is most easily measured by comparing ingested and egested ratios of the food to an inert tracer. The ideal tracer is inseparable from the food except by digestive processes, is not itself digested or absorbed, and is easily quantified with low error. I followed Decho and Moriarty (1990) in using polystyrene microspheres covalently bonded to EPS as tracers of digestion. The method was improved by the use of fluorescent microspheres to facilitate counting.

By attaching an assimilable radiocarbon label to an inert tracer, two independent means of assessing digestion become available. First, the ratio of label to tracer in ingesta and feces produces an estimate of digestion efficiency. Second, the proportion of the total ingested radioactivity that is either respired or retained in the tissues gives an estimate of assimilation efficiency. If all material removed from the ingesta were transported across the gut lining, these quantities would be equal. Although the former method was the focus of this work, estimates of both digestion and assimilation efficiency are presented in this study.

Materials and Methods

Biological materials

Benthic detrital aggregates and juvenile *Penaeus vannamei* were collected from earthen ponds at a commercial mariculture facility (Amorient Aquafarms, Kahuku, Hawai‘i). Detrital aggregates from the sediment-water interface settled
from natural resuspension onto submerged plastic panels and were aspirated into a collecting flask. In the laboratory the detrital aggregates were sieved through a 60 μm Nitex mesh to remove meiofauna and maintained for up to 8 days under fluorescent lights in seawater augmented with 100 µM NO₃, 10 µM PO₄ and 12.5 µM Si. These nutrients and daily resuspension preserved the palatability of the detrital aggregates to the shrimp.

Shrimp were maintained in a recirculating seawater system for up to 15 days and fed daily with detrital aggregates and their accustomed commercial feed (Rangen). Experimental animals were between 0.35 and 2.4 g in mass (39-69 mm total length) and were in molt stages C-D₁ (Robertson et al. 1987).

Production of radiolabelled EPS

Culture and purification of bacterial [¹⁴C] EPS was modified from Decho and Moriarty (1990). *Alteromonas atlantica* (ATCC 43666, American Type Culture Collection) was incubated in an 80% seawater medium containing Bacto-peptone (0.5 mg mL⁻¹, Difco), yeast extract (0.1 mg mL⁻¹), glucose (2 mg mL⁻¹) and [U-¹⁴C] glucose (0.44 MBq mL⁻¹, specific activity 9.25 GBq mmol⁻¹ D-glucose, ICN Biomedicals). This concentration of glucose, one-tenth that used in other studies (Decho and Moriarty 1990, Corpe 1970) was found to support abundant EPS production while minimizing dilution of the radiolabelled glucose. Screwtop tubes of filter-sterilized (0.2 μm) medium were inoculated with exponential-phase cells and incubated at 21 °C on a shaker for 120 hours, approximately 60 hours into
stationary phase. Temperatures above 25 °C inhibited growth. Cells in mature cultures were stabilized with formalin (0.5% final concn, v/v) and pelleted by 30 min centrifugation at 40 000 x g and 4 °C. The overlying medium containing the [\(^{14}\)C] EPS was carefully decanted, ethanol at 0 °C was added to a final concentration of 70% (v/v), and the dissolved EPS allowed to precipitate overnight at 4 °C. The EPS was then centrifuged (1 200 x g, 15 min), redissolved in deionized distilled water (DDW), and dialyzed using Spectra-Por membrane until no residual [\(^{14}\)C] glucose was detectable in the DDW bath. To ensure complete precipitation of EPS following dialysis, divalent cations (Ca\(^{2+}\) 13 mM, Mg\(^{2+}\) 3.2 mM) were added and allowed to cross-link the EPS molecules (Decho and Moriarty 1990) for 1 hr. The EPS was then precipitated with ethanol, centrifuged, redissolved in minimal DDW, frozen at -85 °C and lyophilized for storage.

**Labelling fluorescent microspheres and detrital aggregates**

Fluorescent polystyrene microspheres ("beads") were used as tracers of EPS digestion because they are biologically inert and can be easily counted with an epifluorescence microscope. Particles used to trace digestion in penaeids must be larger than 1 μm to avoid diversion into the digestive gland (Dall *et al.* 1990, p. 159). Covalent bonding of beads to EPS using carbodiimide requires amine groups on the bead surface. Because fluorescent, amine-surfaced beads of this size were commercially unavailable, I developed a procedure to convert carboxylate beads
(Polysciences YG Fluorescbrite, 2.7 µm diameter) to amine beads by linking diaminohexane to the carboxyl surface groups (Bangs 1984).

Beads in the purchased latex suspension were sonicated to insure monodispersivity, pelleted in a microcentrifuge at 13,000 x g, and washed (resuspended in new buffer then pelleted) twice in carbonate buffer (pH 9.6, 0.1 M). The beads were then washed 3 times in phosphate buffer (pH 4.5, 20 mM) and resuspended in this buffer containing 10% (w/v) 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), dissolved within 15 minutes of use. After 3.5 hr on an oscillating shaker, the beads were washed 4 times in phosphate buffer, resuspended in borate buffer (pH 8.5, 0.2 M) containing 20 mM 1,7 diaminohexane, and shaken for an additional 30 min. After 3 washes in borate buffer, the now amine-surfaced beads were sonicated and stored at 4 °C.

Beads were bonded to [14C] EPS using a similar protocol. Lyophilized EPS was first dissolved in phosphate buffer (3 mg EPS mL⁻¹), EDC in phosphate buffer was added dropwise to a final concentration of 10%, and the solution was shaken for 3.5 hours. Beads (3.2 x 10⁷ mg⁻¹ EPS) were then added and shaking continued for 30 min. Higher ratios of beads to EPS caused irreversible precipitation during later steps. Acetate buffer (pH 5, 0.1 M final concn) was added to react with any excess EDC, then ethanolamine (0.25 M in borate buffer, final concn 94 mM) was added to bind to any unreacted COO⁻ sites on the EPS. Without these additions the EPS was insoluble following the next step. The EPS-bead mixture was then precipitated in ethanol, redissolved in minimal DDW, frozen at -85 °C, and lyophilized for storage.
The proportion of $[^{14}\text{C}]$ EPS covalently bound to beads was determined by subjecting the EPS-bead mixture to fluid shear stress in a microcentrifuge. The prepared suspension were sampled for LSC, then spun for 5 min at 13 000 x g. The supernatant was then sampled, removed, the pellet washed 3 times in DDW, resuspended in the original volume and sampled. All samples (triplicate, 50 μl) were counted in a mixture of 150 μl of 0.5 $N$ HCl (to hydrolyse EPS) and 3 mL cocktail (Optifluor, Packard). Counts were corrected using an external standard and a quench curve optimized for these conditions. Subsamples of the original suspension, the supernatant and pellet were examined microscopically after staining with Alcian blue (Gurr 1966).

Benthic detrital aggregates used in feeding experiments were labelled by gently mixing with redissolved EPS-bead mixture for 30 min, then repeatedly suspending and settling the detrital aggregates in 100 volumes of seawater. The settled detrital aggregates were pipetted into another container of seawater and the process repeated in order to ensure that the $[^{14}\text{C}]$ EPS used in feeding experiments was strongly associated with detrital aggregates (Fig. 1). Following this washing procedure, tests showed no measurable dissolution of $^{14}\text{C}$ into ambient seawater over 6 hours. The EPS tracer represented less than 0.5% of the total organic matter in the detrital aggregates.
Figure 1. Detrital Aggregate with Fluorescent Beads. Photo taken using blue-light epifluorescent illumination; orange color is caused by dichroic filters. Diameter of each microsphere is 2.7 μm.
EPS digestion experiment

The digestion of EPS was investigated using a pulse-chase protocol which allowed the complete quantitative collection of radiolabelled feces, and therefore provided the opportunity to use a mass-balance approach to verify digestion calculations based on [14C] EPS:bead ratios.

The experimental apparatus consisted of an array of 1 L polyethylene beakers ("chambers") filled with 600 mL aerated seawater and kept at 24 °C in a circulating water bath. All procedures were conducted in the dark to maximize feeding behavior. A dim red headlamp was used during animal observation and manipulation. Placement of experimental replicates was randomized within the array.

Six *Penaeus vannamei* (0.83-1.3 g, mean 0.98 g) that were actively feeding on detrital aggregates were blotted dry, weighed and placed individually in experimental chambers containing 0.75 mL of detrital aggregates labelled with the EPS-bead mixture. Twenty minutes after an individual began feeding on the labelled detrital aggregates it was transferred to another chamber containing 4 mL unlabelled detrital aggregates where it was allowed to feed *ad libitum*. Preliminary tests had shown that the minimum time for gut transit was 34 minutes, and that all label (14C and beads) cleared the gut after 2.5 hours of feeding on unlabelled detrital aggregates.

Samples of the labelled detrital aggregates were taken prior to feeding and immediately after removal of the animals. After 4.5 hr in the second chamber, the
shrimp were removed and quickly frozen. The dense and compact fecal strings in the second chamber were cleaned of uneaten detrital aggregates using a gentle water stream and also frozen. Tests showed no measurable radioactivity in the residual unlabelled detrital aggregates. Frozen animals were dissected and the digestive gland, midgut, hindgut and a sample of abdominal muscle tissue were removed for LSC. Feces remaining in the gut were removed and added to the fecal sample. The digestive gland from each animal was weighed and dissolved in Scintigest tissue solubilizer (Fisher) at 50 °C for 18 hr. The muscle was weighed and solubilized as above, and the total body burden of 14C exclusive of the digestive gland was calculated using the specific activity of muscle. Although this could underestimate the specific activity of some tissues (Dall et al. 1993), the large proportion of shrimp mass represented by muscle (>60%, Dall et al. 1993) suggests that this estimate would be approximately correct.

The concentration of respired 14CO2 in the chamber seawater proved too low to measure reliably. The total 14CO2 respired was therefore estimated using a factor based on Dall et al. (1993). These authors found that at 23 °C, approximately 2.25% of the total 14C assimilated from dietary lipids was respired by *Penaeus esculentus* in 4.5 hr. Because carbohydrates such as EPS might be oxidized more rapidly than fats, 4% of the measured body burden in *Penaeus vannamei* was assumed to have been respired during this experiment.

The initial and post-feeding samples of detrital aggregates and feces were assayed for both 14C and bead numbers and the results expressed as disintegrations
min⁻¹ (dpm) bead⁻¹. For bead counts, the samples were sonicated until completely disaggregated and subsamples were removed, diluted and filtered onto Nuclepore filters (0.8 μm) prestained with irgalan black. Volumes filtered were selected to yield approximately 200 beads filter⁻¹. Eight replicate filters were prepared for each sample, mounted with Cargille A immersion oil, and frozen to prevent dissolution of the beads. All beads on the surface of each Nuclepore filter were counted under blue-light epifluorescent illumination using a compound microscope.

Simultaneously with the preparation of the bead filters, parallel subsamples were pipetted onto tared GF/F filters (Whatman), dried in a vacuum oven at 60 °C overnight and weighed. These filters were transferred to scintillation vials, moistened with 3 N HCl to hydrolyse the EPS, and left overnight. Next, Scintigest was added and the filters were incubated overnight at 50 °C to digest microbial cells. Scintillation cocktail (Cytoscint, Packard) was then added and the samples were sonicated and counted using a Packard Tricarb 4640 liquid scintillation counter. Counts were corrected for quench using an external standard and an efficiency curve designed for this protocol. Five replicate filters were processed for each sample.

Selectivity experiment

Ingestion selectivity, the ingestion of a food component in a different proportion than it occurs in the offered diet, can affect ratio-based tracer studies if the two ratio components are separable during ingestion. The requirement to collect feces in the digestion experiment meant that the EPS:bead ratio in newly-ingested
foregut contents, obtainable only by dissection, could not be examined simultaneously. A separate experiment was conducted to compare the EPS ratio actually ingested to that present in the labelled detrital aggregates. A wider size range of shrimp was used in order to test for size-dependence of any ingestion selectivity.

Using the same apparatus and general protocol as the previous experiment, 12 shrimp (0.35 g-2.4 g, mean 0.98 g) were introduced into chambers containing 75 μl of labelled detrital aggregates and observed for feeding behavior. As soon as food was visible in the foreguts the animals were removed and frozen immediately to prevent defecation or respiration of label. Samples of pre- and post-feeding detrital aggregates were taken as above. The animals were dissected while frozen and the entire digestive tract, including the digestive gland and all gut contents, were removed and solubilized in Scintiget at 50 °C for 18 hr. This solution was then sonicated and subsamples were diluted and Nuclepore filtered for microscopy as above. After addition of HCl and Cytoscint cocktail the 14C in the remainder was counted via LSC.

Selective ingestion of EPS was quantified using the forage ratio (Lopez and Cheng 1983), defined as \( FR = \frac{r}{p} \), where \( r \) is the proportion of a food in the ingesta, and \( p \) is the proportion in the offered food. Because the beads were not sequestered or absorbed during gut passage and were thus a conserved tracer, both \( r \) and \( p \) were expressed as dpm bead\(^{-1} \), rather than as proportions by weight.
Results

**Ingestion selectivity**

Ingestion selectivity was evident, with all gut contents showing higher EPS ratios (dpm bead⁻¹) than the detrital aggregates in the chambers (Fig. 2). This pattern could result from either biased ingestion of EPS containing fewer than average beads, or rejection of beads prior to or during ingestion. The latter process is consistent with the increase in the EPS ratio of the detrital aggregates following feeding (Fig. 2), which suggested that foraging activity releases beads from the EPS. Manipulation by the mouthparts likely imparted more mechanical stress than did foraging, so ingestion would have tended to increase any shear-related loss of beads from the EPS. Because the shrimp fed upon detrital aggregates with an EPS ratio between the initial and post-feeding values, the arithmetic mean of the two values for each chamber was used as the denominator in calculating forage ratios.

The distribution of forage ratios (Table 1) reflected that of the gut EPS ratios, and was similarly skewed. While most forage ratios were below 2, several were higher. The forage ratios were not significantly correlated with animal weight or total number of beads ingested (both P>0.10), and their distribution may reflect individual variation in feeding behavior. Because the forage ratio represented the degree to which a foodstuff was concentrated during ingestion, it could be used as a correction factor to estimate the ingested concentration when direct measurement of ingesta was impossible, as in the digestion experiment. The median forage ratio
from this experiment was chosen as the most representative value due to the non-normality of the distribution.

**EPS digestion**

As in the preceding experiment, foraging by shrimp during the digestion experiment caused an increase in EPS ratio in the detrital aggregates (Fig. 3), suggesting that the beads were more likely to dissociate from detrital aggregates than was the [\(^{14}\)C] EPS. The feces produced by these shrimp had higher EPS ratios than the food (Table 1), consistent with selective ingestion of EPS over beads. An alternate possibility, that some beads had been sequestered in the digestive tract, was discarded when no beads were found during microscopic examination of the digestive glands and foreguts.
Figure 2. Selectivity Experiment. Ratio of $[^{14}C]$ EPS to beads in detrital aggregates and foregut contents, $n=12$.

Figure 3. Digestion Experiment. Ratio of $[^{14}C]$ EPS to beads in detrital aggregates and feces, $n=6$. Foregut ratios estimated using median forage ratio from selectivity experiment.
Table 1. Ingestion Selectivity and Digestion Efficiency Based on $^{[14]}$C EPS: microsphere Ratios

<table>
<thead>
<tr>
<th>Value</th>
<th>Selectivity Experiment$^a$</th>
<th>Digestion Experiment$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Observed Ratios$^c$</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ingested Food$^d$</td>
<td>0.637 (0.009)</td>
<td>0.299 (0.008)</td>
</tr>
<tr>
<td>Foregut</td>
<td>1.214$^e$</td>
<td>0.564 (0.012)$^f$</td>
</tr>
<tr>
<td></td>
<td>(1.022 - 2.375)</td>
<td></td>
</tr>
<tr>
<td>Feces</td>
<td></td>
<td>0.498 (0.034) $^*$</td>
</tr>
<tr>
<td><strong>Forage Ratio</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.887$^g$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1.598 - 3.559)</td>
<td></td>
</tr>
<tr>
<td><strong>Digestion Efficiency (%)</strong></td>
<td></td>
<td>16.3 (5.52)</td>
</tr>
</tbody>
</table>

* Different from ingesta, P=0.06, Welch t-test (1-tailed)
$^a$n=12
$^b$n=6
$^c$DPM microsphere$^{-1}$; Means (SE) except where noted.
$^d$Ingested food value is mean of initial and post-feeding values.
$^e$Median (range)
$^f$Ingested food ratio x forage ratio
To calculate digestive efficiency in the presence of significant ingestion selectivity, the concentration of the foodstuff that was actually ingested must be known or estimated. As in the above experiment, the EPS ratio in the food was estimated as the mean of the pre- and post-feeding samples from each chamber. Multiplying this value with the median forage ratio yielded the estimated EPS ratio of the ingesta. Use of a constant multiplier necessarily reduced the variability of the ingesta EPS ratios below that which was probably present (Fig. 3). Because the median forage ratio was closer to the minimum than the maximum value (Table 1), the ingesta EPS ratios for this experiment were more likely to underestimate, rather than overestimate, the true value for an individual shrimp. This would have lead to spuriously low digestion efficiency estimates.

*Penaeus vannamei* did digest EPS, as shown by the trend to lower EPS ratio in the feces compared to the estimated ingesta (Fig. 3). Due to the wide variance of the feces values, the calculated digestion was of marginal statistical significance (Table 1). A mean of 16.3% of the labelled EPS was removed during gut passage. This estimate of EPS digestion is supported by the assimilation efficiency calculated from the distribution of ingested $^{14}$C (Table 2). Those data show that 16% of the ingested $^{14}$C was either retained in tissue or respired, while 84% was expelled in the feces. When the shrimp were killed 4.5 hr after feeding, 89% of the label estimated to have been assimilated was contained in the digestive gland.
Table 2. Assimilation of $[^{14}C]$ EPS Estimated from Tissue and Fecal Radioactivity

<table>
<thead>
<tr>
<th>Total $[^{14}C]$ Ingested (DPM)$^a$</th>
<th>% of Total</th>
<th>Digestive Gland</th>
<th>Other Tissues</th>
<th>Respiration</th>
<th>Assimilation Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SE)</td>
<td>26,018 (5,238)</td>
<td>84.0 (0.93)</td>
<td>14.3 (0.97)</td>
<td>1.1 (0.13)</td>
<td>0.6 (0.04)</td>
</tr>
</tbody>
</table>

$^a$Sum of feces, digestive gland, tissue and respiration counts.

$^b_n=5$. One shrimp ingested only 188 dpm and was omitted from this analysis.

Table 3. Association of $[^{14}C]$ EPS and Microspheres Before and After Centrifugation

<table>
<thead>
<tr>
<th>EPS-Microsphere Suspension</th>
<th>DPM</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPS-Microsphere Supernatant</td>
<td>24,831 (612)</td>
<td>100 (2.5)</td>
</tr>
<tr>
<td>EPS-Microsphere Pellet</td>
<td>25,316 (463)</td>
<td>102 (1.9)</td>
</tr>
</tbody>
</table>

$^a$Values are means (SE), n=3.

EPS: Bead binding

Centrifugation of the EPS-bead mixture used to label the detrital aggregates showed that most of the EPS was only loosely associated with beads and could be separated from them mechanically (Table 3). Only 2% of the EPS was apparently covalently bound to the bead surface, where it could be observed as a thin film when stained. Stained samples of my uncentrifuged mixture closely resembled the
micrograph published in Decho and Moriarty (1990), suggesting that this result was not due to differences in the protocols.

Discussion

**EPS as a food in nature**

The bacterial polysaccharide used in this experiment was digested and assimilated into tissues by *Penaeus vannamei*, demonstrating that a penaeid shrimp can utilize a common type of amorphous detritus. Although the EPS used here is well characterized (Dade *et al.* 1990) and seems typical of that found in sediments (Uhlinger and White 1983, Baird and Thistle 1986), EPS in the marine environment is heterogeneous (Ford *et al.* 1991). The assimilation efficiency of EPS occurring naturally in habitats of *P. vannamei* could therefore be higher or lower than the 16% found in this study. The large amounts of EPS available in some systems (Uhlinger and White 1983) makes it a potentially important food even if digestibility is low (Hobbie and Lee 1980). For example, resuspended sedimentary organic matter is thought to be a significant source of energy for scallops, even though the assimilation efficiency is only 9% (Cranford and Grant 1990).

Similar polymers in nature are therefore potential sources of nutrition for this species and other omnivorous fauna. The acidic stomachs of some fishes are thought to increase digestive efficiency of amorphous detritus (Decho 1990), but penaeids, like many detritivorous fishes (Bowen 1976), have nearly neutral gut pH.
and rely on enzymes to digest their food (Dall et al. 1990, p. 160). Although it is unclear which enzymes are responsible for degradation of EPS, the carbohydrates amylase (Lee and Lawrence 1982), β-galactosidase (Chuang 1990) and β-glucuronidase (Chambers et al. 1978) have been reported from Penaeus spp.

Digestive enzymes of penaeids apparently vary little among species (Lee and Lawrence 1982), so an ability to digest EPS is likely to be a general phenomenon in this genus. This ability may also allow recovery of the polysaccharide mucus produced by the shrimps’ mouthparts (McKenzie and Alexander 1990).

In shallow marine ecosystems with abundant, generalized consumers such as penaeid shrimp, EPS in detrital aggregates may transfer more bacterial and microalgal production directly into higher trophic levels than is currently thought. Stable isotope studies of saltmarshes (Peterson and Howarth 1987), mangrove lagoons (Stoner and Zimmerman 1988) and seagrass beds (Fry 1984) have indicated that microalgal production supports most consumers, including penaeids and omnivorous juvenile fishes. In these environments diatom EPS can form suspended and benthic detrital aggregates (Ribelin and Collier 1979) similar to those produced by bacterial action. Although the experiments presented here used bacterial EPS, diatom EPS is broadly similar (Decho 1990). Direct utilization of microbial EPS by macrofauna would bypass intermediate prey and increase food web efficiency. Because fragmented plant detritus is far less digestible that the EPS tested here (Tenore 1981), future research on obligate or facultative detritivory in macrophyte-
dominated marine systems should thoroughly investigate the production and utilization of amorphous detritus.

Microbial EPS is predominantly carbohydrate, with a variable concentration of intrinsic and adsorbed amino-nitrogen (proteins, peptides, amino sugars, free amino acids (Decho 1990)). For example, the EPS of Alteromonas atlantica is only 2.5% protein (Decho and Moriarty 1990), and therefore would be mainly a source of dietary energy. For strict detritivores, the digestible nitrogen content of both morphous (Tenore 1983) and amorphous (Bowen 1979a) detritus limits growth, but omnivores such as penaeids are more likely to be limited by energy intake. Provided ingestion of amorphous detritus such as EPS during foraging does not restrict consumption of encountered prey, the increased energy intake could result in higher growth rates than would a strictly carnivorous diet. A freshwater fish appears to ingest detritus along with prey for this reason (Ahlgren 1990b). When other foods are scarce or absent, even limited energy intake from amorphous detritus can increase survival rates and reduce weight loss in omnivores (Ahlgren and Bowen 1991).

The role of EPS and other types of amorphous detritus in the nutrition of penaeids in the field depends on the rate of energy intake, which is in turn dependent on the digestive efficiency, the gut throughput rate and the concentration of amorphous detritus in the diet. Although the first quantity has been estimated here, measurement of the other variables is problematic. If aggregates of low dietary energy density were provided to shrimp ad libitum in a low-stress environment, the
fecal production over 24 hours should provide an estimate of maximum throughput. Assays of the uronic acid components of EPS, though laborious, could be used to estimate concentrations in field aggregates and foregut samples with two caveats. First, polysaccharides produced by the shrimp (McKenzie and Alexander 1990) potentially confound gut measurements. Second, use of inorganic aggregate components as natural tracers to calculate EPS concentrations in gut contents or feces could be affected by the same process that caused loss of beads from the experimental EPS.

**Methodological issues**

A major limitation of the EPS:bead ratio method is the relatively small fraction of the EPS that is covalently bound to the beads. This allows an unpredictable degree of dissociation between the excess labelled EPS and the beads during foraging and ingestion. The penaeid shrimp used here apparently handled the labelled detrital aggregates much more than did the meiofaunal copepods used to test the original method. The resulting ingestion selectivity was estimated and used as a correction factor in calculations of digestive efficiency, but the accuracy of the resulting efficiency estimate was poor. In this study, the coefficient of variation for the EPS ratio estimate was 83%, compared to 13% for the mass-balance method. Clearly the close agreement of the two efficiency estimates was largely fortuitous.

Ingestion selectivity could be eliminated by removing all but the covalently bound EPS from the beads by centrifugation prior to feeding trials. In the present
case, ensuring the same $^{14}$C signal would have required 50 times more beads in the detrital aggregates, or a 50-fold increase in the radioactivity of the EPS. Because 98% of the labelled EPS is lost during centrifugation, the latter approach would be very expensive unless the number of beads mg$^{-1}$ EPS during the bonding reaction could be greatly increased.

If the problem of ingestion selectivity were solved, the EPS ratio method would provide an estimate of digestion efficiency that did not depend on quantitative collection of feces or the accurate sampling of diverse carbon pools. On the other hand, the older mass-balance method requires no chemical modification of the EPS and allows the fate of the assimilated label to be traced. Both methods require enough label to be ingested and egested to provide adequate and sufficiently replicated LSC counts of $^{14}$C.
CHAPTER 3. MICROBIAL LIPID DIGESTION

Introduction

Penaeid shrimp are common and ecologically important (Salini et al. 1990, Leber 1985) members of coastal tropical and warm temperate ecosystems. The commercial value of penaeid fisheries and aquaculture has spurred numerous studies of dietary requirements (e.g., Sedgwick 1979, Colvin and Brand 1977) as well as of diets in the wild (e.g., Cockcroft and McLachlan 1986, Robertson 1988). Ontogenetic changes in size and habitat correspond to dietary transitions from a mixture of microalgae and meiofauna (Gleason and Wellington 1988) to progressively larger invertebrate prey (Stoner and Zimmerman 1988). At all sizes, however, and especially in prey-poor environments such as mangrove creeks (Robertson 1988) and aquaculture ponds (Hunter et al. 1987), detritus and detrital aggregates (sensu Bowen 1981) are eaten, and can form the bulk of gut contents (Robertson 1988).

Whether the detrital material or the associated microbes are the important food resources is unclear, although $\delta^{13}$C analyses have shown that plant fragments contribute little or no carbon (C) to shrimp which ingest them (Stoner and Zimmerman 1988). In general, the microbial biomass in sediments and detritus is insufficient to meet the C needs of sessile deposit feeders (Levinton et al. 1984), but highly mobile and selective crustaceans may be exceptions (Robertson and Newell 1988).
In addition, microbially-derived specific nutrients may be critical for optimum growth (Phillips 1984). For example, polyunsaturated fatty acids and sterols are necessary nutrients of crustaceans that occur at high levels in some microbial groups, but not in others (Phillips 1984). Laboratory studies have shown that access to detrital aggregates containing microalgae and bacteria increases growth rates of penaeids even when high-quality formulated feed is provided in excess (Moss et al. 1992). Although experiments have demonstrated penaeids' ability to digest microbial cells (Hood and Meyers 1973, Moriarty and Barclay 1981, Condrey et al. 1972), the protocols used make the conclusions difficult to apply to natural diets.

The ability of invertebrates to digest sedimentary and detritus-associated microbes has been investigated by a variety of techniques, but few possess both reproducability and specificity. Analysis of phospholipid, ester-linked fatty acids (PLFA) provides a detailed and quantitative assessment of a microbial community's biomass and composition (Guckert and White 1988). Studies of deposit feeders using this and other techniques have shown wide differences in digestibilities of microbial groups (White et al. 1980, Dobbs and Guckert 1988a, Lopez and Cheng 1983). Although ingestion of sediment or detritus appears to be common in marine (Stoner and Zimmerman 1988, Robertson 1988, Qasim and Easterson 1974) and freshwater (Bowen et al. 1984, Bowen 1979a, Ahlgren 1990b) omnivores, omnivorous benthic fauna have not been included in studies of sedimentary
microbial digestion, and their ability to digest different types of microorganisms is essentially unknown.

The purpose of this study was to determine the degree to which Penaeus vannamei digested components of a natural microbial assemblage from benthic detrital aggregates. Juvenile P. vannamei inhabit shallow embayments on the Pacific coast of Central and South America, and are known to forage on and ingest such aggregates in the wild (Edwards 1977) and in earthen aquaculture ponds (Hunter et al. 1987).

Materials and Methods

Experimental materials and procedures

Experimental animals and detrital aggregates were obtained from commercial shrimp aquaculture ponds at Kahuku, O'ahu. Detrital aggregates, naturally resuspended from the sediment-water interface, accumulated over several days on submerged plastic panels and were collected by gentle suction. Because micrometazoan lipids would confound the analysis of microbial lipids, the detrital aggregates were sieved through 60 µm Nitex mesh to remove meiofauna. Aggregates in a layer less than 4 mm thick were kept for 4 days in shallow trays under fluorescent light in seawater augmented with 100 µM NO₃, 10 µM PO₄, and 12.5 µM Si. These nutrient levels removed the potential for nutrient stress in
microalgae and helped to maintain palatability of the aggregates to the shrimp (personal observation).

Juvenile *Penaeus vannamei* were collected from a similar adjacent pond and maintained in a recirculating seawater tank for 12 days. The experimental shrimp weighed 1.2-2.1 g (56-66 mm total length) and were all in molt stages C-D₁ (Robertson *et al.* 1987), to avoid effects of the molt cycle on feeding behavior and physiology. They were fed twice daily with a mixture of their accustomed shrimp ration (Rangen) and detrital aggregates.

The experiment was conducted in 1 L polyethylene beakers filled with 600 mL aerated seawater kept at 24 °C by a circulating water bath. All procedures were conducted in darkness to maximize feeding behavior. A dim red headlamp was used during periods of animal observation and manipulation.

Six 500 μL samples of detrital aggregates ("initial" treatment) were taken while adding 4 mL of aggregates to each of twelve beakers. Six *P. vannamei*, actively feeding on identical aggregates in a holding tank, were blotted dry, weighed and placed individually in random beakers. The six beakers without shrimp served as controls for the effects of darkness and disturbance, and were stirred every 30 min to simulate shrimp foraging. Shrimp were removed after 4 hours. The "feces" treatment consisted of all fecal material present, cleaned by repeated gentle suspension and removal of residual aggregates. Microscopic inspection showed virtually no adhering contaminants. The "dark" treatment consisted of 500 μL samples of aggregates from each control beaker. Feces and dark treatment samples
were frozen 1 hr after shrimp removal, or 5 hr after the initial samples were taken. All samples were frozen at -20 °C, lyophilized and stored at 4 °C.

**Lipid analysis**

Lipid extraction and fractionation procedures are described in (Dobbs and Findlay 1993). Extraction of samples in methanol:chloroform:50 mM PO₄ buffer, pH 7.4 (1:2:0.8 by volume) was followed by fractionation on silicic acid columns into polar lipid (primarily phospholipid), glycolipid and neutral lipid fractions. Only the first two fractions were analyzed further. Fractions were stored dry under N₂ at -85 °C until analyzed.

The fatty acid constituents of both the phospholipid and glycolipid fractions were prepared for analysis by mild alkaline transesterification (Findlay and Dobbs 1993) of the fatty acids. The resulting fatty acid methyl esters (FAME) were separated and quantified using gas chromatography (GC). Samples were dissolved in hexane containing 19:0 (see below for nomenclature) as an internal standard, and 250 μL aliquots were injected onto a gas chromatograph (HP 5890) equipped with a 60 m non-polar capillary column (Restek Rt-1) and a flame ionization detector.

The separated FAME were quantified by integrated peak area and tentatively identified by retention time relative to known standards. Further identification was accomplished using GC/mass spectrometry as described in Ringelberg et al. (1989). Because digestion lowers the organic content of feces, FAME concentrations were
normalized to ash weight rather than dry weight. Ash weights were obtained by
combusting the extracted samples at 450 °C for 5 hours.

**Fatty acid nomenclature and biomarkers**

Fatty acids and their methyl esters are named using a system of A:BωC,
where A is the number of carbon atoms, B the number of double bonds, and C is the
position of the first double bond from the aliphatic (methyl) end of the molecule.
Thus, 18:4ω3 refers to an 18-carbon chain with 4 double bonds, the first of which is
located between the third and fourth carbons from the methyl end. Double bonds
are presumed to be separated by methylene groups. All double bonds are presumed
cis unless otherwise denoted by a suffix "t" (for trans). The prefixes "a", "i"
indicate anteiso- and iso-branching, respectively. Saturated fatty acids are those
without double bonds, while polyenoic or polyunsaturated fatty acids have at least 2
double bonds.

Iso-17:0 and 17:1 could not be resolved by this system, and are presented
together. Phytol, which is not a fatty acid but a terpenoid component of chlorophyll,
eluted with both polar lipid and glycolipid and is reported as a combined value.

Fatty acids of glycolipids, the primary membrane lipids of chloroplasts and a
minor constituent of Gram-positive bacteria, have not been found useful in
characterizing diverse microbial communities. In contrast, phospholipid fatty acids
(PLFA), found in cell membranes, display a taxonomically complex distribution
among prokaryotic and eukaryotic cell types (Guckert and White 1988). Those
PLFA determined by other workers to be "biomarkers" (Findlay and Dobbs 1993, Vestal and White 1989) were used to assign treatment effects to various microbial groups.

Statistical analyses

Biomass was estimated using total PLFA, total glycolipid fatty acid (GLFA) and phytol concentrations. Box plots (Hoaglin et al. 1983) showed no outliers or consistently non-normal distributions. Because variance heterogeneity among treatments was not reduced by transformation, Welch’s robust analysis of variance (Day and Quinn 1989) was used to test for differences among the 3 treatments. In that procedure, the $W$ statistic is significant if it exceeds the critical value of $F$.

Planned comparisons among treatments were performed using Welch’s t-test (Day and Quinn 1989), which is also robust to heteroscedasticity. This test compares the difference of means to the product of $t$ and the adjusted standard error ($t \times SE'$); a difference greater than this critical value is significant. Values of $F$ and $t$ for the fractional degrees of freedom used in these tests were calculated from the respective distributions using SYSTAT (Wilkinson 1986) software.

Changes in concentration of individual PLFA from initial values were tested using Welch’s t-tests. Twenty-one tests were performed for each of two treatments. In these tests and the planned comparisons above, significance was determined using error rates calculated by the Dunn-Šidák method (Sokal and Rohlf 1981, p. 242).
Thus, to maintain a treatment-wise error rate of 0.05, each change was tested at $P<0.0024$.

Cluster analyses were used to evaluate differences among treatments and to group those PLFA with similar responses to digestion. The euclidean distance metric and a complete linkage (farthest neighbor) clustering algorithm in SYSTAT were used. The response of individual FAME to the feces treatment was evaluated to assess whether functional groups of microbes displayed coherent responses to gut passage. Percentage change from the initial mean was used to weight all fatty acids equally by removing effects of absolute abundance. Clusters were arbitrarily defined as groups with similarity values (SV) of 65% or greater.

Results

Microbial biomass

The reduction in microbial biomass during gut passage was highly significant by all three biomass measures (Table 4). The digestion efficiency of total PLFA indicated that 52% of the initial microbial biomass in the detrital aggregates was absent from the feces. The total PLFA was unaffected by conditions in the dark treatment. Total GLFA was significantly reduced in the dark treatment, so the 47% digestion efficiency of this lipid fraction could have been partly due to factors other than gut passage. The mean concentration of phytol, a component of chlorophyll $a$ and $b$, appeared to be lower in the dark treatment, but because of high variance the
Table 4. Concentrations of Lipid Fractions in Three Experimental Treatments

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Initial</th>
<th>Feces</th>
<th>Dark</th>
<th>Digestion Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLFA</td>
<td>3216 (197)</td>
<td>1542 (72.6)</td>
<td>3117 (52.9)</td>
<td>52.1 (2.3)</td>
</tr>
<tr>
<td>GLFA</td>
<td>2673 (213)</td>
<td>1392 (100)</td>
<td>2003 (100)</td>
<td>47.9 (3.7)</td>
</tr>
<tr>
<td>Phytol</td>
<td>226 (82.2)</td>
<td>140 (3.6)</td>
<td>193 (323)</td>
<td>38.3 (0.5)</td>
</tr>
</tbody>
</table>

Welch ANOVA and Planned Tests:

<table>
<thead>
<tr>
<th>Fraction</th>
<th>A</th>
<th>B</th>
<th>(W_{(2,9.5)})</th>
<th>Digestion Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLFA</td>
<td>5.73x10^-4</td>
<td>9.87x10^-8</td>
<td>147.8</td>
<td>***</td>
</tr>
<tr>
<td>I vs F</td>
<td>df' = 6.34</td>
<td>t(0.001) = 5.75</td>
<td>SE' = 209.9</td>
<td>***</td>
</tr>
<tr>
<td>I vs D</td>
<td>df' = 5.72</td>
<td>t(0.05) = 3.02</td>
<td>SE' = 203.9</td>
<td>ns</td>
</tr>
<tr>
<td>GLFA</td>
<td>2.22x10^-4</td>
<td>1.39x10^-8</td>
<td>17.6</td>
<td>***</td>
</tr>
<tr>
<td>I vs F</td>
<td>df' = 7.09</td>
<td>t(0.001) = 5.37</td>
<td>SE' = 235.0</td>
<td>***</td>
</tr>
<tr>
<td>I vs D</td>
<td>df' = 7.12</td>
<td>t(0.05) = 2.83</td>
<td>SE' = 235.4</td>
<td>*</td>
</tr>
<tr>
<td>Phytol</td>
<td>0.725</td>
<td>0.200</td>
<td>106.2</td>
<td>***</td>
</tr>
<tr>
<td>I vs F</td>
<td>df' = 5.43</td>
<td>t(0.001) = 6.41</td>
<td>SE' = 5.921</td>
<td>***</td>
</tr>
<tr>
<td>I vs D</td>
<td>df' = 7.39</td>
<td>t(0.05) = 2.80</td>
<td>SE' = 12.86</td>
<td>ns</td>
</tr>
</tbody>
</table>

*Values are mean (SE) nmol g^-1 ash weight, n=6. Dry weight:ash weight ratios for the treatments: Initial = 1.281, Feces = 1.240, Dark = 1.276.

PLFA = phospholipid fatty acid, GLFA = glycolipid fatty acid. Phytol summed from both phospholipid and glycolipid fractions.

Digestion efficiency is (1-feces concn/initial mean concn) x 100.

Welch's robust ANOVA and t-tests were used to test for overall differences and treatment effects. Planned comparisons test Initial vs Feces (I vs F, 1-tailed) and Initial vs Dark (I vs D, 2-tailed). Experiment-wise error rates: *** = P<0.001, * = P<0.05, ns = not significant.
difference was not significant. The digestion efficiency of phytol, and by extension microalgae, was lower than that for overall biomass (PLFA). Shrimp weight had no significant correlation (P>0.10) with feces concentrations of PLFA, GLFA or phytol.

**Response of individual PLFA**

Cluster analysis of the relative abundances of PLFA (i.e., proportion of total PLFA represented by each FAME) in the treatment replicates showed that the feces samples differed qualitatively, as well as quantitatively, from the initial and dark samples (Fig. 4). Such differences were not seen in the dark treatment, which clustered among the initial replicates. The wide variation in PLFA profiles exhibited by the initial samples (Fig. 4) was reflected in the large standard deviations associated with several FAME, notably 14:0 and some longer-chain polyenoics (Table 5).

The majority of PLFA in the feces treatment were significantly digested, while only 2 fatty acids changed significantly in the dark treatment (Table 5). Both of these increased in concentration, while they significantly decreased in the feces. The patterns of change in the 2 treatments were distinct (Fig. 5). Fatty acids in the dark treatment showed ungrouped, insignificant decreases or increases, with most of the latter concentrated in the fatty acids with 18 or more carbons. The feces treatment displayed a more coherent pattern, with large decreases in most fatty acids, especially evident in those smaller than 18 carbons. Although 2 fatty acids in the
Figure 4: Dendrogram of Treatment Replicates. Data for cluster analysis were the relative abundances of phospholipid fatty acids in replicates of the initial (I), dark (D) and feces (F) treatments. Clustering was based on euclidean distance using the complete linkage (farthest neighbor) method.
Table 5. Concentrations of Phospholipid Fatty Acids in Three Treatments

<table>
<thead>
<tr>
<th>FAME(^b)</th>
<th>Initial</th>
<th>Feces t-test(^c)</th>
<th>Dark t-test(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>282.3 (105.0)</td>
<td>76.6 (32.5) ns</td>
<td>206.1 (30.1) ns</td>
</tr>
<tr>
<td>15:0</td>
<td>235.6 (46.6)</td>
<td>79.0 (17.6) *</td>
<td>210.8 (14.3) ns</td>
</tr>
<tr>
<td>i15:0</td>
<td>50.2 (9.6)</td>
<td>17.8 (4.9) *</td>
<td>54.8 (5.4) ns</td>
</tr>
<tr>
<td>a15:0</td>
<td>25.4 (5.3)</td>
<td>10.7 (3.5) *</td>
<td>25.5 (3.1) ns</td>
</tr>
<tr>
<td>16:0</td>
<td>1213.6 (156.2)</td>
<td>559.3 (61.9) *</td>
<td>1110.4 (57.8) ns</td>
</tr>
<tr>
<td>16:1(\omega7)</td>
<td>625.1 (87.5)</td>
<td>228.9 (34.0) *</td>
<td>583.5 (32.1) ns</td>
</tr>
<tr>
<td>16:3(\omega4)</td>
<td>21.8 (4.2)</td>
<td>9.8 (1.5) *</td>
<td>21.3 (1.4) ns</td>
</tr>
<tr>
<td>17:0</td>
<td>30.7 (3.2)</td>
<td>19.2 (1.2) *</td>
<td>33.4 (1.4) ns</td>
</tr>
<tr>
<td>i17/17:1</td>
<td>18.9 (2.6)</td>
<td>12.8 (1.6) *</td>
<td>21.8 (1.9) ns</td>
</tr>
<tr>
<td>Poly 17(^d)</td>
<td>10.8 (1.3)</td>
<td>4.6 (2.4) *</td>
<td>9.3 (2.2) ns</td>
</tr>
<tr>
<td>18:0</td>
<td>48.7 (6.2)</td>
<td>39.7 (5.3) ns</td>
<td>51.1 (1.3) ns</td>
</tr>
<tr>
<td>18:1(\omega7)</td>
<td>168.5 (11.3)</td>
<td>119.3 (8.4) *</td>
<td>206.3 (13.5) *</td>
</tr>
<tr>
<td>18:1(\omega9)</td>
<td>61.5 (6.0)</td>
<td>59.2 (7.4) ns</td>
<td>69.5 (4.2) ns</td>
</tr>
<tr>
<td>18:2(\omega6)</td>
<td>36.3 (5.4)</td>
<td>26.3 (4.9) ns</td>
<td>35.1 (1.7) ns</td>
</tr>
<tr>
<td>18:4(\omega3)</td>
<td>17.8 (5.6)</td>
<td>24.0 (3.7) ns</td>
<td>25.6 (3.7) ns</td>
</tr>
<tr>
<td>Poly 19(^d)</td>
<td>9.2 (3.4)</td>
<td>6.1 (1.5) ns</td>
<td>11.8 (1.5) ns</td>
</tr>
<tr>
<td>20:0</td>
<td>23.9 (3.4)</td>
<td>9.7 (2.5) *</td>
<td>32.9 (3.4) *</td>
</tr>
<tr>
<td>20:4(\omega6)</td>
<td>108.8 (47.6)</td>
<td>56.0 (10.2) ns</td>
<td>125.6 (18.8) ns</td>
</tr>
<tr>
<td>20:5(\omega3)</td>
<td>127.1 (63.9)</td>
<td>91.9 (17.0) ns</td>
<td>160.2 (25.1) ns</td>
</tr>
<tr>
<td>22:6(\omega3)</td>
<td>47.7 (19.6)</td>
<td>60.9 (20.3) ns</td>
<td>76.1 (15.3) ns</td>
</tr>
<tr>
<td>24:0</td>
<td>51.2 (5.1)</td>
<td>29.7 (23.0) ns</td>
<td>45.5 (4.5) ns</td>
</tr>
</tbody>
</table>

\(^a\)Values are mean (SD) nmol g\(^{-1}\) ash weight, n=6.

\(^b\)Fatty acid methyl ester.

\(^c\)Differences from initial mean tested using Welch t-tests. Significance (treatment-wise): * = P<0.05, ns = not significant.

\(^d\)Polyenoic; molecular geometry not determined due to low abundance.
Table 1. Changes in phospholipid fatty acid concentrations in feces and dark treatments. Significant (P<0.05) changes indicated with asterisk.

<table>
<thead>
<tr>
<th>Fatty Acid Methyl Ester</th>
<th>Feces</th>
<th>Dark</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>i15:0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a15:0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:1w7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:3w4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>i17/17:1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly 17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:1w7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:1w9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:2w6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:4w3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly 19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:4w6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:5w3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22:6w3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24:0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 5. Changes in phospholipid fatty acid concentrations in feces and dark treatments. Significant (P<0.05) changes indicated with asterisk.
feces appeared to show parallel increases in feces and dark treatments, none of these changes were significant.

Response of functional groups

Cluster analysis showed three distinct groups of fatty acid responses to digestion (Fig. 6). Cluster 1, long-chain polyenoics characteristic of eukaryotes, increased in concentration in the feces. Cluster 2 consisted of bacterial and eukaryotic markers which were moderately reduced following gut passage. Cluster 3 also contained biomarkers for both prokaryotes and eukaryotes, but showed larger decreases in abundance. The distribution of biomarkers among the clusters suggests that the effects of the feces treatment were not uniform within the functional groups. For example, markers for aerobic and Gram-positive bacteria occur in both clusters 2 and 3.

Changes in the functional groups represented by the biomarkers suggest that prokaryotes in general were more affected by gut passage than were eukaryotes (Fig. 7). No differences were apparent between the response of the aerobic and Gram-positive subgroups. Among the eukaryotic markers, 20:4\omega6, characteristic of protozoa, declined strongly while the abundance of the green algae/higher plant marker 18:1\omega9 was virtually unchanged in the feces (Table 6). Polyenoic fatty acids, essentially restricted to eukaryotes, showed a response similar to that of the eukaryotic biomarkers as a whole. Assuming that the biomarker fatty acids of the prokaryotes and eukaryotes are directly proportional to the biomass of these groups,
Figure 6: Phospholipid Fatty Acid Dendrogram. Data for cluster analysis were percentage change (from mean initial concentration) of phospholipid fatty acid concentrations in the feces treatment. Clustering was based on euclidean distance using the complete linkage (farthest neighbor) method. Functional group designations: Eu = eukaryotes, Pr = prokaryotes, ae = aerobic bacteria, di = diatoms, gr = green algae/higher plants, g+ = Gram-positive bacteria, pz = protozoa.
Figure 7. Digestion Efficiencies of Microbial Functional Groups. Poly=Total polyenoics, Eu=Eukaryotes, di=diatoms, gr=green algae/higher plants, pz=protozoa, Pr=Prokaryotes, ae=aerobic bacteria, g+=Gram-positive bacteria. Biomarker FAME in each functional group are listed in Figure 6. Values are means±SE, n=6.
over half of the bacterial biomass was lost during gut transit, while eukaryotes were reduced by less than one third (Fig. 7).

Table 6. Total Concentrations of Functional Group Biomarker PLFA in Two Treatments

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Initial</th>
<th>Feces</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(nmol PLFA g⁻¹ ash weight)</td>
<td>(nmol PLFA g⁻¹ ash weight)</td>
</tr>
<tr>
<td>Total polyenoic</td>
<td>379.8 (136.1)</td>
<td>279.6 (46.0)</td>
</tr>
<tr>
<td>Eukaryotes</td>
<td>421.3 (135.1)</td>
<td>328.2 (53.7)</td>
</tr>
<tr>
<td>Diatoms</td>
<td>127.2 (63.9)</td>
<td>91.9 (17.0)</td>
</tr>
<tr>
<td>Green algae/plants</td>
<td>61.5 (6.0)</td>
<td>59.2 (7.4)</td>
</tr>
<tr>
<td>Protozoa</td>
<td>108.9 (47.6)</td>
<td>56.0 (10.2)</td>
</tr>
<tr>
<td>Prokaryotes</td>
<td>1154.5 (156.9)</td>
<td>487.7 (64.6)</td>
</tr>
<tr>
<td>Aerobic</td>
<td>793.6 (94.9)</td>
<td>348.1 (39.2)</td>
</tr>
<tr>
<td>Gram-positive</td>
<td>94.6 (16.8)</td>
<td>41.4 (9.5)</td>
</tr>
</tbody>
</table>

*Values are mean (SD) nmol PLFA g⁻¹ ash weight, n=6.

*bFunctional group assignments of PLFA are shown in Figure 6.

Discussion

Microbial community biomass and composition

The microbial biomass (PLFA g⁻¹) in the detrital aggregates used here was two orders of magnitude higher than that found in subtidal sands (Findlay et al. 1990a, Dobbs and Guckert 1988a) and at least three times higher than organically enriched muds in Maine (Dobbs and Findlay 1993). This concentration reflects both the distribution of biomass in surface sediments and the origin of the aggregates. Benthic detrital aggregates normally occur as a thin layer at the sediment-water interface, and are mixed with and diluted by underlying inorganic sediment in the
course of most sediment sampling. This flocculent aerobic zone is known to contain much higher biomass than bulk surface sediments (Novitsky and Karl 1986). Until detrital aggregates in diverse environments are assayed for PLFA content, the aggregates used here should not be considered atypically rich in microbial biomass.

Ponds used for semi-intensive shrimp aquaculture receive detrital organic inputs in the form of feed pellets, which either directly or indirectly (as shrimp feces) stimulate growth of heterotrophic microbes (Moriarty 1986). In addition, mineralized nutrients from this same source are suspected to increase microalgal productivity (Schroeder 1978). However, these detrital inputs could simply increase turnover rates with little effect on microbial biomass, rather than raising microbial standing stocks in aggregates.

Bacteria dominated the biomass in the detrital aggregates used here. The total PLFA of the initial samples can be roughly divided into prokaryotic and eukaryotic components using the abundance of polyenoic fatty acids, which with few exceptions are restricted to eukaryotes. If we assume that 50% of the PLFA of eukaryotic microbes are polyenoic (Findlay and Dobbs 1993), then 23% of the total PLFA was due to eukaryotes, and by difference 77% of the PLFA was prokaryotic. Using conversion factors (Dobbs and Findlay 1993), which may only be approximate for the microbial community in this experiment, the respective biomass carbon contributions can be estimated. Assuming 100 μmol PLFA g⁻¹ C for eukaryotic cells and 200 μmol PLFA g⁻¹ C for prokaryotes, eukaryotic microbes
represented 7.4 mg C g\(^{-1}\) sediment (ash wt), or 37% of the total biomass C, and bacteria made up 12.4 mg C g\(^{-1}\), or 63% of the total.

The ratio of 20:4\(ω6\) to 20:5\(ω3\), which is typically 1:5 in photoautotrophs (R. Findlay, pers. comm.), here was near unity, suggesting a eukaryotic community dominated by heterotrophs. This was supported by the apparent absence of a known marker for microalgae, 16:1\(ω13\). Other \textit{trans} fatty acids, which increase in prokaryotes under nutrient or other stresses, were absent as well. Also absent (or present at levels below detection limits) were cyclopropyl fatty acids (cy17:0, cy19:0) characteristic of sulfate-reducing anaerobic bacteria, although other PLFA found in anaerobes (e.g., a15:0, i15:0) were present. Polyenoic 17 and 19 C fatty acids are unusual, and may reflect the presence of sarcodine protozoa (D. Hedrick, pers. comm.).

In summary, the detrital aggregates used in this experiment, derived from the flocculent sediment-water interface of a marine aquaculture pond, were unusually rich in microbial biomass and were dominated by bacteria. Some anaerobic forms were present, but sulfate-reducers were absent. The smaller eukaryotic component was largely heterotrophic, and may have included amoebae.

The biomass distribution in the detrital aggregates used in this study should not be considered representative of conditions in aquaculture ponds or natural nursery habitats of \textit{P. vannamei}. Collection, transport, sieving and removal of meiofauna (harpacticoid copepods and nematodes), and maintenance for 4 days under artificial light with minimal water motion are all likely to have affected the
population levels and metabolic states of the microbiota. Findlay et al. (1990b) sieved sediments to simulate biotic disturbance, and while total PLFA levels were unchanged after 4 days, the functional groups represented in the sediment were strongly affected, with microeukaryotes showing the largest proportionate drop in biomass. If these patterns also occurred in the detrital aggregates, the original aggregates in the ponds may have contained higher relative abundances of aerobic bacteria and eukaryotes. Microscopic examination revealed very numerous, but senescent, pennate diatoms, the decay of which may have elevated the biomass of bacteria and shifted the system towards heterotrophy. Mass mortalities ("crashes") of microalgae are common in aquaculture ponds, but whether this community state preceded or followed collection of the detrital aggregates is unknown.

Because of the rapid responses of microbes to disturbance (Findlay et al. 1990b) and the inevitable changes that occur in even well-designed sediment microcosms (Federle et al. 1986), differences between laboratory and field diets appear to be unavoidable. Therefore conclusions regarding microbial food resources of these animals should be based on absolute and relative abundances measured in the field, integrated with data, such as those presented here, regarding the relative vulnerabilities of functional groups to digestion.

Effects of digestion on microbial populations

The digestion efficiency calculated for total PLFA suggests that approximately half of the microorganisms in detrital aggregates were digested by
*Penaeus vannamei*. Although PLFA, GLFA and phytol all showed significant reductions in the feces, processes other than digestion potentially affect the accuracy of these digestion efficiency estimates. First, selective ingestion of biomass over inorganic material could have raised the concentration of all three lipids in the ingesta above the initial value, resulting in an underestimate of the true digestion efficiency. Without foregut samples this possibility cannot be tested directly, but a nearly identical experiment (Chapter 4) examined the concentration of microalgal pigments, principally from benthic diatoms, in the foreguts of *P. vannamei* fed on similar detrital aggregates. A 6.7% increase in pigment concentration over the initial value was measured, but was not statistically significant. Such an increase in total PLFA concentration in this experiment would yield a digestion efficiency of 55% compared to the calculated 52%, so ingestion selectivity could be considered a potentially minor source of error.

Biomass digestion efficiencies could also have been affected by microbial responses to processes common to the dark and feces treatments, such as darkness and mechanical agitation. The apparent digestion efficiency on total GLFA may be partly due to whatever process caused a significant loss of GLFA in the dark treatment. If most of the GLFA was associated with chloroplasts, the phytol concentration would also be expected to decrease in the dark due to the fixed ratio of chlorophyll *a* to glycolipid in these organelles (Rosenberg 1973). The lack of close coupling between phytol concentrations and total GLFA suggests that sources other than chloroplasts contributed substantially to the glycolipid content of detrital
aggregates. Although the large variance in the dark phytol values makes any conclusion tentative, phytol appears not to have been affected by the dark treatment, implying that the phytol digestion efficiency measures the decline in microalgal biomass.

Most changes in individual PLFA in the dark treatment did not appear to be congruent with the changes observed in the feces, suggesting that the majority of fecal changes were due to some process associated with gut transit. Digestion by the shrimp is the most obvious process. Differential removal of populations containing contrasting PLFA profiles would cause differential changes among PLFA. Digestion could be expected to attack populations of microbes differently based on their cell wall composition or degree of protection from enzymes, due to polysaccharide capsules, attachment to particles, or relative positions within biofilm consortia.

Penaeids are known to digest Gram-positive cell walls (Moriarty 1976), but filamentous cyanobacteria, which are relatively large and have a dense polysaccharide sheath, are assimilated to a lesser extent than other bacteria (Moriarty 1976). Microbes larger than, or attached to particles larger than, about 1 μm might suffer less digestion because they would be prevented from entering the digestive gland by the filter-press in the foregut, and would pass into the feces. Free bacteria <1 μm which enter the digestive gland lumen would be surrounded by digestive enzymes and may have longer gut residence times. Although penaeids can assimilate a variety of Gram-negative and Gram-positive bacteria with efficiencies of
85-99% when they are presented as free cells (Moriarty 1976, Hood and Meyers 1973), most bacteria in sediment environments are attached to particles and are embedded in extracellular polysaccharide (Moriarty and Hayward 1982). These differences between in situ and cultured bacteria may explain the relatively low efficiency (58%) with which Penaeus vannamei digested the prokaryotic functional group in detrital aggregates.

The two functional groups of bacteria did not differ in the degree of digestion by P. vannamei. Eukaryotes as a whole appeared less digestible, and in contrast to the bacteria, functional groups of eukaryotes appeared to differ in digestibility. Although it is tempting to ascribe the contrast between the protozoa and green algae/higher plant functional groups to differences in cellular structure, each group was represented by a single PLFA marker. The relative abundances of such markers in the feces could be affected by processes other than digestion.

The failure of the fatty acids characteristic of functional groups to show coherent responses to digestion (Fig. 6) suggests either that these biomarkers are not confined to the nominal cell types or that some process differentially affected certain marker fatty acids in the feces. The biomarkers used here are a conservative compilation of PLFA observed in culture studies, and have been used with field samples by other several other workers (e.g., Findlay and Dobbs 1993, Vestal and White 1989, Mayer et al. 1991). Several non-exclusive processes could have altered the pattern produced by digestion, including shedding of symbiotic gut microbes into
the feces, growth of surviving sediment bacteria within the fecal strings, and incorporation of shrimp cellular material into the feces.

A distinctive gut flora has been reported from the midgut of *Penaeus setiferus* that was dominated by the Gram-negative genus *Vibrio* (Hood and Meyers 1973). Colonization of the feces by shed cells of these (presumably attached) bacteria could increase the prokaryotic signal and 18:1ω7 (a Gram-negative marker, Dobbs and Guckert 1988b) but would not increase levels of i15:0 and a15:0, which are restricted to Gram-positive bacteria (Dobbs and Guckert 1988b). In fact, 18:1ω7 showed a smaller decline (or a larger recovery) in the feces than the branched acids, consistent with this hypothesis.

Unless bacteria undergo several doublings in the hindgut and feces, it is unlikely that the digestion efficiencies reported previously for penaceids (Moriarty 1976, Hood and Meyers 1973) are realistic in systems such as this. The growth of surviving sediment bacteria within *P. vannamei* feces could strongly decrease the apparent digestion of prokaryotic functional groups if rapid growth occurred before feces were stabilized, in this case by freezing approximately 3 hours post-defecation. Digestion theory predicts that deposit feeders maximizing their rate of nutrient gain in food-rich environments will produce feces containing undigested microbes and unabsorbed soluble digestive products (Plante *et al.* 1990). Bacteria in such an environment can attain high growth rates within the hindgut (Plante *et al.* 1989), but theory suggests that solutes diffuse away from small fecal pellets within minutes of defecation (Jumars *et al.* 1989). Observations of pelagic fecal pellets confirm that
they are poor environments for bacterial growth (Karl et al. 1988). Preliminary
experiments showed that the gut transit time under these conditions was 35-45 min
(unpublished observations). Because bacterial regrowth could only occur during the
latter part of gut transit, and because minimal bacterial doubling times are on the
order of 20 min, at most one doubling could occur within the gut, where most
regrowth is expected to occur (Plante et al. 1989). Diffusion of 90% of solutes from
the feces (approximate diameter 1 mm) should occur in <10 min (Jumars et al.
1989), suggesting that bacterial growth on these substrates would be short-lived after
defecation. In addition, a low growth rate of bacteria in the shrimp feces is implied
by the relatively small standard deviations of the prokaryote functional groups in
Figure 7. The among-animal variance in bacterial markers should be high if these
bacteria were growing exponentially, due to inevitable variations in fecal production
rates and thus ages of feces prior to fixation. Two to 4 post-digestion doublings
would be required to accommodate the 90-99% digestion efficiencies seen in
experiments using cultured, free bacterial cells (Moriarty 1976, Hood and Meyers
1973). However, if the bacteria in feces doubled even once after digestion, the error
in the calculated digestion efficiency would be substantial (i.e., 79% vs 58% for
prokaryotes, Fig. 7).

Despite the potential underestimates of prokaryotic digestion, these digestion
efficiencies were higher than those of the eukaryotic functional groups. This could
be due to the size-selective nature of the penaeid digestive system described above.
Alternatively, contributions from the shrimp themselves could have reduced this
apparent digestion by increasing fecal concentrations of fatty acids characteristic of both eukaryotic microbes and *Penaeus vannamei*. The most likely source would be the digestive gland, because esophageal cells would be digested in the foregut, the foregut itself is lined with cuticle (Dall *et al.* 1990), and the midgut and hindgut are separated from the feces by a peritrophic membrane. Senescent epithelial cells are shed into the lumen of the digestive tubules and may survive digestion to enter the feces, although this has not been shown to occur (Dall *et al.* 1990). The possible contamination of copepod feces by gut-derived lipids has been reported (Prahl *et al.* 1984).

The dominant fatty acids (largely PLFA) of wild *P. vannamei* are, in order of abundance, 20:5ω3, 16:0, 18:1ω9, 18:0, 22:6ω3, 20:4ω6, and 16:1ω7 (Araujo and Lawrence 1993). The major PLFA of penaeids are not strongly influenced by diet (Tinoco 1982, Read 1981), so these fatty acids should dominate any shrimp signal in the feces. The measured decrease in 20:4ω6 constrains the potential contribution of shrimp PLFA. If this fatty acid were actually digested to the extent observed for a similar PLFA not known from *P. vannamei* (59%, 20:0), then a maximum of 11.4 nmol g⁻¹ (20.3%) of the fecal 20:4ω6 could be due to shrimp cells. Proportionate contributions of the other major shrimp PLFA could represent from 4% (16:0) to 41% (18:0) of their feces concentrations. Although this contamination has the potential to bias the digestion efficiencies of some PLFA and of all the eukaryotic subgroups, it could not account for the difference between the protozoa and green algae/higher plant digestion efficiencies. Also, other fatty acids not reported from
*P. vannamei* also had low digestion efficiencies (18:1ω7, 18:2ω6, 18:4ω3, Poly 19; Fig. 5). Thus a contribution of shrimp cellular PLFA does not explain the generally lower digestion of eukaryotic markers relative to those from bacteria.

Phytol, derived from chlorophyll, acted as an independent marker for microalgae and confirmed that this functional group was digested with low efficiency. Phytol was reduced 38% in the feces while the diatom marker 20:5ω3 had a digestion efficiency of 28%. Due to the high variance of the latter estimate these efficiencies are comparable.

**Implications for shrimp nutrition**

Detrital aggregates, because they contain large amounts of inorganic material, are far less energetically efficient for consumers than are prey animals. For *Penaeus vannamei*, this limitation is compounded by the low digestibility of the microbial biomass contained in the aggregates. This inefficiency may be causally linked to the inorganic content of aggregates because of the functioning of the shrimps' digestive system.

In general, the digestion efficiency an animal exhibits on a foodstuff is an inverse function of rate of movement through the digestive portion of the gut (Penry and Jumars 1987), which in penaeids is the foregut. Whether the digestion efficiencies determined in this study are similar to those in nature depends on whether this throughput rate is representative. Throughput rate would be expected to vary with the proportion of organic matter in the food and with food availability.
relative to processing rate (Cammen 1980b). Penaeid foreguts are small and are filled rapidly during foraging (Dall et al. 1990), suggesting that, especially with a ubiquitous food such as detrital aggregates, processing rate rather than ingestion rate would be limiting. Assuming that these detrital aggregates are typical in organic content (21%, Table 4, note a), the throughput rate in this study should be maximal and representative of *Penaeus vannamei* feeding on detrital aggregates.

As noted above, deposit feeders maximizing nutritional gain are predicted to egest some undigested microbes. The digestion efficiencies measured here are minimum estimates due to the potential errors discussed above, most of which should be small. Two major uncertainties are the extent of bacterial growth in the midgut and the contribution of shrimp cell fragments to the eukaryotic PLFA in the feces. For PLFA analysis to become routinely applicable to digestive studies, the magnitude of contamination of feces by digestive cell fragments must be assessed. In the case of penaeids, this could be done by feeding the animals a compounded, lipid-free food and assaying the resulting feces.

The relative digestibilities of prokaryotes and eukaryotes imply that in this case bacterial resources (C, protein) were much more readily available than were the essential nutrients (sterols, polyunsaturated fatty acids) found in eukaryotic cells. In the aggregates used here, bacteria were almost two-thirds of the biomass C and were digested over twice as efficiently as eukaryotic cells, resulting in over 4 times as much prokaryotic C (and presumably amino N) available for absorption by the shrimp. If bacterial regrowth in feces obscured the actual digestion efficiency, the
disparity in resource values could be even greater. Thus, the relative concentrations of major and minor nutrients in detrital aggregates does not correspond to their availability to consumers.
CHAPTER 4. PIGMENT DIGESTION

Introduction

Penaeid shrimp are important components of tropical coastal ecosystems (Edwards 1977, Salini et al. 1990, Robertson 1988), and are widely exploited in commercial fisheries and aquaculture. Recent studies have shown that the seagrass meadows, mangroves and salt marshes that support rapid growth of juvenile penaeids have high rates of microalgal production that may support much of the animal biomass (Boesch and Turner 1984, Kitting et al. 1984, Fry 1984). Penaeid postlarvae and juveniles in salt marshes are known to assimilate benthic, planktonic and epiphytic diatoms (Gleason and Wellington 1988, Gleason 1986), but the route through which microalgal carbon reaches juveniles in mangrove and lagoon systems is unclear.

Benthic diatoms are often components of flocculent detrital aggregates (Bowen 1981), that can be sites of high microbial biomass and turnover (Karl and Novitsky 1988). Detrital aggregates and associated microalgae are known to contribute to the growth of penaeids in culture (Moss et al. 1992, Hunter et al. 1987). The ability of shrimp to utilize this autochthonous production may be a key factor in the success of shrimp culture in earthen ponds (Anderson et al. 1987), because abundance of prey in such ponds is low. The regular occurrence of detrital aggregates and fine sediments in gut contents of juvenile and adult penaeids in
nature (Robertson 1988, Dall et al. 1990) may indicate continued consumption of benthic microflora as components of aggregates.

The utility of microalgal resources associated with detrital aggregates would depend upon the ability of penaeids to digest such cells within the organic and inorganic matrix of the aggregates. Penaeids are known to digest microalgae when presented as purified foods (Moriarty 1976). Other crustaceans that consume microalgae present in an inorganic matrix display ingestion selectivity, i.e., prior to ingestion they increase biomass several-fold using specialized morphology or sorting behavior (Cammen 1980b). This ability has not been assessed for penaeids, although observations suggest the possibility (Condrey et al. 1972).

*Penaeus vannamei* Boone is native to the Pacific coast of tropical America from Mexico to Peru, and is an important species in aquaculture. The juvenile stages are passed in extensive shallow lagoons and mangrove areas, where diatoms form a significant component of the shrimps' gut contents (Edwards 1977).

I investigated the ability of juvenile *P. vannamei* to utilize benthic microalgae by analyzing the pigments present in natural detrital aggregates and in subsequent foregut contents and feces. Analysis of pigments using high performance liquid chromatography (HPLC) allowed characterization of the microalgal community and precise estimates of biomass concentration and digestion. In addition, HPLC provides insight into the digestive process through characterization of pigment degradation products.
Materials and Methods

Experimental materials

Juvenile *Penaeus vannamei* and benthic detrital aggregates were obtained from earthen mariculture ponds at Amorient Aquafarms, Kahuku, Hawai‘i. Detrital aggregates characteristic of the sediment-water interface were allowed to accumulate over several days on submerged plastic panels and were collected by gentle suction. The aggregates were sieved through 60 μm Nitex mesh to remove meiofauna and spread in shallow trays to a depth of 3 mm. Seawater augmented with 100 μM NO₃, 10 μM PO₄ and 12.5 μM Si was added to the trays, which were maintained for 24 hours under fluorescent light.

Shrimp were collected during routine harvest of nursery ponds and maintained in a running seawater tank for 8 days. They were fed twice daily with a mixture of their accustomed pelleted shrimp feed (Rangen) and detrital aggregates, maintained as above. Animals were observed to ingest both foods readily. To avoid effects of the molt cycle on feeding behavior (Hill and Wassenberg 1992), shrimp selected for the experiment were in molt stages C-D₁ (Robertson *et al.* 1987). Shrimp sizes are expressed as wet weight, which scales with gut volume. Corresponding linear measure and dry weight conversion equations are listed in Menz and Bowers (1980).
Experimental procedure

The experimental apparatus consisted of an array of 1 L polyethylene chambers filled with 600 mL aerated seawater kept at 24 °C in a circulating water bath. All procedures were conducted in the dark to eliminate possible burrowing and maximize feeding behavior (Moctezuma and Blake 1981). A dim red headlamp was used during periods of animal observation and manipulation.

Six *Penaeus vannamei* (1.0-1.8 g, mean 1.4 g) were blotted dry, weighed and placed individually in experimental chambers containing 4 mL of detrital aggregates. All animals were actively feeding on aggregates when selected. Shrimp were allowed to produce feces over a 4 hr period, after which feces and uneaten aggregates were pipetted into light-tight vials. These samples were processed after the remainder of the experiment was completed, about 3 hr. To obtain foregut samples, the shrimp were transferred to clean chambers and their foreguts were observed to empty (approximately 30 min). One mL of fresh detrital aggregates were then added to the chambers, and after 2 min of feeding the animals were placed in plastic bags, immediately killed by immersion of the bags in -1.5 °C brine, and held at that temperature for dissection.

Sample processing

Initial samples (500 μL) of detrital aggregates were taken during addition to the experimental chambers. The dense and compact fecal strings were washed free of the uneaten aggregates by gentle agitation in filtered seawater. The initial
samples and the feces from each animal were centrifuged at 13,000 x g for 2 minutes and the water decanted. The foregut of each animal was dissected free under dim red illumination, opened and the contents flushed onto a preweighed, combusted filter (Whatman GF/F) with 5 mL seawater under a gentle vacuum. Foregut contents were exposed to digestive enzymes at room temperature for a maximum of 8 min. All samples were quick frozen and stored at -85 °C for 25 days prior to extraction.

To extract the pigments, samples were thawed in the dark at 4 °C in 3 mL 100% acetone, sonicated to disrupt pellets and held for 15 hrs at -20 °C. An internal standard (canthaxanthin) was added, the samples were vortexed and centrifuged, and the supernatant liquid was removed for analysis by reverse-phase high performance liquid chromatography (HPLC). Pellets and filters were lyophilized for dry weight measurements, then combusted at 450 °C for 5 hrs, reweighed, and the organic content calculated by difference.

Pigment analysis followed Bidigare et al. (1989) with minor modifications. Five hundred µL of each sample were injected automatically via a refrigerated autosampler into a Spectra-Physics SP8800 HPLC fitted with a Radial-Pak C18 column (8 x 100 mm, 5 µm particle size, Waters Chromatography). Eluting peaks were detected by absorbance (436 nm, Waters model 440 detector) and fluorescence (ex=408 nm, em=662 nm, Waters model 470 detector) spectroscopy. Routine identification of peaks was based on retention time relative to standards. The absorption spectra of some unknown peaks were obtained using diode array
spectroscopy (Waters model 990 detector, 400-750 nm). Carotenoids and chlorophylls were quantified from absorbance peak areas, while pheopigments were determined from fluorescence peaks. Pigment concentrations were calculated using external standards. All chlorophyll $a$-related pigment concentrations were expressed as chlorophyll $a$ equivalents.

**Statistical analysis**

Ash weights were used to calculate pigment concentrations because of the potentially confounding effect of digestion on the organic component of dry weight. Absorption of the inorganic clay matrix by the shrimp during gut transit was assumed to be negligible.

Chlorophyll $a$ and its derivatives are potentially conserved tracers of microalgal biomass (Shuman and Lorenzen 1975). Total chlorophyll $a$-related pigment concentrations were examined using the procedures of Hoaglin *et al.* (1983), and outlying replicates were deleted. Because the unequal variances of the treatments violated a key assumption of parametric analysis of variance (ANOVA), the robust Welch test (Welch 1951) was used to test overall significance (Day and Quinn 1989). Welch’s $t$-tests, with Satterthwaite’s adjusted degrees of freedom (Day and Quinn 1989), were used to test hypotheses of ingestion selectivity and pigment conservation. Experiment-wise error rates were held at 0.05 with the Dunn-Sidák method (Sokal and Rohlf 1981). Critical values of $F$ and $t$ for fractional degrees of freedom were calculated using SYSTAT (Wilkinson 1986).
Chlorophyll and carotenoids can be converted to non-absorbing and non-fluorescing degradation products (Kiorboe and Tiselius 1987, Owens and Falkowski 1982, Head and Harris 1992, Lopez et al. 1988), so reductions in pigment concentration during gut transit did not confirm uptake by the gut. Percent decrease in pigment concentrations were therefore termed "digestion efficiency", while reductions in total organic matter implied absorption and were termed "assimilation efficiency".

Results

Ingestion selectivity

Total chlorophyll a-related pigment concentrations were used to test whether ingestion selectivity occurred and whether pigments were conserved during gut transit. Selective concentration of microalgae relative to inorganic material was not evident; although the mean foregut concentration was 6.7% above the initial mean, the difference was not significant (Table 7). Initial concentrations were therefore used in subsequent calculations of pigment digestion. Significant loss of total chlorophyll a-related pigments occurred during gut transit (Table 7). Over 18% of total chlorophyll a-related pigments initially present were unaccounted for by breakdown products in the feces (Table 8).
Table 7. Overall Test and Planned Contrasts of Total Chlorophyll a-Related Pigment Concentrations

<table>
<thead>
<tr>
<th>Test</th>
<th>Parameters</th>
<th>Significance&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Welch ANOVA</td>
<td>A = 2.95x10^-3  B = 3.66x10^-6</td>
<td>W(2,6.4) = 5.70 *</td>
</tr>
<tr>
<td>FG vs I&lt;sup&gt;b&lt;/sup&gt;</td>
<td>df' = 4.30   t&lt;sub&gt;(0.05)&lt;/sub&gt; = 3.37</td>
<td>SE' = 100.4 ns</td>
</tr>
<tr>
<td>F vs I</td>
<td>df' = 5.39   t&lt;sub&gt;(0.05)&lt;/sub&gt; = 3.08</td>
<td>SE' = 98.0 *</td>
</tr>
</tbody>
</table>

<sup>a</sup>: P<0.05; ns: not significant.

<sup>b</sup>Planned contrasts (Welch t-tests) test Foregut vs Initial (FG vs I) and Feces vs Initial (F vs I), both 2-tailed.

**Microalgal community present**

The pigments present in the initial samples support the visual observation that pennate and centric diatoms dominated the microalgal assemblage in the detrital aggregates. Absorbance and fluorescence chromatograms of the initial samples (Figs. 8a, 9a) were dominated by fucoxanthin, diadinoxanthin, diatoxanthin, β-carotene, and chlorophyll a, its epimer and allomers. Chlorophyll c was less than 4% of total chloropigments. Approximately 7% of the initial chlorophyll a-related pigments were present as degradation products (chlorophyllide a, pheophytin a, pheophorbide a; Table 8), which suggests that most pigments present were constituents of living cells. Although microscopic examination showed scattered dinoflagellates and cyanobacteria, the lack of detectable peridinin or zeaxanthin indicates that these groups contributed relatively little biomass.
Table 8. Pigment Concentrations in Experimental Treatmentsa

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Initial</th>
<th>Foregut</th>
<th>Feces</th>
<th>Digestion Efficiency (%)b</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chloropigments*c</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorophyll c</td>
<td>56.3 (0.9)</td>
<td>48.1 (2.9)</td>
<td>18.4 (1.5)</td>
<td>67.3 (2.7)</td>
</tr>
<tr>
<td>Chlorophyll a²</td>
<td>1620 (17.9)</td>
<td>655.6 (44.9)</td>
<td>597.4 (30.5)</td>
<td>63.3 (1.9)</td>
</tr>
<tr>
<td>Chlorophyllide a</td>
<td>7.4 (0.3)</td>
<td>1.4 (0.9)</td>
<td>3.6 (0.6)</td>
<td>51.2 (7.9)</td>
</tr>
<tr>
<td>Pheophytin a</td>
<td>54.3 (1.0)</td>
<td>111.0 (15.6)</td>
<td>145.0 (19.0)</td>
<td>-167 (35)</td>
</tr>
<tr>
<td>Pheophytin a-like*</td>
<td>30.4 (3.1)</td>
<td>463.6 (34.7)</td>
<td>438.3 (89.6)</td>
<td>-1339 (294)</td>
</tr>
<tr>
<td>Pheophorbide a</td>
<td>37.3 (1.5)</td>
<td>644.5 (84.3)</td>
<td>250.6 (38.4)</td>
<td>-527 (103)</td>
</tr>
<tr>
<td><strong>Total Chl a-related Pigments</strong></td>
<td>1759 (19.1)</td>
<td>1876 (98.5)</td>
<td>1435 (96.2)</td>
<td>18.4 (5.5)</td>
</tr>
<tr>
<td><strong>Carotenoids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fucoxanthin</td>
<td>759.7 (7.2)</td>
<td>517.8 (33.3)</td>
<td>416.2 (20.8)</td>
<td>45.2 (2.7)</td>
</tr>
<tr>
<td>cis-Fucoxanthin</td>
<td>112.8 (0.6)</td>
<td>65.5 (3.1)</td>
<td>65.6 (4.8)</td>
<td>41.8 (2.7)</td>
</tr>
<tr>
<td>Diadinoxanthin</td>
<td>105.9 (1.1)</td>
<td>66.0 (4.0)</td>
<td>52.7 (2.2)</td>
<td>50.2 (2.1)</td>
</tr>
<tr>
<td>Diatixanthin</td>
<td>23.7 (0.5)</td>
<td>3.4 (1.6)</td>
<td>25.4 (2.7)</td>
<td>-7.2 (11.5)</td>
</tr>
<tr>
<td>β-carotene</td>
<td>56.2 (0.8)</td>
<td>30.1 (1.8)</td>
<td>17.6 (0.9)</td>
<td>68.7 (1.7)</td>
</tr>
</tbody>
</table>

aValues are mean (SE) µg pigment g⁻¹ ash weight, n=6, except chloropigments in foregut, n=5.
bCalculated as (1-feces/initial mean) x 100. Negative digestion values indicate production during gut transit.
cAll except chlorophyll c expressed as chlorophyll a equivalents.
dIncludes allomers and chlorophyll a'.
ePeaks with retention times of 17.5, 21.8, and 25.4 min.
Alteration of pigments by digestion

Maceration by the mouthparts and foregut and an 8-min exposure to digestive enzymes strongly affected the pigment profiles (Figs. 8b, 9b; Table 8). Chlorophyll c, chlorophyll a, chlorophyll a allomers, and chlorophyllide a decreased in concentration in the foregut, while pheophorbide a, pheophytin a and pheophytin a-like pigments increased. The low concentration of chlorophyllide a indicates that essentially all chlorophyll a exposed to the digestive process was converted to pheopigments through loss of Mg\(^{2+}\). Carotenoids were also degraded in the foregut, with concentrations 32% to 46% lower than initial values.

Fecal pigment concentrations reflected more prolonged exposure to digestive enzymes as well as potential effects of gut passage and fecal leaching. Beta-carotene was more completely degraded (68%) than were the xanthophylls fucoxanthin, cis-fucoxanthin and diadinoxanthin (41-50%; Table 8). Diatoxanthin displayed an anomalous increase in concentration between the foregut and feces, which was not explicable as interconversion with diadinoxanthin (Table 8). No absorption peaks attributable to carotenoid degradation products were observed in the foregut or feces.

Sixty-three percent of the initial chlorophyll a was digested (Table 8). Pheophytin a, which formed in the foregut through the demetallation of chlorophyll a, increased slightly in the feces but remained a minor breakdown product. Pheophorbide a, formed by removal of the phytol side chain from pheophytin a, was abundant in the foregut and declined to less than half of this value in the feces.
Figure 8. Absorbance Chromatograms. Samples were of detrital aggregates at various stages of passage through *Penaeus vannamei* guts: (a) initial (uneaten); (b) foregut; (c) feces. Peak identifications: s=solvent peak, 1=chlorophillide *a*, 2=chlorophyll *c*, 3=fucoxanthin, 4=pheophorbide *a*, *b*=pheophorbide *a*-like pigment, 5= cis-fucoxanthins, 6=diadinoxanthin, 7=diatoxanthin, 8=chlorophyll *a* allomers, 9=chlorophyll *a*, 10=chlorophyll *a*’, *p*=pheophytin *a*-like pigment, 11=β-carotene, 12=pheophytin *a*, 13=pheophytin *a*’.
Figure 9. Fluorescence Chromatograms. Samples were of detrital aggregates at various stages of passage through *Penaeus vannamei* guts: (a) initial (uneaten); (b) foregut; (c) feces.
Peaks representing unidentified chlorophyll $a$ derivatives were initially present at low concentrations, but became prominent in the foregut and feces (Fig. 9b, 9c). These were labelled as pheophytin $a$-like on the basis of retention times and absorption spectra. The concentration of one derivative was highly variable among fecal replicates (Fig. 10), as was pheophorbide $a$. The proportion of fecal pheopigment present as these pheophytin $a$-like pigments was inversely correlated with the proportion represented by pheophorbide $a$ ($r=-0.96$, $n=6$, $P<0.01$).

**Assimilation of organic matter**

Bulk organic matter in the aggregates was significantly assimilated by *Penaeus vannamei*, but at a low efficiency relative to pigments such as chlorophyll $a$ (Table 9). The assimilation rate of this organic material was a product of the assimilation efficiency and the rate of processing of ingesta. Due to the episodic nature of penaeid feeding, the rate at which ash was egested was a more accurate, time-averaged measure of the processing rate of detrital aggregates than was ingestion rate. For example, the mean ingestion rate measured from foregut filling was 0.81 mg ash min$^{-1}$, over 4 times the egestion rate of 0.19 mg ash min$^{-1}$. Although the experimental animals maintained full guts throughout the experiment (pers. obs.), stress could have reduced the foraging rate, yielding a biased mean rate of organic assimilation (Table 9). The maximum egestion rate observed, 17.9 mg ash hr$^{-1}$, yielded a digestion rate of 0.734 mg organic matter hr$^{-1}$. There were no
Figure 10. Differences among Fecal Fluorescence Chromatograms. Feces from these two animals show extremes of variation in concentrations of pheophorbide $a$ and pheophytin $a$-like pigments. Peak identifications as in Figure 8.
significant correlations between animal weight, egestion rate and assimilation efficiency (all $P>0.05$).

### Table 9. Assimilation of Organic Matter in Detrital Aggregates

<table>
<thead>
<tr>
<th></th>
<th>Initial</th>
<th>Feces</th>
<th>Calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass ratio</td>
<td>0.281 (0.002)</td>
<td>0.240 (0.003)</td>
<td>$P&lt;0.01^b$</td>
</tr>
<tr>
<td>(organic:ash)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assimilation</td>
<td>...</td>
<td>...</td>
<td>14.60</td>
</tr>
<tr>
<td>efficiency (%)</td>
<td></td>
<td></td>
<td>(0.96)</td>
</tr>
<tr>
<td>Egestion rate</td>
<td></td>
<td>11.66</td>
<td>...</td>
</tr>
<tr>
<td>(mg ash hr$^{-1}$)</td>
<td></td>
<td>(1.46)</td>
<td></td>
</tr>
<tr>
<td>Assimilation</td>
<td></td>
<td>...</td>
<td>0.478</td>
</tr>
<tr>
<td>rate (mg organic hr$^{-1}$)</td>
<td></td>
<td></td>
<td>(0.06)</td>
</tr>
</tbody>
</table>

*Values are means (SE), $n=6$.  
^bOne-tailed t-test.  
^cCalculated using mass ratios as $(1 - \text{feces/initial mean}) \times 100$.

### Discussion

**Alteration of pigments during digestion**

*Penaeus vannamei* appears similar to planktonic copepods (Kiorboe and Tiselius 1987, Conover *et al.* 1986, Lopez *et al.* 1988) in that digestion of microalgae lead to a loss of total chlorophyll $a$-related pigments. This phenomenon has been attributed to digestive conversion of the chlorin ring to a non-fluorescing form (Conover *et al.* 1986), although the enzymatic pathway has not been specified. However, destruction of up to 17% of total chlorophyll $a$-related pigments by
intracellular enzymes has been reported to immediately follow disruption of diatoms (Owens and Falkowski 1982). If endogenous microalgal enzymes were responsible for much of the chlorophyll transformation seen in this experiment, any actual ingestion selectivity might have been masked, and loss of chlorophyll $a$ during gut transit could overestimate the actual digestion of biomass by the shrimp. However, in $P. \text{vannamei}$ feces pheophorbide $a$ was disproportionately reduced, opposite the pattern produced by endogenous algal enzymes (Owens and Falkowski 1982). This indicates that the loss of total chlorophyll $a$-related pigments was at least partly due to digestion by the shrimp.

The variability among $P. \text{vannamei}$ in the production of pheophytin $a$-like pigments is consistent with the large individual variation in gut enzyme activities reported for penaeids (Lee et al. 1984, Lee and Lawrence 1982), and suggests that shrimp digestive enzymes dominated pigment degradation. The reciprocal relationship between the pheophytin $a$-like pigments and pheophorbide $a$ implies that the former are intermediate or alternative degradation products. A similar inference has been drawn from ratios of pheophytin $a$ and pheophorbide $a$ associated with zooplankton grazing (Vernet and Lorenzen 1987).

The absence of detectable breakdown products of carotenoids is surprising in light of the numerous derivatives reported from large-bodied planktonic grazers (Nelson 1989). The overall loss of carotenoids is consistent with the destruction of these pigments observed in studies of copepods, euphausids and fish (Head and Harris 1992, Repeta 1989). Non-specific esterases, reported from penaeids (Lee et al. 1984, Lee and Lawrence 1982), and suggests that shrimp digestive enzymes dominated pigment degradation. The reciprocal relationship between the pheophytin $a$-like pigments and pheophorbide $a$ implies that the former are intermediate or alternative degradation products. A similar inference has been drawn from ratios of pheophytin $a$ and pheophorbide $a$ associated with zooplankton grazing (Vernet and Lorenzen 1987).

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al. 1984), are known to degrade carotenoids (Jacobs et al. 1982). However, fucoxanthin was only slowly degraded by these enzymes (Jacobs et al. 1982), which may explain the lower digestion efficiency of xanthophylls relative to β-carotene and the chlorophylls.

The diatoxanthin concentration in microalgae is known to decrease in the dark (Brunet et al. 1993) but to increase during cell senescence (Klein 1988). Neither process corresponds to the observed increase in fecal levels of this pigment. It is possible that an unknown carotenoid degradation product coeluted with the feces diatoxanthin peak, leading to a spurious value.

Resource value of microalgae

Not all of the microalgal cells in detrital aggregates were available to Penaeus vannamei. Chlorophyll a was 63% degraded, indicating that approximately 37% of the microalgal biomass remained in intact cells. The difference between these results and the 87% digestion efficiency reported previously for penaeids feeding on diatoms (Moriarty 1976) is more likely related to the mechanism of ingestion than to differences in digestive processes. In this experiment most changes in pigment concentrations were evident in the foregut samples and therefore reflected cell disruption associated with ingestion. Unconsolidated and soft foods, such as detrital aggregates, may be swallowed by penaeids with little grinding by the mandibles (Hunt et al. 1992), whereas algin-bound clumps of diatoms (Moriarty 1976) could stimulate more mandibular processing. Microalgae in detrital
aggregates that survived ingestion may have been protected from digestive attack by resistant capsules of extracellular polysaccharides (Decho 1990).

The ability of *Penaeus vannamei* to utilize microalgae in detrital aggregates was also limited by the lack of selective feeding. The concentration of total chlorophyll *a*-related pigments indicates that *Penaeus vannamei* did not significantly select microalgae from the detrital aggregates tested here. Shrimp were observed to sweep in detrital aggregates with the third maxillipeds and to manipulate them into a bolus prior to ingestion. Unlike sandy substrata, detrital aggregates have fine mineral grains and a gelatinous texture (Bowen 1981) which may preclude rapid separation of embedded diatoms. Fiddler crabs (*Uca* sp.) can achieve a tenfold concentration of organic matter from sand but this ability essentially disappears when they feed on mud substrata (Teal 1962). Detailed examination of penaeids mouthparts (Alexander and Hindley 1985) have shown that they lack the specialized setae which are used by some other decapods to concentrate sedimentary biomass (Robertson and Newell 1982b). However, preparation of these aggregates removed all particles >60 μm. Because penaeids can reject sand particles in food, while the relative proportions of organic matter in ingesta should be stable, some selection of organic over inorganic material is probable in the field.

Microalgae have been implicated as nutritional resources for juvenile penaeids both as free cells (Gleason and Wellington 1988, Cockcroft and McLachlan 1986) and as components of detrital aggregates (Moss *et al.* 1992, Robertson 1988, Hunter *et al.* 1987). Stable isotope profiles of juvenile penaeids are consistent with
epiphytic and benthic microalgae as the major carbon source in several systems (Stoner and Zimmerman 1988, Kitting et al. 1984, Anderson et al. 1987). This carbon could be acquired from algal-dependent prey or directly, but larger juveniles probably lack the ability to grasp dispersed microalgal cells. The results presented here suggest that because microalgae are ingested at ambient densities and are only partly digestible, significant direct consumption of microalgal carbon would be dependent on the availability of high-biomass aggregates or algal mats.

Juvenile *Penaeus vannamei* could survive and grow on a diet of detrital aggregates such as those used here. In a review of deposit-feeder studies spanning three phyla, Cammen (Cammen 1980b) found that ingestion rate at 15°C was essentially a function of body size only, and was described by the equation:

\[
C = 0.381 \times W^{0.742}
\]

where \(C\) is mg organic matter ingested day\(^{-1}\) and \(W\) is mg dry body weight. This model, corrected for the 9 °C temperature differential using a \(Q_{10}\) of 2, predicts that a 1.4 g shrimp, with a dry weight of approximately 280 mg (Menz and Bowers 1980), should ingest 45 mg of sedimentary organic matter day\(^{-1}\). If we assume that the maximum egestion rate in this experiment (17.9 mg ash hr\(^{-1}\)) approximates an undisturbed feeding rate in the field, then it would take 8.9 hours for *P. vannamei* to ingest this amount from these detrital aggregates. The assimilation efficiency on
organic matter found here approximates the 15% average of the deposit feeders used
to derive the model (Cammen 1980b).

As well as being quantitatively adequate, the organic matter assimilated was
probably nutritionally adequate to support growth. The digestion of microalgae can
account for most, if not all, of the organic matter assimilated from these detrital
aggregates. Microalgae, especially diatoms, are known to be excellent food for
macrofauna because they contain high concentrations of protein, energy and essential
fatty acids (Hunter et al. 1987, Phillips 1984). Assuming a minimum
phytocarbon:chlorophyll a ratio of 30 for benthic diatoms (deJonge 1980) and an
organic matter:phytocarbon ratio of 1.98 (Condrey et al. 1972), microalgae initially
represented at least 0.096 mg organic matter mg\(^{-1}\) ash, or 34% of the organic matter
in detrital aggregates. If assimilation of microalgal cell contents equalled the
degradation of chlorophyll a, 63% of the microalgal biomass was assimilated, which
would leave a fecal mass ratio of 0.220 mg organic matter mg\(^{-1}\) ash. Although this
figure is lower than the measured value in Table 9, it is probably an underestimate
because it assumes complete assimilation of digested biomass and ignores
contributions of shrimp organic matter, such as peritrophic membrane, to the feces.
These calculations suggest that the non-algal organic matter in the aggregates was
relatively refractory.

Accessible microalgae are attractive foods even for larger juvenile penaeids
that are considered predominantly carnivorous (e.g., Penaeus aztecs; Kitting et al.
1984). Detrital aggregates rich in microalgae reflect the high ratio of protein to
energy (P:E) of these cells (Hunter et al. 1987), but the admixture of less digestible detritus and inorganic matter reduces the density of food energy per unit weight or volume.

Aggregates containing microalgae are thus nutritionally adequate but dilute foods. Penaeids are unusual in being able to forage and ingest small items simultaneously (Hindley 1975), so handling costs of small aggregates may be minimal. The guts of penaeids often contain a mix of prey and less nutritive material (Stoner and Zimmerman 1988, Chong and Sasekumar 1981, Marte 1980). As long as prey are not displaced, whenever prey capture rate is insufficient for a foraging penaeid to maintain a full gut, consumption of encountered detrital aggregates would benefit the animal by increasing the rate of food assimilation.
CHAPTER 5. DISCUSSION

The discussions presented in each chapter have reviewed the experimental results in the context of similar research by other workers, and have discussed implications of the data without reference to experiments in other chapters. This chapter integrates these results while addressing the overall focus of study framed in the introduction: to what extent are each of the dominant organic components of detrital aggregates potential nutritional resources for omnivorous consumers?

EPS digestion and the potential utility of amorphous detritus to omnivorous consumers

The first hypothesis, proposing that bacterial EPS was digested and assimilated by Penaeus vannamei, was corroborated in Chapter 2. The fact that penaeids can utilize microbial EPS suggests that these and other omnivorous consumers could shunt significant microbial production directly to higher trophic levels. While previous work indicated that this abundant resource was assimilated by unpalatable detritivores (Baird and Thistle 1986) and meiofauna (Decho and Moriarty 1990), penaeids and similar generalists are abundant and favored prey for larger predators (Salini et al. 1990). The potential nutritive role of this type of amorphous detritus appears to be much greater than that of morphous detritus (Mann 1988), which penaeids, for example, are unable to assimilate (Gleason 1986, Stoner 85
Assessment of the importance of this finding to trophic structure in shallow coastal ecosystems depends upon two major unknowns: the degree to which bacterial EPS models amorphous detritus available to abundant omnivorous consumers, and the abundance of amorphous detritus in their diets.

Amorphous detritus in shallow, vegetated marine habitats has been inadequately sampled (Mann 1988) and may or may not be primarily of microbial origin. In coastal environments with low or variable salinity and freshwater input, flocculation of freshwater DOC could make abiotically-generated amorphous detritus available to animals that feed on suspended and benthic particles. Such flocculation occurs up to salinities of 25‰ (Sholkovitz 1976) and has been suggested to underlie food web patterns in some temperate embayments (Simenstad and Wissmar 1985). However, most freshwater DOC does not precipitate (Mulholland 1981) and the compounds which do are in some cases humic substances (Sholkovitz 1976, Mulholland 1981) of poor food value (Ahlgren and Bowen 1991). In contrast, condensed leachates from phytoplankton and angiosperms in the immediate environment of the consumer are less likely to have undergone chemical transformation and are thus more likely to be digestively labile. Although these materials have been produced experimentally (Riley 1963, Baylor and Sutcliffe 1963, Jensen and Sondergaard 1982, Camilleri and Ribi 1986) their presence in natural systems is difficult to confirm or quantify.

Amorphous detritus composed primarily of EPS should be relatively common in shallow benthic and epiphytic microhabitats. Where tidal action exposes and
covers mudflats or sandy areas, EPS from the benthic diatom population can be lifted off and form aggregates that either remain suspended or settle to the bottom (Ribelin and Collier 1979, Hicks 1988). Epiphytic microalgae on substrata such as Spartina include diatoms (Gleason 1986) known to exude EPS. These epiphytes form mats that are grazed by penaeids and other omnivores (Kitting et al. 1984). When sediments are disturbed by animal foraging or wave action the microbial EPS binding the sediments is released as particulate material (Watling 1988). Senescent phytoplankton can release copious EPS as they aggregate and settle to the sediment surface in shallow waters (Kranck and Milligan 1988).

The EPS used here can be considered a realistic model for the amorphous detritus available to penaeids and other benthic marine macrofauna. The EPS of Alteromonas atlantica closely resembles that in sediments (Uhlinger and White 1983, Baird and Thistle 1986). Research cited above suggests that in shallow vegetated habitats, particularly those without extensive freshwater inputs, microbial EPS is an important if not the dominant type of amorphous detritus. As discussed in Chapter 1, adsorption of dissolved organics to EPS may create amorphous detritus containing features of both EPS and abiotically-condensed DOC. Digestion of the EPS polymer, in addition to pH changes in the guts of consumers, should facilitate desorption and assimilation of EPS-associated organic compounds by consumers. Therefore the ability of penaeids to digest bacterial EPS potentially affects the availability of adsorbed detrital compounds as well.
The quantitative importance of amorphous detritus in the gut contents of macrofauna is difficult to assess for several reasons. First, the concept of amorphous detritus itself is barely a decade old and is still unfamiliar to many marine scientists. Consequently emphasis has been placed on identifiable prey and morphous detrital particles. Except when it attracts attention by predominating in gut contents, amorphous detritus likely has been frequently overlooked. Second, some methods of quantifying gut contents are biased against transparent, fragile food items in that they employ sieves and repeated washing (Carr and Adams 1972). Third, no chemical constituent is common to all amorphous detritus, obviating a standardized assay. Fourth, in crustaceans, grinding of food by molar-like mandibles and the chitinized foregut makes a large proportion of the ingested biomass into an amorphous mass (Dall et al. 1990, p. 316).

Species which are known to ingest amorphous detritus are chiefly those that ingest large amounts and therefore appear unusual, such as menhaden (Lewis and Peters 1984), tilapia (Bowen 1979a), Prochilodus (Bowen et al. 1984), and juvenile Penaeus merguensis (Robertson 1988). Because amorphous detritus appears to be generally digestible, many more species probably ingest lesser amounts during omnivorous feeding. To determine the extent of feeding on EPS-based amorphous detritus, the gut contents of omnivores could be screened using a stain specific for acid polysaccharides, such as alcian blue (Gurr 1966). Quantification of EPS content would require GC/MS analysis (Fazio et al. 1982). A better approach might be to use these methods to determine which environmental substrata, plant surfaces,
aggregates and sediment types were rich in EPS or other amorphous detritus and then to observe which animals fed upon them (Kitting et al. 1984). A possible technique for separating amorphous detritus from sediment or other samples is high-speed density gradient centrifugation using an inert, viscous agent such as Percoll-sorbitol (Alongi 1990).

Indirect evidence for the ingestion of amorphous detritus could be obtained by examining gut contents for indigestible correlates of amorphous detritus or by assaying the consumer animal for some signature due to assimilation. Fine sediment particles or plant fragments, if reliably coated or associated with amorphous detritus during environmental sampling, might be used for preliminary screening of gut contents but would not yield quantitative information. Assays for stable isotope signatures may be feasible if the amorphous detritus present had a different δ¹³C or δ¹⁵N signature than the animals’ other food items. Although bacterial EPS would reflect the dissolved substrates metabolized (Coffin et al. 1989) and diatom EPS the ambient dissolved inorganic carbon value, condensed DOC might possess a signature distinctive enough to be traceable in consumer tissues. It is known, however, that flocculent material flowing into estuaries changes its δ¹³C signature as it moves seaward (Eisma 1986). Another potential assay is for chromium (Cr) metal, specifically Cr(III). This and other metallic ions can bind reversibly to microbial EPS (Bremer and Loutit 1986). However, like stable isotopic signatures, metals can be acquired through predation as well as primary consumption, and can only suggest
ingestion of amorphous detritus. In addition, EPS-bound metals could be absorbed by consumers without digestion or assimilation of the EPS substrate.

**Digestion of bacteria and microalgae by *Penaeus vannamei***

The measurements of microbial digestion in Chapters 3 and 4 falsified the hypothesis, based on previous research (Hood and Meyers 1973, Moriarty and Barclay 1981, Condrey et al. 1972), that bacteria and microalgae were efficiently digestible by *Penaeus vannamei*. When present in detrital aggregates, these cells were apparently less vulnerable to the digestive processes of penaeids than was the case in more artificial trials. Possible reasons for these lower digestion efficiencies involve intrinsic differences in the cells and atypical digestive processing by the shrimp. The bacteria presented to the shrimp by Hood and Meyers (1973) and Dobbs and Guckert (1988b) were cultured in liquid media, which reduces or eliminates (Costerton et al. 1981) the EPS capsules and biofilms characteristic of sedimentary bacteria (Moriarty and Hayward 1982). These capsules may afford some protection against the osmotic and ionic environment of the digestive tract (Decho 1990). Judging by the low digestion efficiency of EPS, cells surrounded by a capsule or biofilm would be partly protected from enzymatic attack as well.

The feeding protocol for these earlier experiments could also have artificially raised the digestion efficiency. Cells were presented in a highly digestible binder and the animals were given no other foods for many hours after the feeding (Hood
and Meyers 1973, Moriarty and Barclay 1981). Under these conditions, digestive throughput (i.e., fecal production) slows or stops and the pH of the foregut can fall to unusually low levels (Hood and Meyers 1973). Because juvenile shrimp in the field feed more or less continuously (McTigue and Feller 1989) on material with a large fraction of indigestible material (Marte 1980, Stoner and Zimmerman 1988), the digestion estimates from my experiments probably more nearly represent the situation in nature.

An interesting difference between the lipid and pigment experiments was the discrepancy in the digestion efficiency estimates for microalgae. Destruction of chlorophyll \(a\) indicated a 63% digestion efficiency of microalgae (Chapter 4), but concentrations of phytol and the diatom marker PLFA 20:5\(ω3\) decreased less than 40% and 30%, respectively (Chapter 3). Because phytol is a constituent of chlorophyll \(a\) and is not present in penaeids, pigment and phytol values would be expected to be comparable even if the biomarker PLFA were affected by a contribution of shrimp lipids to the feces. Three potential explanations for the different estimates involve experimental protocol, digestive processing rates and the microalgal populations involved.

The major procedural variation was that the feces in the pigment experiment was two hours older that the lipid feces prior to being frozen. Pigment degradation during this time is unlikely to have caused the higher digestion estimate because most of the pigment changes occurred immediately after ingestion (Table 8). A slower rate of digestive processing is associated with higher digestion efficiencies in
invertebrates feeding on microbes (Plante et al. 1990). However, although the rates of processing of detrital aggregates did differ between the experiments, it was in the direction opposite that predicted from this effect. The *P. vannamei* in the pigment experiment produced a mean (±SD) of 37.3 (±9.7) mg feces ash hr⁻¹, whereas the animals in the lipid experiment processed 12.5 (±3.9) mg feces ash hr⁻¹. These values are significantly different (P=0.005, t-test) and indicate that the microbes in the lipid experiment were exposed to the digestive process longer than were the pigment samples.

The most plausible explanation for the difference in digestive efficiencies is that the microbial communities in the two experiments were quite different. Microscopic examination of the lipid aggregates indicated that most diatoms were moribund, consistent with the low estimate for eukaryotic biomass. It is possible that surviving microalgae were more resistant to disruption or digestion by virtue of size or taxonomic group. Green algae, for example, are known not to be assimilated by penaeids (Gleason 1986).

**Detrital aggregates as food**

Detrital aggregates are formed by the accretion of small particles rendered sticky by coatings of microbial EPS or other type of amorphous detritus (Eisma 1986, Logan and Wilkinson 1990). Most aggregates sampled in marine systems, even those produced abiotically, are soon colonized by bacteria (Camilleri and Ribi
As producers of EPS, microbes such as bacteria and diatoms are integral to the formation of macroscopic detrital aggregates. Animals eating detrital aggregates therefore necessarily ingest amorphous detritus and microbes together. In addition to being a digestible resource, EPS makes microbial biomass accessible to animals unable to harvest free cells from the water column or sediment. These aggregates are readily eaten by *Penaeus vannamei*, and appear to support growth in penaeids (George 1974), fish (Bowen 1979a) and other animals. Their potential value as foods is dependent on their content of protein, energy and micronutrients (although strictly speaking animals require the subunits of protein, amino acids, in their diet, I will use protein to include peptides and free amino acids).

Food consumption in animals under normal circumstances is thought to be regulated specifically to supply energy needs (Bowen 1979a). Assuming an animal is able to ingest sufficient food to satisfy those needs, the amount of digestible protein consumed depends on the ratio of digestible protein to digestible energy in the diet. Thus, the basic nutritional value of a given diet depends on its ratio of protein (P) to energy (E) (Bowen 1987). The optimum P:E ratio varies slightly but is remarkably similar across phyla (Bowen 1987). When the dietary ratio is below this optimum, satisfaction of energy needs results in insufficient protein for maximum growth. A supraoptimal ratio can result in reduced growth, presumably as a result of the energetic cost of protein digestion and metabolism (Sedgwick 1979).

For animals that ingest detritus containing a high concentration of carbohydrate (e.g., cellulose, EPS) to microbial protein, efficient digestion of the
carbohydrate can and does result in malnutrition based on insufficient protein (Bowen 1979a). Conversely, metazoans that feed exclusively on protein-rich microbial cells could be incurring a metabolic penalty. It is possible that the highly efficient digestion of bacterial EPS by a bacterivorous harpacticoid copepod (Decho and Moriarty 1990) allows this animal to more closely approach an optimal P:E. Although it is possible that micronutrients such as essential fatty acids and vitamins could limit growth in nature, evidence for such limitation is lacking (Bowen 1987).

The energy density (kJ mg\(^{-1}\)) of a food determines the amount that must be ingested to satisfy the energy requirements of an organism. Lipids in food are assumed to be readily digestible. Because much of the carbohydrate in plants and morphous detritus is indigestible by metazoans, techniques such as bomb calorimetry reveal little about the utility of detrital energy. Standard methods to measure digestible carbohydrates apply chiefly to mammals and fish with acid stomachs (Bowen 1979a) and are of limited value in assessing the actual resource available to animals, such as penaeids, which employ enzymatic digestion.

Few researchers have accurately measured the digestible protein and energy (i.e., carbohydrate, lipid) content of detrital aggregates. Some (Qasim and Sankaranarayanan 1972) have estimated protein from total N, which may yield spurious values because of the sometimes high proportion of indigestible N compounds in detritus (Rice 1982). Based on limited data, the P:E of detrital aggregates appears to vary greatly among, and even within, environments (Table 10; Bowen 1979a). Samples from marine environments appear to have higher P:E
values than those from lakes, which are reported to have few associated microbes (Bowen 1979b). These freshwater aggregates appear to be predominantly amorphous detritus (Bowen 1979b), but their mode of formation has not been described in detail. Pure EPS, such as that used in the experiment in Chapter 2, has a low P:E (Decho and Moriarty 1990, Corpe 1970), although EPS in aggregates could have a higher ratio due to adsorbed compounds (Decho 1990). Because microbial cells have a high P:E (Table 10), differences in the relative content of biomass and amorphous detritus can vary the P:E of detrital aggregates over a wide range (Bowen 1979a).

Detrital aggregates in diets of omnivores

Penaeids, like goldfish (Bowen 1987) and probably many other animals, have an optimum dietary P:E of approximately 17 mg kJ\(^{-1}\) (Table 10). Omnivores such as penaeids are potentially able to achieve this optimum and simultaneously minimize the amount of prey to be captured by mixing animal prey, which have a much higher P:E than optimum, with plant or detrital foods containing a suboptimal P:E. This optimizing process, termed "protein sparing" because it allows protein to be used for growth rather than energy, has been criticized as unlikely to occur in nature (Dall et al. 1990, p. 169). However, *Penaeus esculentus* have been observed to preferentially ingest the energy-rich seeds of *Zostera capricorni* when they are
Table 10. Nutritional composition of some potential foods of penaeid shrimp

<table>
<thead>
<tr>
<th>Source</th>
<th>Protein Energy (mg kJ⁻¹)</th>
<th>Energy Density (kJ g⁻¹)</th>
<th>Protein</th>
<th>CHO⁺</th>
<th>Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimum Penaeid P:E ²</td>
<td>17.1</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Plant material</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Planktonic diatom³</td>
<td>35.4</td>
<td>11.3</td>
<td>40.0</td>
<td>4.7</td>
<td>2.6</td>
</tr>
<tr>
<td>Shrimp feed MR-25⁴</td>
<td>13.3</td>
<td>10.8</td>
<td>14.4</td>
<td>27.4</td>
<td>6.8</td>
</tr>
<tr>
<td>Zostera seeds⁵</td>
<td>7.2</td>
<td>14.0</td>
<td>10.0</td>
<td>60.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Animal material</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crustacean⁶</td>
<td>33.1</td>
<td>8.2</td>
<td>27.0</td>
<td>3.0</td>
<td>3.2</td>
</tr>
<tr>
<td>Bivalve²</td>
<td>30.3</td>
<td>18.9</td>
<td>57.2</td>
<td>20.4</td>
<td>4.6</td>
</tr>
<tr>
<td>Detrital aggregates (DA), etc.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shrimp pond DA⁶</td>
<td>34.3</td>
<td>4.3</td>
<td>14.8</td>
<td>1.1</td>
<td>1.6</td>
</tr>
<tr>
<td>Mangrove DA⁶</td>
<td>17.7</td>
<td>6.1</td>
<td>10.9</td>
<td>7.1</td>
<td>6.0</td>
</tr>
<tr>
<td>Lake, site 1 DA³</td>
<td>3.9</td>
<td>5.9</td>
<td>2.3</td>
<td>9.5</td>
<td>9.5</td>
</tr>
<tr>
<td>Lake, site 2 DA³</td>
<td>3.2</td>
<td>3.7</td>
<td>1.2</td>
<td>6.6</td>
<td>5.8</td>
</tr>
<tr>
<td>Alteromonas EPS¹</td>
<td>2.3</td>
<td>11.0</td>
<td>2.5</td>
<td>60.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Penaeid diets</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Zostera seed⁷</td>
<td>29.2</td>
<td>--</td>
<td>74.4</td>
<td>11.1</td>
<td>14.5</td>
</tr>
<tr>
<td>With Zostera seed⁷</td>
<td>27.1</td>
<td>--</td>
<td>66.0</td>
<td>21.0</td>
<td>13.0</td>
</tr>
<tr>
<td>Shrimp pond guts⁸</td>
<td>19.6</td>
<td>4.9</td>
<td>9.6</td>
<td>4.2</td>
<td>4.8</td>
</tr>
</tbody>
</table>

⁺Carbohydrate.
²Sedgwick (1979)
³Hunter et al. (1987)
⁴Dall et al. (1992)
⁵Penaeus vannamei, Hunter et al. (1987)
⁶Mytilus edulis, Sedgwick (1979)
⁷Qasim and Sankaranarayanan (1972). Published protein value divided by 3 to correct for humic substances (Bowen 1979).
⁸Bowen (1979)
⁹Decho and Moriarty (1990), Corpe (1970)
available (Dall et al. 1992). Budget calculations of diets with and without seeds show that protein sparing takes place in this instance (Dall et al. 1992, Table 10). Because of the low protein content of EPS (Decho and Moriarty 1990) and other types of amorphous detritus (Bowen 1979b), omnivores that efficiently digest amorphous detritus could use this resource for dietary energy if captured prey supplied adequate protein. This appears to be the basis for the mixing of detritus and invertebrate prey in the diet of the freshwater fish *Catostomus commersoni* (Ahlgren 1990b). The benefit from ingestion of such detritus is presumably that, compared to prey, detritus and detrital aggregates are freely available with little or no investment in search or capture behavior (Ahlgren 1990b).

The digestibility of the organic components of detrital aggregates determines the available P:E and energy density. The latter will be some fraction of the total caloric energy measured chemically, but the P:E may be higher or lower than the analyzed value, depending on the relative digestibilities and relative abundance of the component microbes and detritus. Only if all components had equal digestion efficiencies would the available P:E correspond to the analytically-determined value. The digestion efficiencies measured for *Penaeus vannamei* suggest that aggregates eaten by shrimp will have an available P:E higher than that measured chemically. Roughly 60% of the microbial biomass and 16% of the EPS were digested in these trials, meaning that EPS would have to be 3.75 times more abundant than microbes in aggregates in order for an equivalent mass to be digested. The P:E ratios for detrital aggregates in aquaculture ponds and mangrove lagoons (Table 10) suggest
that relative to freshwater aggregates, those in penaeid habitats are low in EPS. For animals with low digestion efficiencies on amorphous detritus, the available P:E of these detrital aggregates will exceed the dietary optimum, and ingestion of aggregates will not offer an opportunity for protein sparing. In terms of macronutrient balance, such aggregates will be similar to prey animals such as crustaceans and bivalves (Table 10).

Although detrital aggregates may have a high available P:E, the admixture of inorganic material and indigestible morphous and amorphous detritus ensures that the available energy density is low for consumers such as penaeids. Depending on whether the dietary energy is predominantly in amorphous detritus or microbial biomass, the available energy in aggregates would be between 16% and 60% of that determined by analysis, placing aggregates below other potential foods in energy density (Table 10). *Penaeus vannamei* was apparently unable to compensate for low energy density by concentrating biomass during ingestion (Chapter 4).

In terms of food value and energy density, detrital aggregates can be conceived of as "dilute prey" which can substitute for, rather than energetically complement, actual prey. Penaeids could ingest only detrital aggregates and not encounter protein-deficiency malnutrition, unlike some fish (Bowen 1979a). In fact, penaeids will survive for indefinite periods on such a diet (Qasim and Sankaranarayanan 1972). However, reliance on detrital aggregates alone would probably hinder growth due to restriction of energy intake. The survival and eventual reproduction of penaeids is closely tied to growth rate (Haywood and
Staples 1993). For species such as *Penaeus vannamei*, in which larger juveniles are strictly nocturnal (Moctezuma and Blake 1981), detrital aggregates may have too little digestible organic matter to meet energy requirements during a restricted foraging period (Chapter 4).

Omnivores such as penaeids are not restricted to detrital aggregates, but can and do ingest prey when available. If basic energy requirements can be met, a combination of prey and detrital aggregates is probably more efficient than a strictly carnivorous diet. Shrimp forage by walking and testing the substratum with multiple appendages, which can independently probe and bring small particles to the mouth (Hindley 1975). Thus detrital aggregates can be encountered, tasted (Hindley and Alexander 1978), and ingested with little or no interruption of predatory search behavior, and with apparently low energy expenditure in buccal processing (Hunt et al. 1992). Unless aggregates are eaten to such an extent that they preclude ingestion of encountered prey, there appears to be little cost and definite benefit to ingestion of aggregates during foraging. Experiments with *Penaeus vannamei* or *Penaeus merguensis* (Robertson 1988) on diet mixing and food preferences, similar to those performed on fish by Ahlgren (1990a), would help to determine the behaviors underlying the dietary patterns observed in nature and in culture.
Implications for aquaculture and penaeid diets

Juvenile penaeids in nature (Robertson 1988) and in culture (Hunter et al. 1987) can ingest large amounts of detrital aggregates in addition to more nutritious food items. Such aggregates alone are sufficient for survival and growth of penaeids (Qasim and Easterson 1974), although the realized growth rates are probably low. That the microbial biomass and amorphous detritus in these aggregates are available and valuable foods is revealed by stable isotope studies of aquaculture ponds (Lilyestrom and Romaine 1987, Schroeder 1987, Anderson et al. 1987), which have shown that a half or more of the carbon in omnivorous fish and crustaceans is produced by pond microalgae. The gut contents of *Penaeus vannamei* in aquaculture ponds clearly resemble detrital aggregates more than they do applied feed (Table 10; Hunter et al. 1987).

Because detrital aggregates are very dilute sources of energy for penaeids, large amounts of aggregates in gut contents might inversely correlate with energy intake and thus growth rate. Due to circadian differences in feeding behavior and activity (Vance 1992), researchers performing diet studies should integrate gut contents over 24 hr periods before constructing food budgets. Unless food is available *ad libitum* (Hill and Wassenberg 1987), feeding by penaeids appears to be continuous during foraging (McTigue and Feller 1989). Consequently, assuming prey are generally preferred over aggregates, the proportion of aggregates or other...
detritus in the gut (e.g., Stoner and Zimmerman 1988) can be used as a crude index of the rate of prey (or feed pellet) encounter during foraging.

For aquaculture managers, the high proportion of detrital aggregates commonly observed in guts indicates that the carefully formulated feed is not encountered at a sufficient frequency to be the dominant food. Until this problem is solved by improvements in feed application techniques, aquaculturists could take advantage of the properties of detrital aggregates in feed formulation. Specifically, feeds could have lower than optimal P:E in order to take advantage of the superoptimal P:E of detrital aggregates. This would not be the case if the biomass component of aggregates were dominated by indigestible organisms such as chlorophytes or cyanobacteria (Gleason 1986). The presence of such microbes would lower both the available P:E and the energy density of aggregates.

To more easily explore the dietary value of amorphous detritus and microorganisms to omnivores and deposit feeders, an in vitro model system of invertebrate enzymatic digestion should be developed. Analytically-derived values for protein, lipid and carbohydrate composition of potential foods presently are poor indicators of the value of these foods to animals such as penaeids. Until such a system is developed, potentially useful estimates of actual food value could be obtained from the nucleic acid ratio method, in which short term growth responses to foods can be measured (Moss 1993).


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