



Padilla Bay

National Estuarine Research Reserve

Reprint Series No. 25
Reprinted May 1996

**MANILA CLAM (VENERUPIS PHILIPPINARUM)
UTILIZATION OF RADIOLABELLED DIETS COMPOSED
OF PHYTOPLANKTON OR DETRITAL PARTICULATES**

Sandra V. Palm

February 1996

Publication No. 96-105

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DETRITAL PARTICULATES

A Thesis

Presented to

The Faculty of

Western Washington University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Sandra V. Palm

Bibliographic citation: Palm, Sandra V. 1996. Manila clam (*Venerupis philippinarum*) utilization of radiolabelled diets composed of phytoplankton or detrital particulates. Master's Thesis. Western Washington University, Bellingham, Washington. 76 pp. Padilla Bay National Estuarine Research Reserve Reprint No. 25, Reprinted May 1996.

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**By
Sandra V. Palm**

**Accepted in Partial Completion
of the Requirements for the Degree
Master of Science**



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MANILA CLAM (VENERUPIS PHILIPPINARUM)
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ABSTRACT. Laboratory feeding experiments with ^{14}C tracers demonstrated that adult Manila clams (*Venerupis philippinarum* (Adams & Reeve, 1850)) could ingest and absorb detrital particulates from both *Ulva fenestrata* and *Zostera marina*. Only about one-third the quantity of *Ulva* particulates were ingested compared with phytoplankton diets consisting of *Chaetoceros gracilis* or *Isochrysis galbana*. About one-eighth the quantity of detrital particulates from *Ulva* or *Zostera* were absorbed into tissue compared with both phytoplankton diets. No statistical difference between treatments was detected for dietary contributions to either respiration or egestion. Patterns of gut passage time for all diets were statistically similar. Carbon budgets assessed relative contributions of diets to tissue, respiration, and egestion. The contribution of carbon to tissue was significantly different between treatments; however, contributions of carbon to respiration and egestion were not statistically different. This research quantifies that in the laboratory, adult Manila clams can utilize detrital particulates and implies participation in detrital food webs of nearshore estuarine habitats associated with mud flats and eelgrass.

ACKNOWLEDGEMENTS

This research was made possible through the generous support from several sources. Padilla Bay Foundation awarded a research assistantship. Washington Sea Grant contributed funding for a multi-channel peristaltic pump and radioisotope. Doug Doolittle and Dennis Bohrer of Biology, Ruth Schoonover of Chemistry, and Nancy Elkins of Huxley College of Environmental Studies provided equipment, supplies, and support. Staff of the Instrument Center constructed a multiple station magnetic stirrer, water bath, and other unique equipment. Dr. Gisèle Muller-Parker contributed advice, scintillation vials, and scintillation cocktail. Dr. Dave Schneider offered support and suggestions during several phases of experimental work. Dr. Emily Peele provided guidance, laboratory space, and use of equipment. Dr. Bert Webber assisted with funding, project definition, laboratory space, and piloted the process to completion.

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INTRODUCTION

The inland marine waters of Washington State nurture a diverse flora and fauna within many habitats. These waters also consist of a series of estuaries that support mud flats and eelgrass meadows in many protected areas. Within estuarine habitats, nearshore food webs unite living biota in a complex interaction between living food sources and once-living or detrital components (Simenstad *et al.*, 1979:32-34). Carbon flow is one way to trace interactions between components of food webs. In 1985, Simenstad and Wissmar, traced carbon flow in estuarine detrital food webs of Washington using the stable isotope ^{13}C . They found evidence of detrital enrichment in oysters, mussels, and native littleneck clams, all suspension feeding bivalves.

Potential food sources for suspension feeding bivalves include phytoplankton, bacteria, rotifers, and organic detritus (Jorgensen, 1955:391; Chew and Ma, 1987:12; Kozloff, 1990:427; and Sorokin and Giovanardi, 1995). The classic definition of detritus was provided by Darnell (1967:374): "all types of biogenic material in various stages of microbial decomposition which represent potential energy sources for consumer species." In contrast, other researchers consider detritus as any nonliving organic matter, primarily consisting of particulates but also including dissolved organic matter (Moriarty and Pullin, 1987). Bacteria, fungi, protozoans, and meiofauna also might form detrital associations, but they are not detritus (Moriarty and Pullin, 1987). Detrital particulates are one component of seston consisting of all particles suspended in the water column (Levinton, 1982:45; and Muschenheim, 1987). Seston includes both organic and inorganic particles. Inorganic components include particulate inorganic matter and suspended particulate matter such as sediment (Muschenheim, 1987; and Ruckelshaus, 1988:47). Organic components include, for example, phytoplankton, detritus, particulate organic carbon, and particulate organic nitrogen (Muschenheim, 1987; and Ruckelshaus, 1988:47).

Some researchers interchange the terminology for suspension feeding bivalves and filter feeders (Jorgensen, 1955:395). However, animals such as urochordates have a pharynx that serves as a filtering apparatus and suspension feeding bivalves do not (Kozloff, 1990:820). Because of this distinction, some zoologists only use the terminology of

suspension feeding when referring to eulamellibranch bivalves (Kozloff, personal communication, 1991).

The Manila clam, *Venerupis philippinarum* (Adams & Reeve, 1850) is a suspension feeding bivalve living in the intertidal region of local protected estuaries. Originally from Sendai Bay in northern Japan, the Manila clam was accidentally imported during the 1930's to the inland marine waters of Washington, along with intended imports of commercially important oyster spat, *Crassostrea gigas* (Menzel, 1991:235). During the 1950's, the clam became commercially significant and now is the major hard shell clam in Washington mariculture. The importance of the clam to world aquaculture is evidenced by cultivation in France, Italy, Japan, Canada, and the United States (Arakawa, 1989:2; Gouletquer *et al.*, 1989; and Sorokin and Giovanardi, 1995).

The scientific name of the Manila clam has a history of revision. One article documented twenty-nine synonyms for the scientific name (Ponurovsky and Yakovlev, 1992). Once called *Tapes japonica*, official documents refer to the clam as *Tapes philippinarum* or *Ruditapes philippinarum* (Adams & Reeve, 1850) (Bernard, 1983a:56; Kozloff, 1987:282; and Turgeon *et al.*, 1988:45). Recently, Carlton (1992) referred to the clam as *Venerupis philippinarum*. The name is in revision again and *Venerupis philippinarum* (Adams & Reeve, 1850) may become the official name, although *Ruditapes philippinarum* will continue as a recognized alternate (Coan, personal communication, 1993; and Paulay, personal communication, 1993).

Presently, detrital food web relationships of the estuarine Manila clam are inconclusive. Chew and Ma (1987:12) suggest detrital use by a related species, *Protothaca staminea*, although data are lacking. Using the stable isotope ^{13}C , Ruckelshaus (1988:46) documented possible enrichment of Manila clams by eelgrass (*Zostera marina*). Other research findings concerning dietary use of *Ulva* or eelgrass (*Z. marina*) by suspension feeding bivalves are limited. Stable isotope δC^{13} ratios for the bay scallop (*Argopecten irradians*) suggested that up to 30% of carbon in the diet originated from particulates generated in nearby *Z. marina* meadows (Thayer *et al.*, 1978). In 1986, Stephenson *et al.* determined that suspension feeding bivalves (*Mytilus edulis* and *Modiolus modiolus*) obtained

little carbon from *Z. marina*. Nitrogen from detrital aggregates of macrophytes (*Ulva lactuca* and *Z. marina*), was utilized by the bay scallop *A. irradians* (Alber and Valiela, 1995). By comparison, contributions of *Spartina alterniflora* detritus to suspension feeding bivalves of the eastern United States are well documented and include the ribbed mussel *Geukensia demissa* (Kreeger *et al.*, 1988), the oyster *Crassostrea virginica* (Newell and Langdon, 1986; Crosby *et al.*, 1986; Crosby *et al.*, 1990; Langdon and Newell, 1990), and the mussel *M. edulis* (Widdows *et al.*, 1979; Lucas *et al.*, 1987). Dietary use of detrital kelp is documented for the ribbed mussel *Aulacomya ater* (Stuart *et al.*, 1982), the oyster *Crassostrea gigas* (Simenstad and Wissmar, 1985), the butter clam *Saxidomus giganteus* (Simenstad and Wissmar, 1985), the jingle shell *Pododesmus cepio* (Duggins *et al.*, 1989) and the mussel *M. edulis* (Simenstad and Wissmar, 1985; Duggins *et al.*, 1989).

Padilla Bay, Washington contains a rich source of detritus originating from the green alga, *Ulva fenestrata*, and eelgrass, *Zostera marina*. Padilla Bay is part of the National Estuarine Research Reserve system and contains a 3,200 hectare seagrass meadow primarily composed of *Z. marina* and to a lesser degree, *Zostera japonica* (Bulthuis, 1991:8). Macroalgae occur throughout the bay with *U. fenestrata* and *Enteromorpha intestinalis* dominating. In a summer 1989 study, eelgrass covered most of the Padilla Bay reserve; however, at all stations, biomass for macroalgae was greater than eelgrass biomass (Bulthuis, 1991:8).

The plentiful supply of eelgrass and macroalgae in Padilla Bay suggests the possibility that both contribute to detrital loads within the bay, and these loads may contribute to natural diets of suspension feeders. A clam bed containing Manila clams is located in Padilla Bay (Webber, personal communication, 1991). The clam bed is situated near potential sources of detritus, is not subjected to the variables incurred through pressures of recreational or commercial harvest, and provides an abundant population for research.

Feeding and digestion in suspension feeding bivalves

An external view of the Manila clam is illustrated in Figure 1a. Figure 1b illustrates major features of the digestive system. Movement of cilia located on ctenidia creates currents

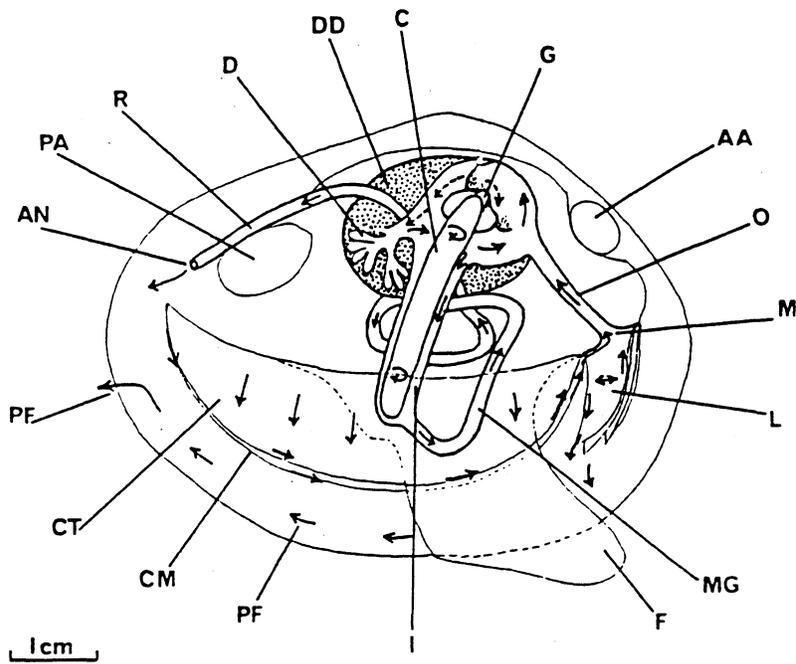
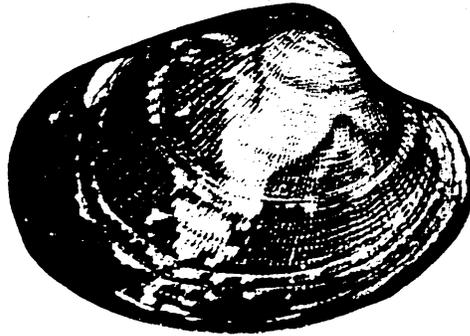


Figure 1: External view and digestive anatomy of a Manila clam.

a: External view (modified from Kozloff, 1983:293). **b:** Digestive anatomy (modified from Reid, 1982:235). Dimensions of the digestive system are exaggerated and currents of ctenidial cilia are simplified. Abbreviations:

- | | | |
|-----------------------------------|----------------------------|--------------------------------|
| aa = anterior adductor muscle | dd = digestive diverticula | m = mouth |
| an = anus | e = esophagus | mg = mid-gut |
| c = crystalline style | f = foot | pa = posterior adductor muscle |
| cm = marginal groove of ctenidium | g = gastric shield | pf = movement of pseudofeces |
| ct = ctenidium | i = intestinal groove | r = rectum |
| d = digestive tubule | l = labial palp | |

that bring prospective food, by way of the inhalant siphon, into the mantle cavity (Jorgensen *et al.*, 1986). Other ctenidial cilia discard excess particles, entrapped in mucus, as pseudofeces or direct food through food grooves terminating with the labial palps. Labial palps also can reject food masses as pseudofeces or direct food to the mouth for ingestion (Purchon, 1977: 117-129). Food moves with ciliary action through the esophagus to the digestive gland (Purchon, 1977:226).

The digestive gland of the eulamellibranch bivalve consists of a stomach with three caeca, a crystalline style located in one of the caeca, and digestive diverticula that connect to the caeca with tubules (Reid, 1982). Digestion is both extracellular and intracellular. Extracellular digestion is assisted by enzyme secretions from the crystalline style (Morton, 1983:68). As the crystalline style grinds against a chitinous plate, the gastric shield, enzymes are released and food particles are mechanically reduced in size (Purchon, 1977:227). Over twenty-one enzymes are found in the bivalve stomach and include lipases, amylase, peptidases, and the occasional cellulase (Reid, 1982). Enzyme attack of food is the first process in extracellular digestion. Large particles may continue to be ground, may be ingested by phagocytes in the caeca, or may pass via a ciliated intestinal groove to the mid-gut (Purchon, 1977:229).

Small particles pass toward the digestive diverticula through progressively smaller ciliated tubules. With each decrease in tubule size, counter currents help move large particles back to the mid-gut and move small particles towards the digestive diverticula (Purchon, 1977:232). The digestive cells are located within tubules of the diverticula and perform four functions: particles are ingested, particles are digested with enzymes, solutes produced by actions of the crystalline style are absorbed, and unwanted material is packaged in mucus for export to the mid-gut (Purchon, 1977:238-239). Most ingested food is considered to be absorbed and digested by digestive cells (Morton, 1983:89, 99).

Cilia within the intestine help move material through the regions of midgut, hindgut, rectum, and anus (Morton, 1983:101). The midgut and hindgut serve two functions. Feces are consolidated (Purchon, 1977:241) and additional absorption and digestion can occur (Morton, 1983:102). Feces can be composed of rejected food from sorting in digestive

tubules or from exocytic material formed after processing by the digestive diverticula (Reid, 1982). After feces have passed into the mantle cavity, currents generated with ctenidial cilia flush waste products out the exhalant siphon.

Although much is understood about bivalve digestive anatomy and function, ingestion mechanics are in question. The role of mucus in particle capture and transport has ramifications for particle selection (Beninger *et al.*, 1991). The traditional view of particle ingestion is expressed by Purchon (1977:121) who suggests the crystalline style helps pull mucus bound particle strings, formed on the ctenidia, into the stomach. However, Jorgensen (1955) suggests that mucus bound particles would impair the sorting mechanism of the labial palps and then goes on to suggest that particle movement is a function of hydrodynamic action of cilia alone (Jorgensen, 1993). Several researchers have reaffirmed the role of mucus assisted particle transport through observations made with endoscopes (Gallager, 1988; Beninger *et al.*, 1991 and 1992; and Ward *et al.*, 1991 and 1993). In any case, particles enter the bivalve mouth for subsequent digestion and absorption.

Ingestion, absorption, gut passage time, and carbon budgets

Ingestion addresses how much food enters the bivalve mouth for subsequent processing. Ingestion can be assessed by presenting a known quantity of food particles to an animal, accounting for production of pseudofeces, and counting the number of food particles remaining after a period of time. Perez-Camacho *et al.* (1994) used this method to determine optimum concentrations of algal cells for larvae of Manila clams. This process is suitable in closed systems, but other methods are necessary for flow through systems. Food labelled with radioactive isotopes can be accounted for in tissue, respiration, and feces. Combining these components results in ingestion, an assessment of food that is available for digestion. Ingestion also reflects particle selectivity.

Early research by C.M. Yonge concerning bivalve feeding suggested that no selection occurred, provided food particles were of the size that could be manipulated with ctenidial cilia and labial palps (Reid, 1982). Foster-Smith (1975a) found that inorganic particles were not rejected by labial palps of mussels (*M. edulis*) and hard shell clams (*Ceratoderma edule*

and *Venerupis pullastra*). Instead, the inorganic particles were ingested along with phytoplankton and did not interfere with digestion. Subsequent research demonstrated that particle selection by larval and adult bivalves does occur. Gallager (1988) observed that larvae of the hard shell clam (*Mercenaria mercenaria*), increased rejection of food particles when sated. The sea scallop (*Placopecten magellanicus*) was able to select chlorophyll containing particles from seston and selection was not based on particle size (MacDonald and Ward, 1994). Baldwin and Newell (1995) suggest that oyster larvae (*C. virginica*) vary ingestion rates depending on food volume and quality, not particle concentration nor size.

Absorption follows digestive processes and is the amount of food that becomes usable for animal functioning after diets have moved to unspecified tissues. The term absorption, as used in this study, is consistent with researchers whose modified methods provided experimental procedures (Kreeger *et al.*, 1988; and Kreeger *et al.*, 1990). By comparison, incorporation identifies specific tissues such as muscle or mantle, or tissue fractions such as lipid or protein (Kreeger *et al.*, 1988; and Kreeger *et al.*, 1990). Because of the complexity associated with bivalve digestion, the time needed for absorption can begin immediately or last for several hours (Reid, 1982). The intricate digestive diverticula can contain food that is present but not digested, absorbed, nor rejected. This food constitutes unabsorbed gut material and, given sufficient time, this material will be utilized or rejected.

Gut passage time or residence time is thought to maximize absorption and can improve nutritional benefits of lesser quality food. Gut passage time is related to gut fullness and can be a mechanism of physiological compensation (Bayne *et al.*, 1988). Bayne *et al.* (1987) found that after two weeks on an experimental diet, mussels fed diets of algae and silt increased gut passage time. This increase was thought to occur because of changes in surface area within tubules of the digestive diverticula or because of increased absorption capacity. Adaptive changes in enzyme production were suggested by Seiderer *et al.* (1982) when mussels in kelp-dominated areas increased carbohydrases to utilize available detritus.

Absorption, respiration, and fecal production relate to how food is utilized and together form a carbon budget. A carbon budget is a way to assess energy flow or energy exchanges between organisms and the environment (Kofoed, 1975b). A carbon budget also

can facilitate relative data comparisons when measurement units are different. Several researchers have quantified carbon partitions by using ^{14}C tracers (Kofoed, 1975a; Hawkins and Bayne, 1985; and Crosby *et al.*, 1989). Other researchers have evaluated energy flow by comparing ash content or joules of energy in food, tissue or waste products (Newell, 1982; and Stenton-Dozey and Brown, 1994). A carbon budget is a way of understanding relative contributions of energy to various sectors. A theoretical approach to carbon budgets can provide insight on ecological relationships. A practical approach to carbon budgets, for example in aquaculture, can evaluate food quality. Pertinent to dietary treatments, this study contains elements of the theoretical and the applied.

Experimental approach

Several approaches are available to examine dietary effects and trophic relationships. Thesis research used a ^{14}C radioactive tracer. In addition to tracers, other approaches to evaluate dietary effects include animal growth and animal biochemical content. Whereas growth and biochemical analyses focus on nutrition, tracer results can provide implications for nutrition, as well as ecological relationships. All approaches have advantages and disadvantages relative to Manila clams. Following is a summary of approaches used with bivalve research concerned with feeding, together with examples of investigatory results.

Several researchers have monitored growth. For bivalves, growth assessment typically involves measuring weight or valve dimensions for a time period in which dietary treatments are provided. Epifanio (1982) evaluated growth of juvenile oysters (*C. virginica*) given diets of single species of phytoplankton, mixed phytoplankton diets, and phytoplankton diets supplemented with yeast. Langdon (1982) assessed growth of larval oysters (*C. virginica*) that were fed experimental food capsules and found slightly less growth than resulted from phytoplankton diets. Laing *et al.* (1990) found comparable growth for juvenile Manila clams fed dried and living phytoplankton diets. Because growth of adult Manila clams is slow, considerable time is required to evaluate treatment responses. Resultant information pertains to differences in overall growth, growth rate, and provides insight to long term responses to dietary treatments. This approach can not address other aspects of food use

such as animal lipid content, allocations to respiration or egestion, or inference about mechanisms involved with food transfer.

Biochemical analyses related to bivalve nutrition usually assess tissue lipid content or amounts of particular fatty acids. Because of the role in cell structure and energy reserves, lipid content is used to evaluate animal vitality, prospective reproduction, or the nutritional value of food. Dietary treatments must be provided for a time period after which tissue is evaluated for lipid or fatty acid content. Webb and Chew (1982) detected the presence of suitable long chain fatty acids in phytoplankton food sources and tissues of juvenile oysters (*C. gigas* and *C. virginica*). Laing *et al.* (1990) assessed long chain fatty acids in phytoplankton food sources and tissues of juvenile Manila clams. Based on fatty acid analysis, Ackman (1982) evaluated the suitability of various phytoplankton as diets for a suite of bivalves (*C. gigas*, *C. virginica*, *M. edulis*, *Ostrea edulis*, and *Pecten maximus*). Advantages to this method include the ability to localize treatment response in specific tissues, and to evaluate tissue changes over time. Implications from this approach are similar to the growth approach.

To assess bivalve feeding, most tracer studies use the stable isotopes ^{13}C and ^{15}N , and the radioactive isotope ^{14}C . In the nearshore marine environment, contributions by photosynthesizers to ^{13}C are organism dependent and originate with phytoplankton, macroalgae, eelgrass, and terrestrial plants. All organisms contain ^{13}C and it is possible to compare the isotopic composition of prospective food sources with what occurs in animal tissues. High correlation suggests use of the food source in question; however, the presence of multiple sources of ^{13}C can confound decisive conclusions (Simenstad and Wissmar, 1985; and Mann, 1988).

$\delta^{13}\text{C}$ analyses provide insight to food web relationships in the field. Results of $\delta^{13}\text{C}$ analyses showed a better correlation between suspension feeding bivalves (*Argopecten irradians* and *Chione cancellata*) and suspended particulates originating from sediments in eelgrass meadows than from eelgrass detritus (Thayer *et al.* 1978). Work in Maine correlated tissue of suspension feeding bivalves (*M. edulis*, *M. mercenaria*, *G. demissa*, *Modiolus modiolus*, and *Mya arenaria*) and terrestrial plant sources, but could not explain the

mechanisms (Incze *et al.*, 1982). These researchers also quantified plasticity in feeding response by cross planting suspension feeders to other rivers and comparing $\delta^{13}\text{C}$ signatures. In New Zealand, a bivalve (*Chione stutchburyi*) located in several different types of estuaries had $\delta^{13}\text{C}$ ratios similar to the estuary of residence, but ratios differed between estuaries (Stephenson and Lyon, 1982). Simenstad *et al.* (1985) used $\delta^{13}\text{C}$ analyses to explore food web relationships in Washington estuaries. They found that suspension feeding bivalves (*C. gigas*, *M. edulis*, and *Saxidomus giganteus*) showed minimal direct enrichment from detritus originating in eelgrass meadows. Similar results were obtained for mussels (*M. edulis* and *M. modiolus*) in Nova Scotia when phytoplankton, but not detritus from *Zostera marina* or macroalgae, were found to be the major sources of carbon (Stephenson *et al.*, 1986). Duggins *et al.* (1989) found that mussels (*M. edulis*) on some islands in Alaska ate detritus from kelp. In 1993, Simenstad *et al.* reflected that variability in $\delta^{13}\text{C}$ studies was probably due to unidentified nearshore carbon fluxes.

Radioactive isotopes can isolate prospective food sources, but ^{14}C studies must occur in the laboratory. Tracer work with trophic relationships began in the 1950's. Early trophic studies contained computational errors because isotope recycling was occurring during feeding experiments (Conover and Francis, 1973). Since that time, advances in analytical equipment and resolution of experimental design problems have increased confidence associated with tracer results. Major advantages of tracer work include sensitivity, ability to target specific tissues, ability to perform short term experiments, and the ability to regulate experimental components that can confound treatment responses. Laboratory work with radioactive tracers assumes that results reflect field relationships; however, this might not be the case. Although regulated conditions in the laboratory assist with experimental precision, they also may provoke animal responses that do not exist in the field. The most definitive understanding of trophic relationships combines both laboratory and field analyses.

Much research with ^{14}C examines bivalve consumption of detritus from macroalgae and plants. Oysters (*C. virginica*) absorbed ^{14}C labelled lignocellulose from *Spartina alterniflora* (Newell and Langdon, 1986). In 1988, Kreeger *et al.* demonstrated lignocellulose consumption by mussels (*G. demissa*). Crosby *et al.* (1989) explored the role of refractory

carbon from *S. alterniflora* in the carbon budget of oysters (*C. virginica*) and found it to provide less than 1% of carbon demand. Kreeger *et al.* (1990) found increased utilization of detritus (*S. alterniflora*) when mussels (*G. demissa*) were exposed to tidal fluctuations. More recently, Charles (1993) showed use of fresh detritus from macroalgae and seagrass by a deposit feeding bivalve (*Abra ovata*). ^{14}C can be used to assess partitioning of food sources into specific tissues, such as the digestive gland or mantle tissue (Hawkins and Bayne, 1985). Carbon budgets can be assessed because carbon is present in tissue, respiration, and egestion (Kreeger *et al.*, 1988; Kreeger *et al.*, 1990; and Crosby *et al.*, 1990).

Assessment of bacterial contributions to detrital complexes often uses ^{15}N . Alber and Valiela (1995) showed that aggregates of dissolved organic matter and bacterial protein were assimilated by bay scallops (*A. irradians*) more than particulate detritus colonized by bacteria.

Some researchers use multiple labels to provide additional insight to animal trophic relationships. Seasonal differences were demonstrated for food partitions to mussel (*M. edulis*) tissues by use of ^{15}N and ^{14}C (Hawkins and Bayne, 1985). Results with ^{14}C and ^{15}N experiments showed that mussels (*G. demissa*) and oysters (*C. virginica*) utilized more carbon and nitrogen from detritus (*S. alterniflora*) augmented with bacteria than from bacteria alone (Crosby *et al.*, 1990; and Langdon and Newell, 1990). Peterson *et al.* (1985) used $\delta^{13}\text{C}$, ^{15}N , and ^{34}S to determine relative contributions of detritus (*S. alterniflora*) and plankton to mussels (*G. demissa*) depending upon location within a salt marsh.

This research used a ^{14}C tracer for several reasons. Because questions did not relate directly to bacterial augmentation of detrital food sources, use of a ^{15}N tracer was not required. Equipment was available locally to do ^{14}C analyses and experiments could be conducted in relatively short time periods. A carbon tracer could evaluate dietary contributions to tissue, a factor that could not be assessed directly by other experimental methods. Lastly, considerable literature was available that addressed the contribution of detritus, determined with tracers, to suspension feeding bivalves.

Thesis questions

Although several studies identify utilization of suspended detritus by suspension feeding bivalves, no conclusive studies are available for the Manila clam. This study intended to establish and quantify, in the laboratory, detrital use by this suspension feeding bivalve. The nutritional and ecological role of detrital particulates for Manila clams was examined by addressing the following questions. (1) Can Manila clams absorb detrital particulates from macrophytes, and if so, how does absorption compare with phytoplankton diets? (2) Are there differences in gut passage time between dietary treatments that might explain utilization? (3) How do carbon budgets compare between dietary treatments?

METHODS

Organisms

During August 1992 and May 1993, Manila clams (*Venerupis philippinarum*) were gathered from Padilla Bay in Skagit County, Washington (Figure 2). Clams with shell lengths of 30 mm to 50 mm were transported to Shannon Point Marine Center in Anacortes for maintenance in seawater tables served by a flow through seawater system. Clam valves were allowed to gap without restriction by rubber bands or substrate, consistent with Bernard (1983b:19) who found that Manila clams could maintain valve closure without a compression force. Following weekly cleaning of seawater tables, clams were fed variable quantities of phytoplankton (*Chaetoceros gracilis* and *Isochrysis galbana*) and dried particles of macrophytes (*Ulva fenestrata* and *Zostera marina*) to supplement naturally occurring food.

Eelgrass (Figure 3) was gathered from Padilla Bay in Skagit County and Marine Park in Bellingham (Whatcom County). During October 1992, storm-dislodged plants were gathered from the high tide line at Marine Park in Bellingham. Blades were processed into detrital particles, as described in a following section. A second group of plants was gathered during April 1993 from the northeast tip of March Point in Padilla Bay. These plants were grown in the laboratory and radiolabelled with a ^{14}C isotope.

Ulva was obtained from the Shannon Point Marine Center and Carolina Biological Supply. *Ulva fenestrata* (Figure 3), gathered from the shore at the Shannon Point Marine Center during October 1992, was processed into detrital particles. *Ulva linzea* germlings, obtained from Carolina Biological Supply during November 1992, were grown and radiolabelled in the laboratory with ^{14}C isotope.

Phytoplankton was obtained from stocks at the Shannon Point Marine Center and Sound Seafarms near Bellingham (Figure 4). A flagellated species, *Isochrysis galbana* (Prymnesiophyta, Prymnesiophyceae, Isochrysidales), was acquired from the Shannon Point Marine Center. Although the alga was grown at the laboratory for years, the source of the original stock is unknown. A centric diatom, *Chaetoceros gracilis* (Bacillariophyta, Bacillariophyceae, Centrales) was obtained from Sound Seafarms, Bellingham. The source of the original stock is unknown.

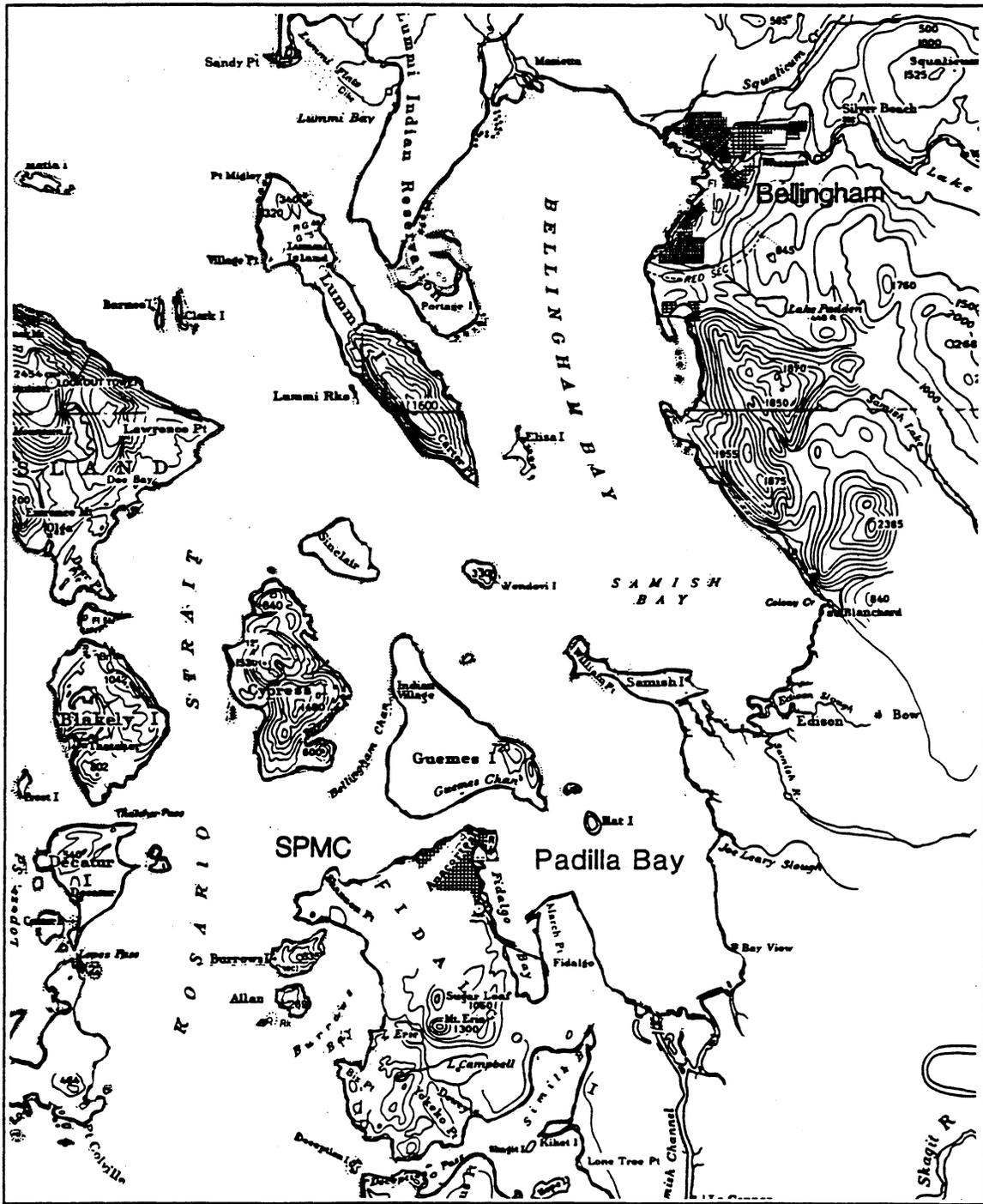


Figure 2: Vicinity map of Bellingham and Padilla Bay, Washington.
 SPMC means Shannon Point Marine Center.

North ↑

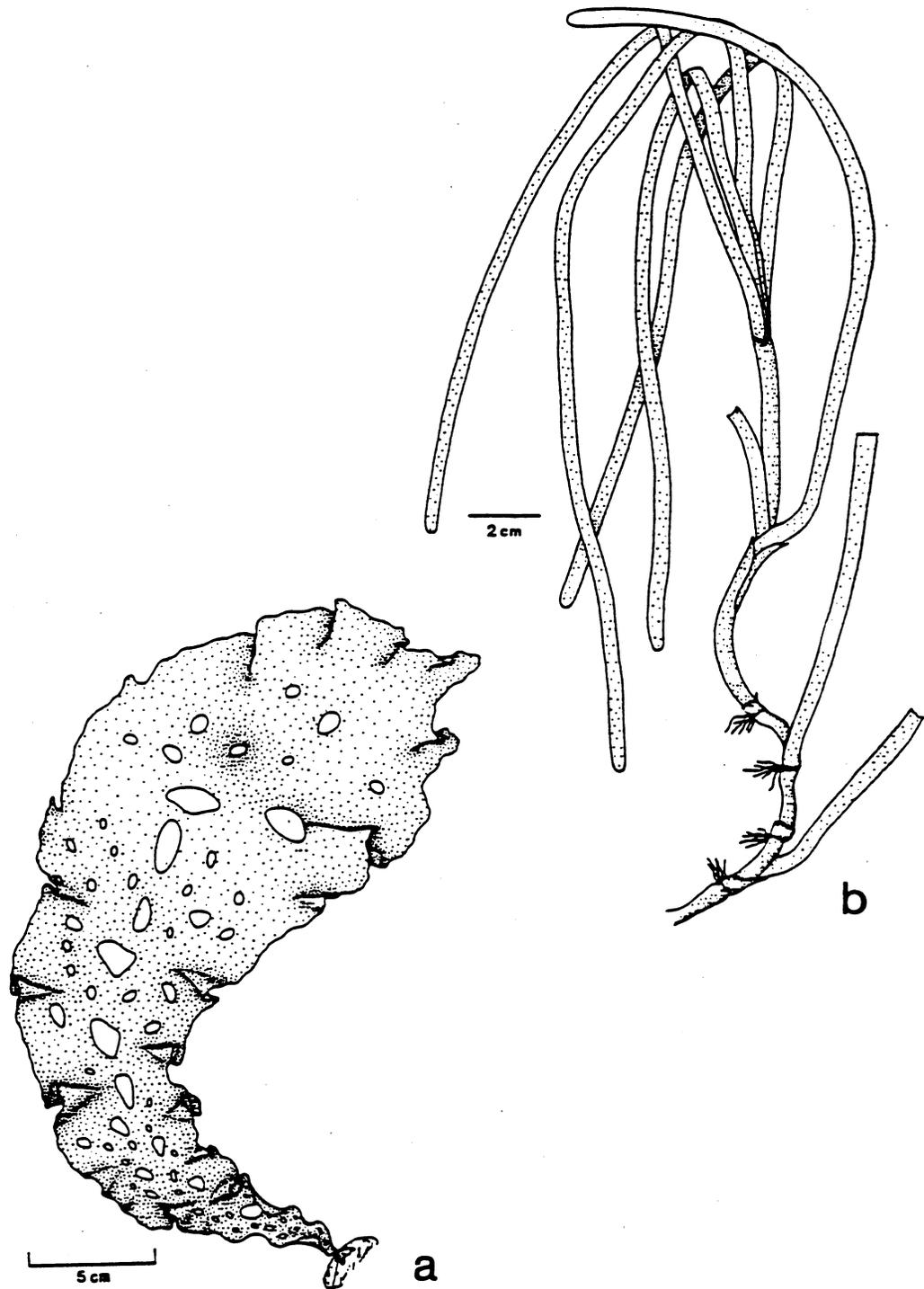


Figure 3: Macrophytes used as sources for detrital particulates.

a: *Ulva fenestrata* (Chlorophyta) Drawing modified from Scagel (1967:49).

b: *Zostera marina* (Anthophyta) Drawing modified from Phillips and Meñez (1988:32).

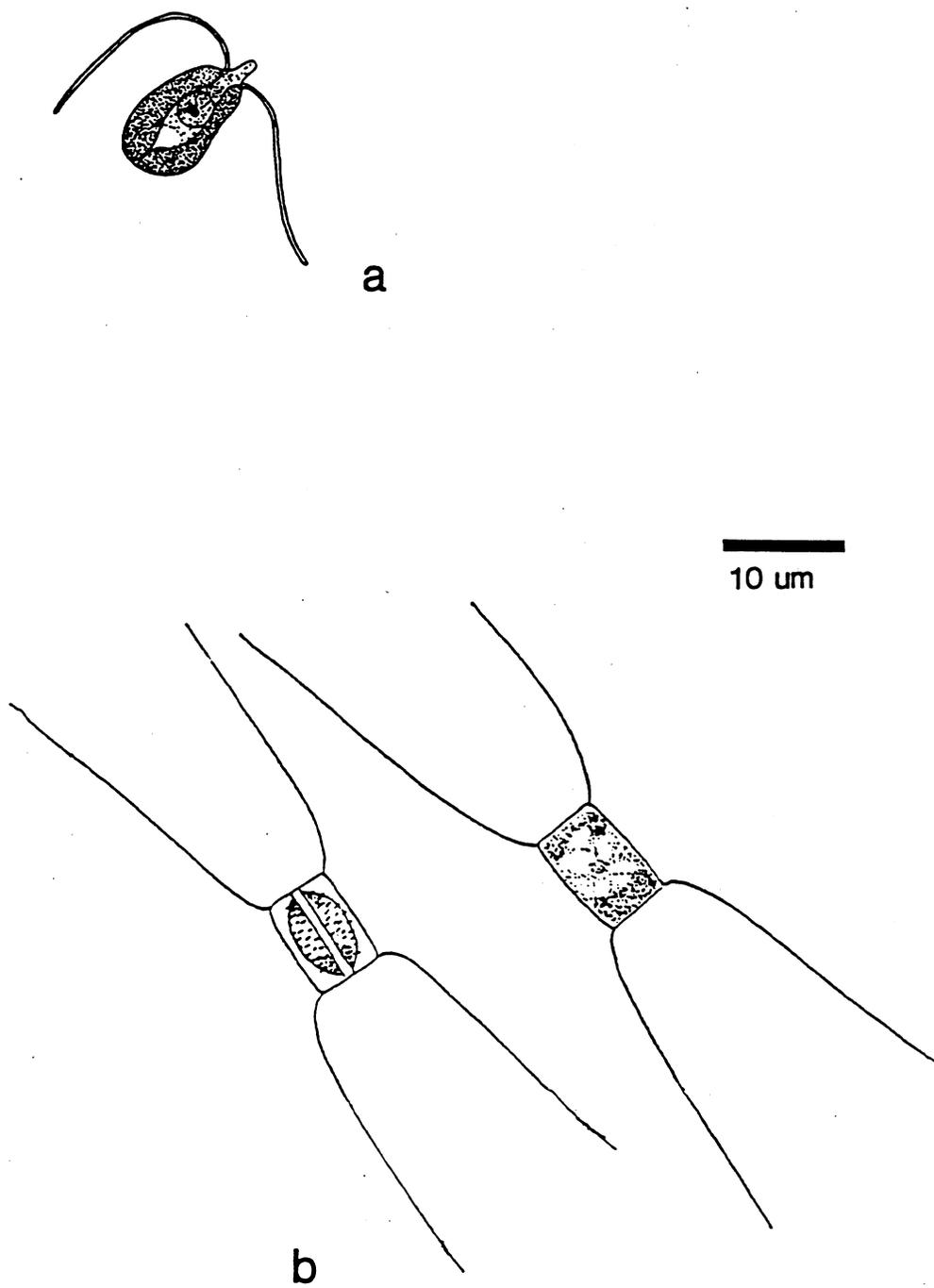


Figure 4: Phytoplankton used in experiments.

- a: *Isochrysis galbana* (Prymnesiophyta). Drawing modified from Lee (1989:460).
- b: *Chaetoceros gracilis* (Bacillariophyta) Drawing modified from Hendeby (1964:136).

Laboratory culture of phytoplankton

Phytoplankton and eelgrass were cultured at 21°C in a Percival incubator (Model 5 LL) equipped with cool white fluorescent bulbs. The light cycle was 16 hours of light and eight hours of darkness. Photosynthetically active radiation (PAR) was measured using a LiCor Quatum/Radiometer/Photometer (Model LI-185) and a quantum sensor (Model LI-190S). Instantaneous light readings of 25 to 85 $\mu\text{mol m}^{-2} \text{s}^{-1}$ were obtained, depending on proximity to the light source.

Seawater for growing eelgrass and algae was obtained at the Shannon Point Marine Center from an in-line cartridge system consisting of a 5 μm glass fiber and activated charcoal filter. Filtered seawater was collected in amber glass jugs and autoclaved at 15 pounds pressure (125°C) for 30 minutes. Prior to use, autoclaved seawater was filtered through a 15 μm nylon filter to remove precipitates. Seawater for growing phytoplankton and eelgrass was supplemented with Guillard's F/2 nutrient (Appendix A) at an amount of 2 ml l⁻¹. In addition to F/2 nutrient, diatoms were given a supplement of sodium metasilicate ($\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$) at an amount of 30 mg l⁻¹ of seawater.

Phytoplankton was cultured in 1000 ml Erlenmeyer flasks. Prior to use, flasks were autoclaved at 15 pounds pressure (125°C) for 30 minutes. Algal cultures were aerated with aquarium pumps and air was delivered to flasks through 1 ml disposable serological pipettes connected to latex hoses with in line fiber air filters. Phytoplankton cultures were divided when cell density reached approximately 10⁸ cells ml⁻¹. Part of the culture was used to inoculate new cultures, part was used as unlabelled food for experiments, and the remainder was used as a dietary supplement for clams maintained at the Shannon Point Marine Center.

Preparation of unlabelled detrital particulates

Field gathered *Ulva fenestrata* and *Zostera marina* were stored in a 5°C cold room until processed. *Ulva* thalli were rinsed in fresh seawater before drying. *Zostera* blades were manually stripped of epiphyton, then rinsed in fresh seawater before drying. Material was dried at 60°C for 18 to 24 hours. Dried material was refrigerated until ground to less than

500µm with a Thomas Wiley laboratory mill. Ground material was pulverized to less than 50µm using a Spex shatterbox then stored in a freezer at -5°C.

The average number of particles per dry milligram was determined for both *Ulva* and *Zostera* by adding 5 mg pulverized particles to 5 ml seawater. Particle aliquots were weighed with a Mettler analytical balance. Ten hemacytometer fields (Improved Neubauer Bright Line) from ten different aliquots were counted while using a microscope (Zeiss Standard WL) at 250x power.

$$\frac{\text{particles}}{\text{division}} * \frac{25 \text{ divisions}}{0.1 \text{ mm}^3} * \frac{1,000 \text{ mm}^3}{\text{cm}^3} * \frac{\text{cm}^3}{1 \text{ ml}} * \frac{1 \text{ ml}}{1 \text{ mg}} = \text{average particles mg}^{-1}$$

Mean particle density for *Ulva* was 5.4×10^6 particles mg^{-1} and particle density for *Zostera* was 8.5×10^6 particles mg^{-1} .

Laboratory culture of ^{14}C labelled algae and eelgrass

Radiolabelled sodium bicarbonate was packaged in 1 ml ampoules (ICN Biomedicals, Inc.) at a concentration of 20µCi or 4.44×10^7 dpm (disintegrations per minute) per milliliter. Ampoule contents were transferred to an autoclaved volumetric flask. Each ampoule was rinsed several times with sterile seawater and rinse water was transferred to a flask. Depending on the material that was to be labelled, radiolabelling solutions contained multiples of one ampoule (1 ml) $\text{NaH}^{14}\text{CO}_3$, 0.5 ml F/2 nutrient, and 248.5 ml sterile seawater. Replicate samples of the radioactive growth medium were assayed for liquid scintillation spectroscopy before adding algae or eelgrass.

After adding algae or eelgrass to the medium, daily replicate samples of the medium were assayed until a negligible amount of activity remained, approximately 600 dpm ml^{-1} . It was assumed that the decline in activity of the growth medium represented photosynthetic uptake by algae or eelgrass.

To measure $\text{NaH}^{14}\text{CO}_3$ activity present in the growth medium, 2 ml of solution was filtered with a 0.2 µm membrane filter. Replicate 0.5 ml aliquots of filtered solution were transferred to replicate 6 ml polyethylene scintillation vials to which 5.5 ml of scintillation cocktail (Fisher Scinti-Safe Plus 50%) were added with an adjustable bottle top dispenser. Radioactivity was determined with a liquid scintillation counter (Packard Tricarb LSC Model

1900 CA) using a ^{14}C efficiency tracing dpm (ET- DPM) protocol. Remaining solution was returned to culture flasks.

Ulva linza was radiolabelled by placing approximately 95 grams wet weight of algae in each of two 1000 ml autoclaved Erlenmeyer flasks that contained 500 ml of labelling solution. After four days in the growth medium, algal biomass was separated from filtrate with a 250 μm nylon mesh filter and the filtrate was discarded. Labelled *Ulva* was placed in glass petri dishes and air dried in a hood. Dried *Ulva* was ground with a mortar and pestle, ground with a tissue grinder, and placed in glass scintillation vials to which autoclaved distilled water was added. Specific activity was measured by adding 20 μl of labelled material to 6 ml of scintillation cocktail. Dried and labelled material was frozen (-5°C) until used.

Eelgrass was cultured using a method modified from McMillan (1980:60-61). Eelgrass gathered from Bellingham Bay was cut to form starts with 4 cm turions and 4 cm rhizomes, as measured from the first node on the rhizome. Eelgrass obtained from Padilla Bay was freed of sediments and cut to make starts having 6 cm turions and 5 cm rhizomes. Starts from Padilla Bay were placed in a 1% sodium hypochlorite solution for one minute, then rinsed in sterile seawater. Plastic lid liners were punched with a slot for the turion and holes for circulation. These served as friction fit collars to maintain eelgrass at the bottom of 38 x 200 mm Pyrex test tubes to which sterilized seawater and F/2 nutrient were added. Cultured eelgrass was not aerated.

Zostera marina was labelled after individual starts were stabilized for five days in culture. Unlabelled seawater was drained and replaced with radioactive growth medium. Daily samples of growth medium were taken until specific activity was constant for two days. Old solution was drained and eelgrass starts were immersed in a new mixture of radioactive growth medium. The process was repeated until sufficient eelgrass biomass was obtained. New growth obtained during labelling was clipped, air dried in a hood, ground with a mortar and pestle, ground with a tissue grinder, and placed in glass scintillation vials to which autoclaved distilled water was added. Specific activity of each vial was measured by adding 20 μl of labelled material to 6 ml of scintillation cocktail. Dried and labelled material was frozen (-5°C) until used.

To radiolabel either species of phytoplankton, 1 ml of $\text{NaH}^{14}\text{CO}_3$ was transferred to 123.5 ml of sterile seawater to which 0.5 ml F/2 nutrient and 125 ml of cells/seawater were added. Each day, replicate samples of filtered solution were taken until activity was no longer decreasing. Labelled cells were separated from filtrate by centrifuging for 10 minutes at 2,250 rpm. Supernatant was discarded and the pellet was resuspended in autoclaved seawater. Specific activity was determined by adding 0.5 ml of resuspended cells to 5.5 ml scintillation cocktail for liquid scintillation counting. Concentrated cells were refrigerated until activity was determined and living cells could be used.

Experimental apparatus

The experimental apparatus (Figure 5) was a flow through system that delivered diets to individual animals at a uniform flow rate, constant temperature, and with a constant density of suspended particles. Each experiment used ten feeding chambers (Figure 6) to which one of two diets was delivered. Five feeding chambers received one diet while the other five chambers received the second diet.

Each diet was placed in a 2000 ml Erlenmeyer flask that was positioned on a heater/magnetic stirrer. Particle and phytoplankton diets were kept in suspension by using a magnetic stir bar rotating at approximately 750 rpm.

A multi-channel peristaltic cassette pump (Manostat), fitted with ten cassettes and ten 3/16" ID silicon tubing elements, moved diets from source flasks through 3/16" ID vinyl distribution lines to feeding chambers. Each cassette plate for the peristaltic pump was individually adjusted to deliver a volume of 200 ± 20 ml per hour. Flow was measured before each experiment by pumping seawater through each distribution line for fifteen minutes. Discharge volume was measured to the nearest milliliter with a graduated cylinder then multiplied to obtain hourly flow.

Uniform water temperature was maintained by placing each feeding chamber in a water bath positioned on the ten-place magnetic stirrer. To maintain feeding chambers at 15°C , hoses circulated cooled water from a heating/cooling recirculating pump (Forma-Temp Jr. Masterline) to the water bath.

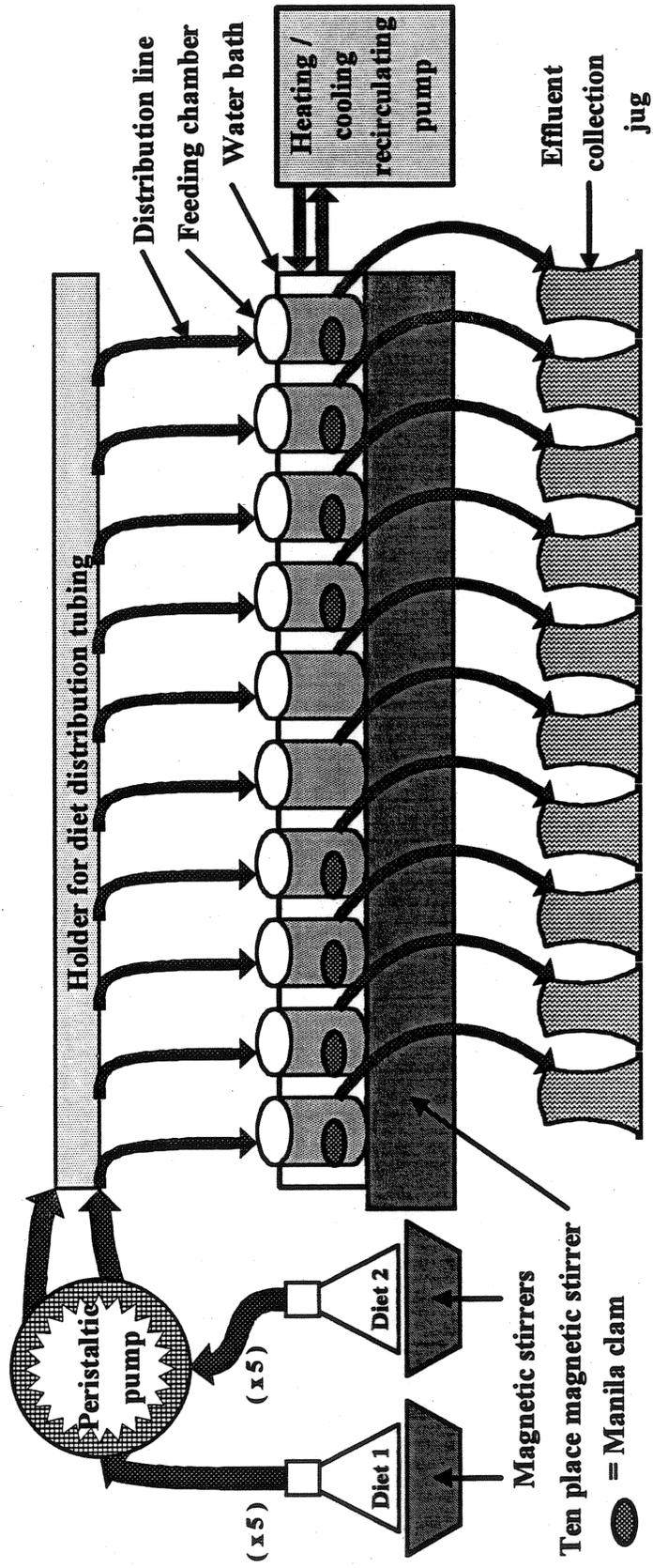


Figure 5: Schematic of the experimental apparatus and flow through system of diet delivery.

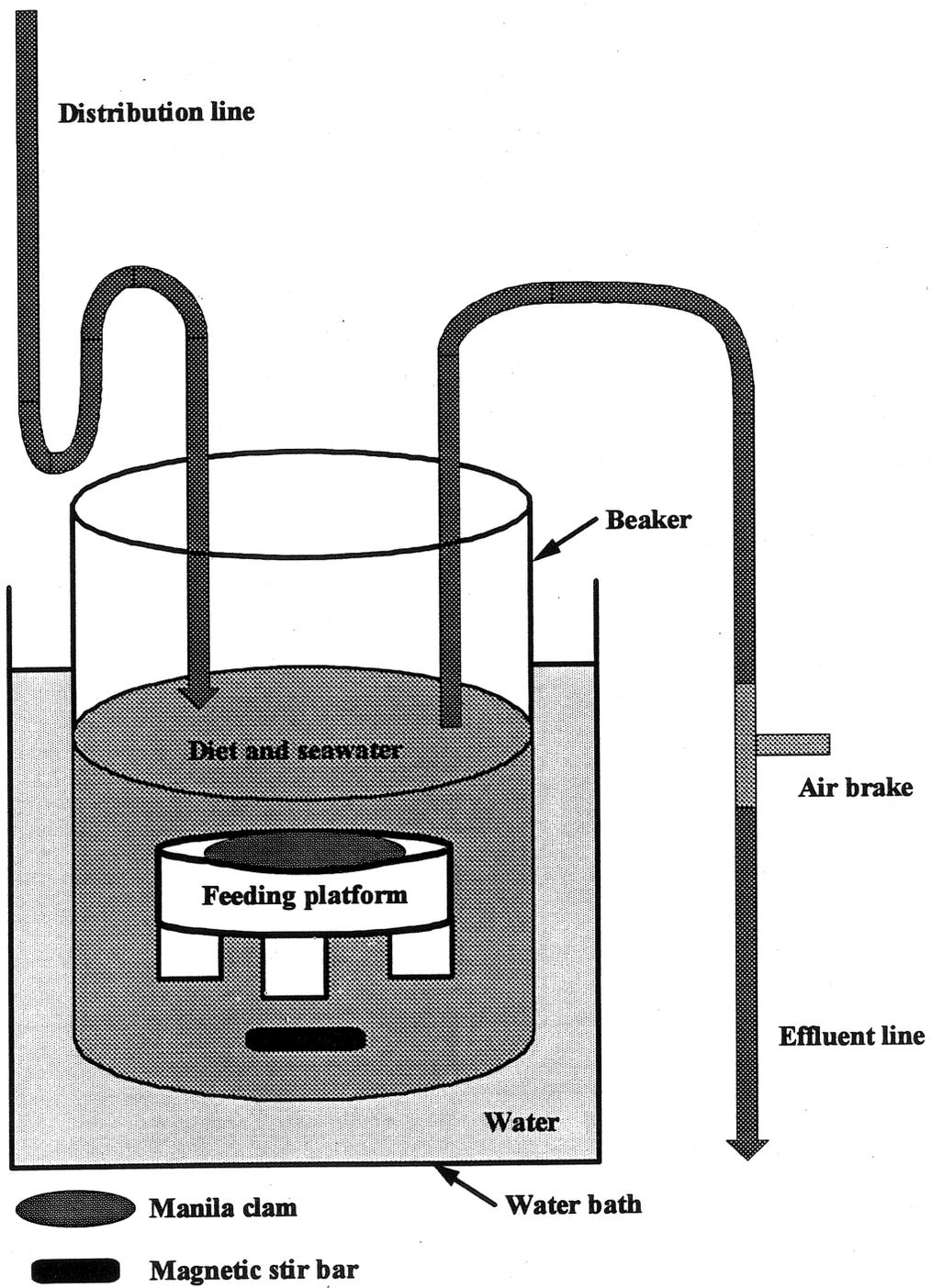


Figure 6: Detail of a feeding chamber.

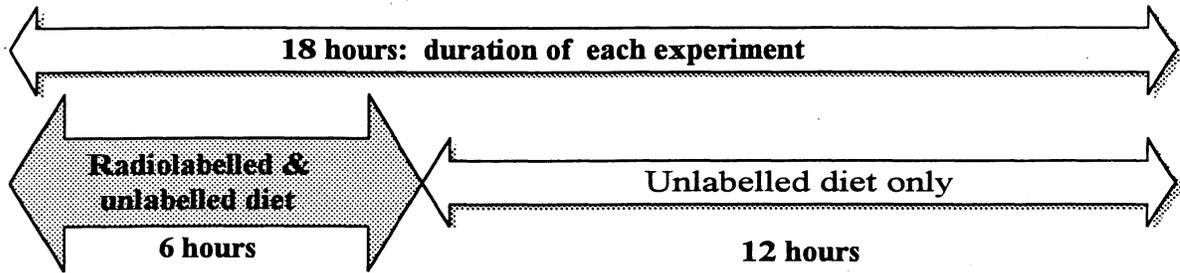
Each feeding chamber was a 600 ml Pyrex beaker that contained one Manila clam placed on a platform constructed of PVC and nylon mesh. Platforms and the motion of magnetic stir bars were designed to maintain phytoplankton and detrital particles in uniform suspension. The PVC platform structure was 70 mm in diameter and 15 mm high, with 15 mm legs. The platform base was made of 250 μ m nylon to permit water motion to resuspend particles that might settle. A magnetic stir bar was placed under each platform and was rotated at approximately 750 rpm with a ten-place belt driven magnetic stirrer.

The distribution line to each chamber terminated with a bent glass fitting and a back flow trap. Approximately 300 ml of seawater and diet were maintained in each chamber by a glass outflow line that ran to an inverted plastic T. One end of the plastic T served as an air vent that automatically maintained chamber fluid level once a siphon was started. Effluent from each feeding chamber was collected in an amber glass jug. One ml of 25% NaOH (6.25 N) was added to each collection jug to increase the pH of effluent water to ≥ 9.5 and to buffer seawater so $^{14}\text{CO}_2$ would remain in solution. Formaldehyde (1% final concentration) was added to each collection jug to control bacterial activity.

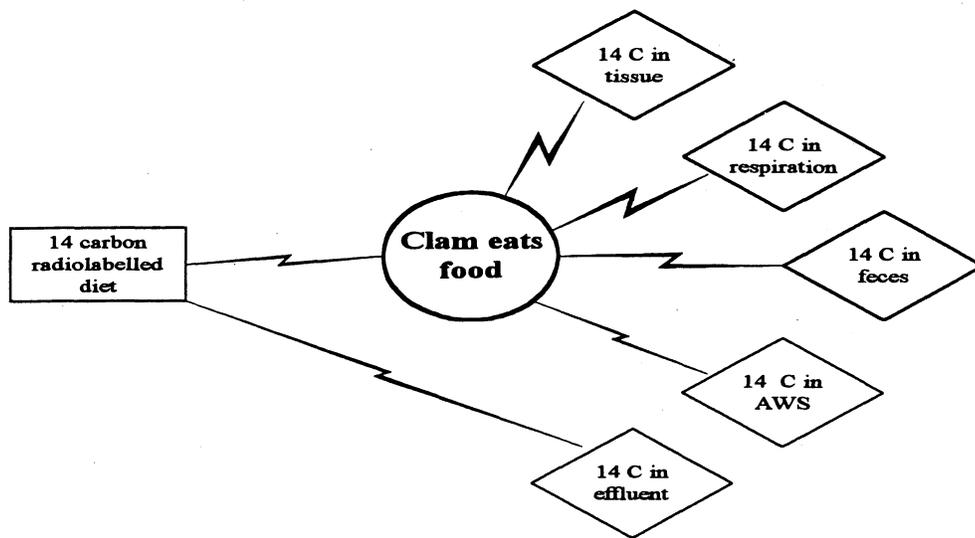
Delivery and preparation of diets used in experiments

Each experiment lasted eighteen hours and used a chase system of diet delivery (Figure 7). For six hours, diets consisted of a mixture of approximately 95% unlabelled particles or algal cells, and 5% radiolabelled particles or algal cells. During the subsequent twelve hours, diets consisted of 100% unlabelled particles or algal cells. The chase system assists diet passage through clam digestive systems.

During each experiment, diets were prepared as needed in one liter batches to minimize bacterial activity. Diet density was approximately 1.0×10^5 particles or algal cells ml^{-1} . Filtered (5 μ m) seawater was sterilized with ultraviolet light immediately before diet preparation.



a.



b.

Figure 7: Schematic of carbon flow during diet delivery. a: Chase system of diet delivery. b: Carbon flow from dietary treatments to components traced with liquid scintillation counting. AWS means acid wash supernatant.

Approximately 20 mg of unlabelled *Ulva* or *Zostera* particulates were added to each sterilized liter of seawater. The mixture was filtered with a 20 µm nylon mesh filter to remove large or coagulated particles. Particle density was verified by calculations using a hemacytometer. Unlabelled particulate diet was poured into a 2000 ml Erlenmeyer delivery flask.

Phytoplankton diets were prepared in one liter batches. Initial phytoplankton cell density was obtained using a hemacytometer. Phytoplankton was diluted to one liter with UV sterilized seawater to obtain 1.0×10^5 cells ml⁻¹. Cell density was verified using a hemacytometer. Unlabelled phytoplankton diet was placed in a 2000 ml Erlenmeyer delivery flask.

Radiolabelled phytoplankton or particles, identical to the unlabelled component, were added directly to each feeding chamber every half hour during the first six hours of each experiment. Because a microscope and hemacytometer were unavailable for use with radioisotopes, the quantity of radiolabelled cells or particles was assumed to be equal to what was determined for unlabelled species. Specific activity for radiolabelled phytoplankton or particles was approximately 2,000 dpm ml⁻¹.

Experimental design

Experimental treatments assessed dietary utilization by Manila clams of two species of phytoplankton and detrital particulates from two species of macrophytes. A total of forty-eight Manila clams received one of four treatments with twelve animal replicates per treatment.

Experiments used the statistical model of a partially nested one-way ANOVA with four treatments (T), three runs (R) nested in each treatment, and four replicates (ε) per run. Alpha was set at 0.05. When ANOVA resulted in statistical differences between treatments, Tukey multiple comparisons tests were used to distinguish treatment effects.

$$Y_{ijk} = \mu + T_{(i)} + TR_{i(j)} + \epsilon_{ij(k)}$$

Dietary treatments were considered fixed, in that the same diets would be selected if experiments were repeated. The runs and replicates were considered to be random because

dietary treatments could be randomly assigned to any run, and replicate animals were randomly selected from all animals maintained in seawater tables.

Each run randomly was assigned two of four possible diets. After all experiments were completed, each diet was used for three runs. During every experiment, one of two diets was delivered to four feeding chambers, each containing a single clam, and one chamber that lacked an animal. The other diet was delivered to four occupied feeding chambers and one additional unoccupied chamber. Diets were randomly assigned to feeding chambers. The sequence of experiments and treatments are described in Appendix B.

Carbon partitioning and associated calculations

At the end of experiments, ^{14}C in diets was traced to tissue absorption, respiration, feces, acid wash supernatant, and effluent (Figure 7). Because of differences in available equipment, methods to assess carbon partitions were modified from Newell and Langdon (1986), Kreeger *et al.* (1988), Crosby *et al.* (1989), and Kreeger *et al.* (1990). Clams were frozen at the end of each experiment then thawed, and whole animal tissue was dissected from the shell. Clam bodies were blotted and wet weighed to the nearest milligram using a Mettler analytical balance.

Animals used in experiments were wet weighed because a drying oven for radioactive materials was unavailable. A calibration curve was prepared to obtain a ratio of wet tissue to dry tissue weight. Clams not used in experiments ($n=94$) were frozen, thawed, dissected, and shells were discarded. Valve length ranged from 3.15 to 5.14 cm, with a mean of 4.53 ± 0.492 cm, and was similar to valve size for experimental animals. After blotting and wet weighing to the nearest milligram, clam bodies were dried at 60°C for 24 hours and weighed again. The following equation expressed the linear relationship between wet and dry weights and provided weight specific units in dpm per gram dry tissue weight (DTW). Using regression analysis, the equation was statistically different from zero ($p < 0.0000$, $r^2 = 0.9141$).

$$\text{grams dry tissue weight (DTW)} = 0.1637 \times \text{wet tissue weight (grams)} - 0.1666$$

Ingestion

Ingestion was determined by combining data for tissue absorption (T), respiration (R), acid wash supernatant (A), and egestion (E) to form the equation: $I = T + R + A + E$. Resulting data were log transformed to normalize treatment distributions prior to statistical analyses.

Acid wash supernatants

A hot acid treatment was modified from Newell and Langdon (1986), Kreeger *et al.* (1988), Crosby *et al.* (1989), and Kreeger *et al.* (1990). The hot acid wash preceded a hot alkali treatment, and together both processes assessed absorption of labelled diets into tissue, as well as diets digested but not absorbed (Newell and Langdon, 1986:109). Acid wash supernatant contained unmeasured fractions of undigested gut material and tissue. By comparison, supernatant from the hot alkali treatment contained only tissue because undigested gut material was precipitated as a pellet. To conservatively estimate dietary contributions to tissue absorption resulting from thesis experiments, data from acid and alkali treatments were differenced.

Clam tissue was liquefied in a blender mini-container to which 5 ml of 0.95% H_2SO_4 were added. Resultant liquid was transferred to a 50 ml polypropylene centrifuge tube. The blender container was rinsed two more times with 5 ml aliquots of 0.95% H_2SO_4 and rinse liquid was added to the centrifuge tube. Contents of centrifuge tubes were heated for 30 minutes using a water bath maintained at 95°C. Tubes were centrifuged for 25 minutes at 2,250 rpm. Volume of the supernatant was measured to the nearest 0.1 ml and transferred to glass vials. Pellets were retained in centrifuge tubes for subsequent hot alkali processing. For each clam, a 0.5 ml sample of supernatant and 5.5 ml of scintillation cocktail (Fisher Scinti-Safe Plus 50%) were placed in a 6 ml polyethylene scintillation vial. Each sample was counted with a liquid scintillation counter (Packard Tri-Carb LSC Model 1900 CA) using the ^{14}C ET-DPM protocol.

Specific activity for acid wash supernatants were determined with the following equation. Data were expressed in disintegrations per minute (dpm) per gram dry tissue

weight (DTW). Data were log transformed to normalize treatment distributions prior to statistical analyses.

$$\frac{dpm}{sample (0.5 ml)} * acid\ supernatant (ml) * \frac{1}{gram\ DTW} = \frac{dpm}{gram\ DTW}$$

Tissue absorption

A hot alkali treatment, modified from Newell and Langdon (1986), Kreeger *et al.* (1988), Crosby *et al.* (1989), and Kreeger *et al.* (1990), determined the amount of ¹⁴C present in clam tissue. Consistent with terminology used by Kreeger *et al.* (1988) and Kreeger *et al.* (1990) results from thesis experiments refer to data from the hot alkali treatment as tissue absorption.

Ten milliliters of 12.5% NaOH were added to the pellet remaining after the hot acid wash. Centrifuge tubes were heated for 30 minutes at 95°C then centrifuged at 2,250 rpm for 25 minutes. Supernatant volume, measured to the nearest 0.1 ml was transferred to glass vials. Resultant pellets, consisting of undigestible dietary material, were discarded and were not measured for specific activity. For each clam, a 0.5 ml sample of supernatant and 5.5 ml of scintillation cocktail were placed in a 6 ml polyethylene vial and counted with LSC using the ¹⁴C ET-DPM protocol. Specific activity in tissue was determined with the following equation. Data were expressed in dpm per gram DTW. Data were log transformed to normalize treatment distributions before statistical analyses.

$$\frac{dpm}{sample (0.5 ml)} * alkali\ supernatant (ml) * \frac{1}{dry\ tissue\ wet (gr)} = \frac{dpm}{gram\ DTW}$$

Respiration

Respired carbon was assessed by acidifying a sample from each effluent jug. Acidification drove off ¹⁴CO₂ that was maintained in solution. After each experiment, effluent was measured to the nearest milliliter with a graduated cylinder. A 102 ml sample from each jug was placed in a 250 ml Erlenmeyer flask. A 2 ml sample from each flask was filtered with a 0.2 µm membrane filter. For each clam, 5 ml of scintillation cocktail, 0.5 ml

of filtrate, and 0.5 ml of methanol were placed in a 6 ml polyethylene scintillation vial for counting with LSC using the ^{14}C ET-DPM protocol. The remaining filtrate was discarded. Results provided pre-acidification data for respiration.

$^{14}\text{CO}_2$ traps were made from glass vials containing 1 ml 25% (6.25 N) NaOH. A trap was suspended in each 250 ml flask containing 100 ml of effluent. To drive off $^{14}\text{CO}_2$, contents of each flask were acidified to $\leq\text{pH}$ 1.5 by adding 500 μl of 37% HCl. Flasks were immediately stopped and placed in a hood for ten days. After this period, a 2 ml aliquot from each flask was filtered using a 0.2 μm membrane filter. Each 0.5 ml sample of filtrate was mixed with 50 μl 37% HCl and 5.5 ml of scintillation cocktail. Results provided post-acidification data.

Bacterial respiration was differentiated from animal respiration. During each experiment, one feeding chamber per diet lacked a clam. Effluent from this chamber was processed in the same way as effluent from feeding chambers with clams. Resulting data represented bacterial respiration. Data for bacterial respiration were scaled based on effluent volumes and subtracted from raw respiration data for clams. After correction for bacterial respiration, clam respiration was determined by subtracting post acidification data from pre-acidification data, consistent with the following equation. Data were log transformed to normalize treatment distributions prior to statistical analyses.

$$\frac{\text{corrected } dpm \text{ (pre)}}{\text{sample (0.5 ml)}} - \frac{\text{corrected } dpm \text{ (post)}}{\text{sample (0.5 ml)}} \cdot \text{total effluent (ml)} \cdot \frac{1}{\text{dry tissue wet (gr)}} = \frac{dpm}{\text{gram DTW}}$$

Egestion

Clams produced both feces and pseudofeces. During experiments, pseudofeces were removed when produced, placed in effluent jugs, and were not analyzed for specific activity. Feces were transferred with Pasteur pipettes and placed in glass vials for three hour increments. At the end of each 18 hour experiment, feces for each animal were contained in six vials. ^{14}C content of feces represented food that was ingested by each animal and passed through the intestine without being absorbed nor respired. Feces were homogenized using a sonifier fitted with a microtip. A 0.5 ml sample from each vial was mixed with 5.5 ml

scintillation cocktail for LSC. Volume of each glass vial was recorded to the nearest milliliter. ^{14}C allocated to feces was calculated with the following equation. Data in dpm per gram DTW for each three hour increment were square root transformed to normalize treatment distributions before statistical analyses.

$$\frac{\text{dpm}}{\text{sample (0.5 ml)}} * \text{vial volume (ml)} * \frac{1}{\text{gram DTW}} = \frac{\text{dpm}}{\text{gram DTW}}$$

Data for three hour periods were used to assess incremental differences in gut passage time. Overall gut passage time was determined by inspecting the graph plot.

Total fecal production for each dietary treatment was determined by combining data for three hour increments. Data for total fecal production in dpm per gram DTW were log transformed before statistical analyses.

Carbon budgets

Carbon budgets are a way to determine relative partitions of food. Many researchers use different methods to evaluate carbon budgets, energy budgets, or food balance. Carbon budgets enable relative comparisons to data expressed in different units. This research used methods modified from Kreeger *et al.*(1988) and Kreeger *et al.* (1990). Carbon budgets in this research differ from ingestion, in that allocations are only considered for tissue absorption (T), respiration (R), and egestion (E). Ingestion also included data from acid wash supernatants and related contributions to tissue absorption were not clearly discernible. Contributions to each component were calculated as follows.

$$T \% = ((T) / (T+ R + E)) * 100$$

$$R \% = ((R) / (T+ R + E)) * 100$$

$$E \% = ((E) / (T+ R + E)) * 100$$

After arcsin transformations of square root ratios, differences in budget allocations were assessed. Differences between dietary treatments for contributions to tissue absorption and respiration were determined with Tukey multiple comparisons tests.

RESULTS

Experiments evaluated several factors including ingestion, tissue absorption, acid wash supernatants, respiration, egestion, gut passage time, and carbon budgets. Data for each factor were based on disintegrations per minute (dpm) equated to gram dry tissue weight (DTW). Several quality control factors were evaluated that could have confounded experimental findings. Included were total dpm available to animals, dpm available to animals during the sixth hour of experiments, hourly flow rates, animal wet weights, and animal dry weights. Appendix C contains ANOVA tables for all data. Appendix D summarizes treatment means and standard deviations for each factor.

Ingestion

Ingestion quantified how much food was consumed by Manila clams. Ingestion was calculated by combining data for tissue absorption, respiration, acid wash supernatants, and egestion. Ingestion was significantly different between dietary treatments ($F_{\text{calc } 3, 8} = 7.17$, $p < 0.0118$). The Tukey multiple comparison test found two groups of similar and overlapping patterns. One group included both phytoplankton diets (*Chaetoceros gracilis* and *Isochrysis galbana*) and *Zostera marina* particulates, the other group consisted only of particulate diets from *Ulva fenestrata* and *Zostera*. The Tukey test was unable to distinguish into what group *Zostera* effects should be placed (Zar, 1984:190). It is possible that a Type II error occurred (false acceptance of the null hypothesis). Statistical power might have been improved with more runs and attendant increased sample size. Because each animal was offered only one diet, an animal could choose to eat or not eat. As such, ingestion reflected animal responses to provided diets. Based on means, phytoplankton diets were ingested approximately three times more than *Ulva* particulates (Figure 8).

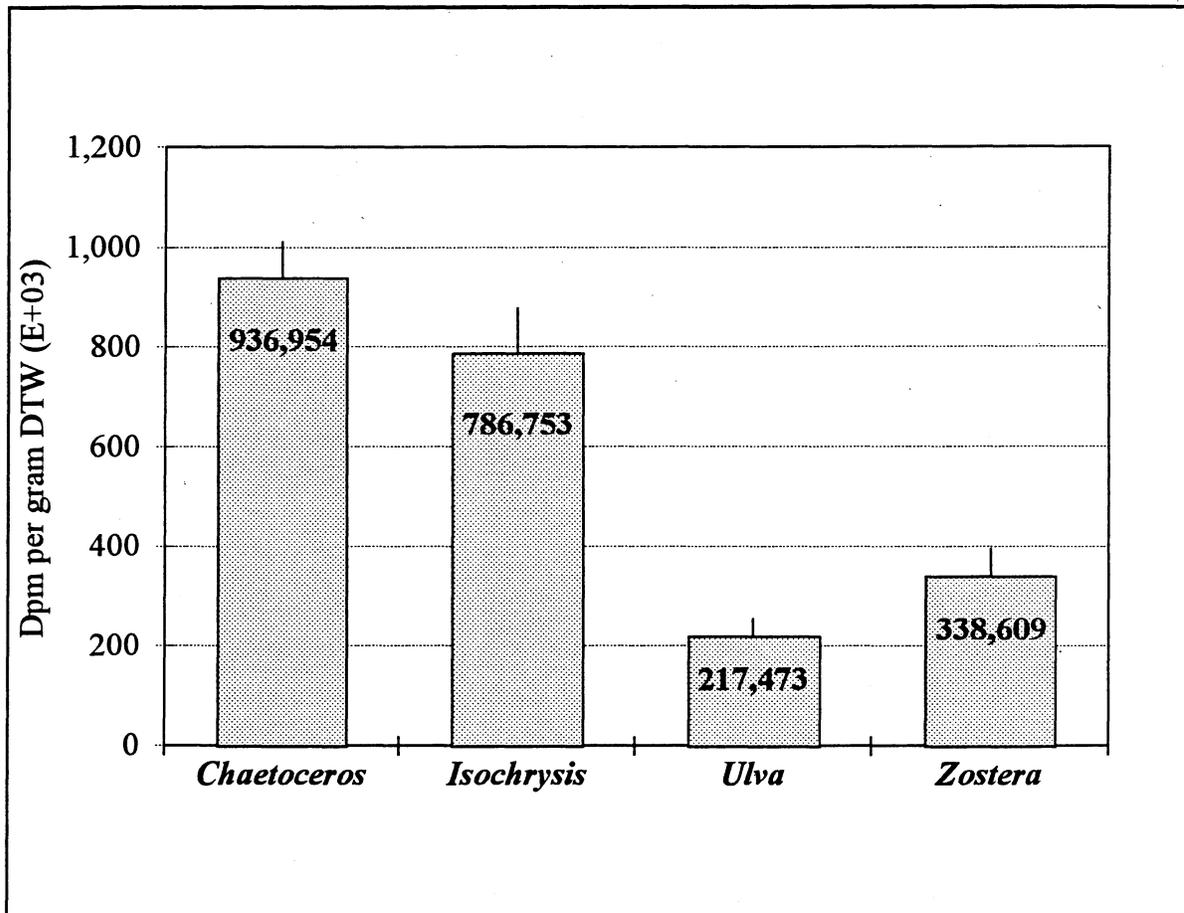


Figure 8: Ingestion by dietary treatment.

Treatment means are enumerated based on grams dry tissue weight (DTW).

Vertical lines (|) are standard errors.

Treatments were significantly different ($F_{\text{calc } 3, 8} = 7.17, p < 0.0118$).

Tukey multiple comparison test: *Chaetoceros Isochrysis Zostera Ulva*

Tissue absorption

Tissue absorption assessed the amount of food that was present in animal tissue at the end of experiments. The terminology tissue absorption is used rather than assimilation to be consistent with literature from which experiments were modeled (Kreeger *et al.*, 1988; and Kreeger *et al.*, 1990). All diets composed of phytoplankton or detrital particulates were absorbed by Manila clams; however, tissue absorption occurred in statistically different quantities ($F_{\text{calc } 3, 8} = 11.72, p < 0.0027$). A Tukey multiple comparison test revealed that phytoplankton diets of *Chaetoceros* and *Isochrysis* were absorbed in similar quantities. Detrital particulate diets of *Ulva* and *Zostera* were absorbed in similar quantities but significantly less than phytoplankton diets (Figure 9). Based on means, phytoplankton diets were absorbed into tissue approximately eight times more than diets composed of detrital particulates.

Acid wash supernatants

Acid wash supernatants consisted of tissue and undigested gut material that remained in animals at the end of experiments. Although acid wash supernatants contained tissue, unknown quantities of undigested gut material also were present. Data from acid wash supernatants were separated from tissue absorption data to conservatively estimate dietary contributions to tissue. Although radiolabelled supernatants from acid washes differed statistically between treatments ($F_{\text{calc } 3, 8} = 4.67, p < 0.0361$), a Tukey multiple comparison test was unable to detect differences between treatment means (Figure 10).

Respiration

Respiration was produced when animals consumed and utilized dietary treatments. Respiration (Figure 11) was not statistically different between dietary treatments ($F_{\text{calc } 3, 8} = 1.45, p > 0.3000$). Although means for respiration differed between treatments, standard deviations often exceeded mean values (Appendix D). The range of standard deviations probably reflects variable animal responses to diets and contributed to lack of statistical differentiation between treatments.

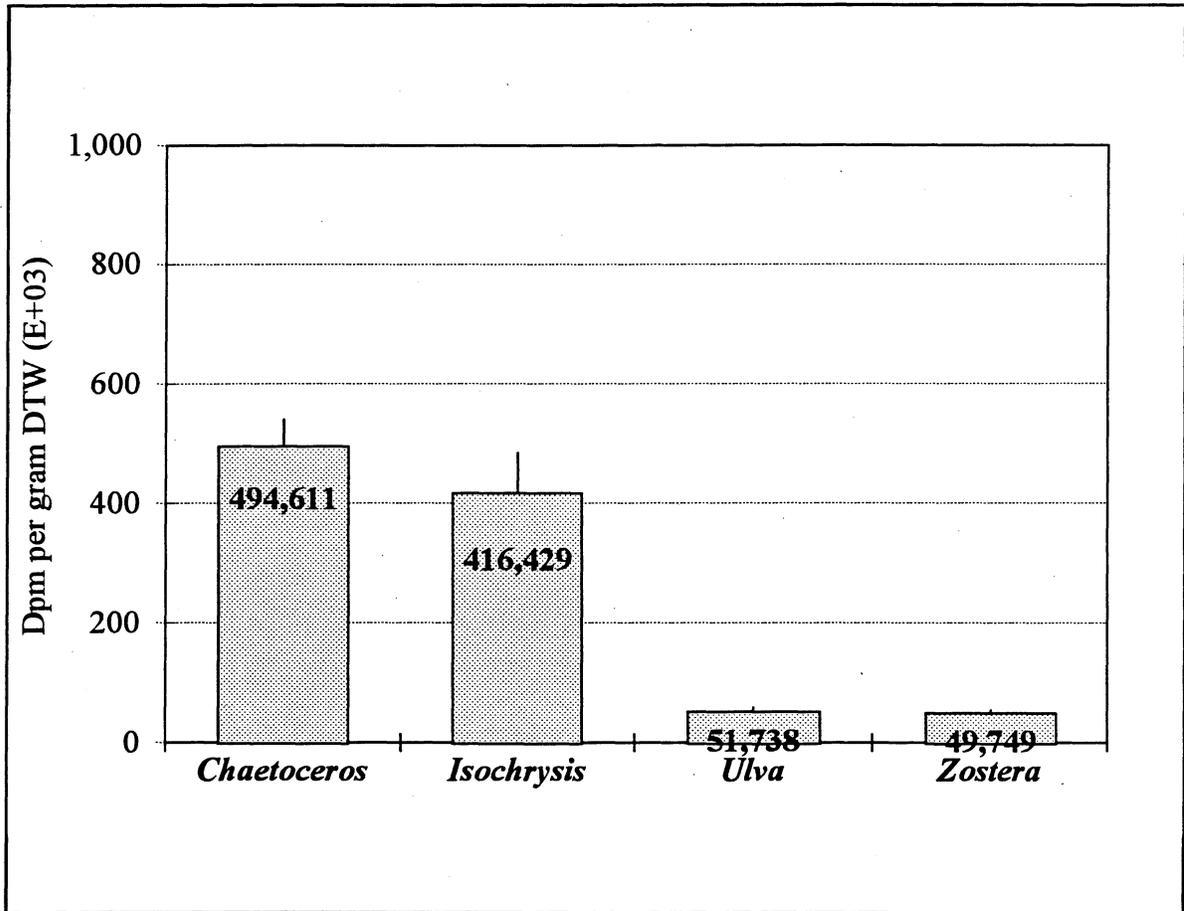


Figure 9: Tissue absorption of diets based on treatments.

Treatment means are enumerated based on grams dry tissue weight (DTW).

Vertical lines (|) are standard errors.

Treatments were significantly different ($F_{\text{calc } 3, 8} = 11.72, p < 0.0027$).

Tukey multiple comparison test: *Chaetoceros* *Isochrysis* *Ulva* *Zostera*

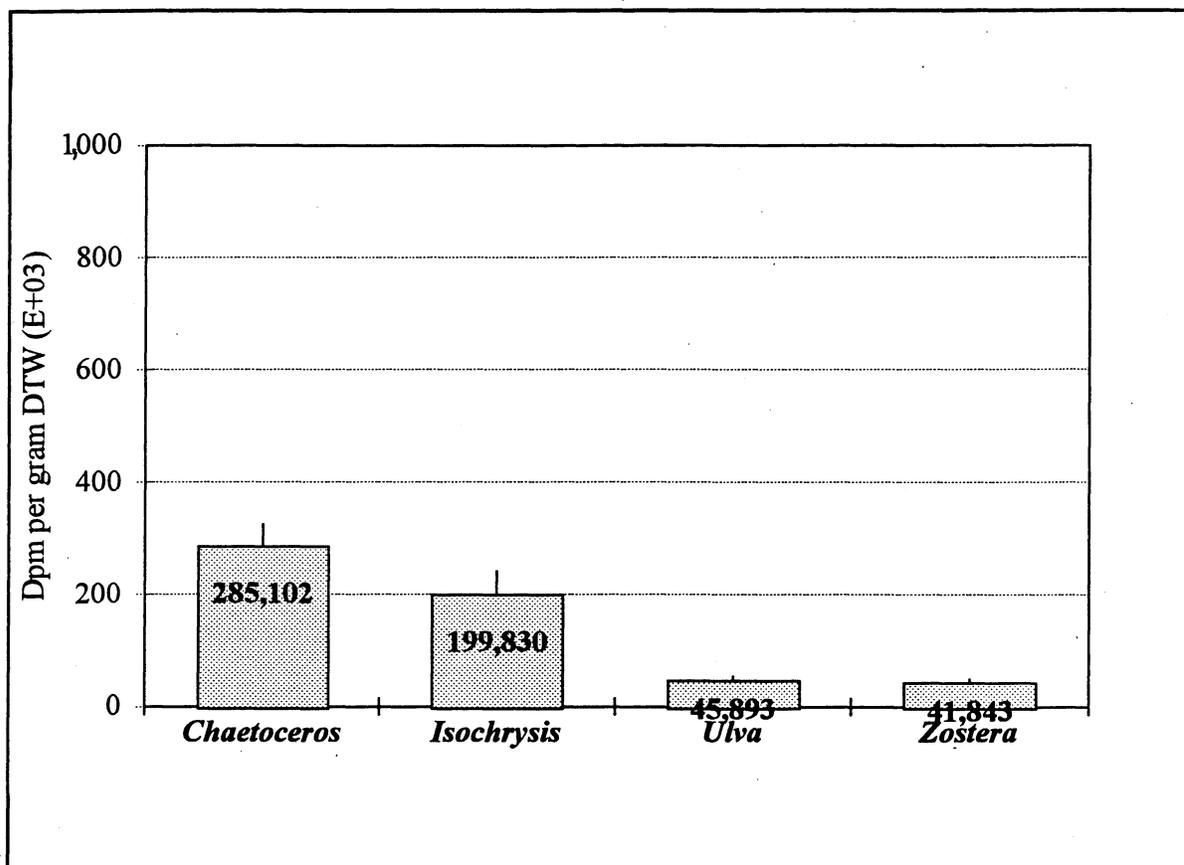


Figure 10: Acid wash supernatants resulting from dietary treatments.

Treatment means are enumerated based on grams dry tissue weight (DTW).

Vertical lines (|) are standard errors.

Treatments were significantly different ($F_{\text{calc } 3, 8} = 4.67, p < 0.0361$).

Tukey multiple comparison test: unable to detect differences between treatment means.

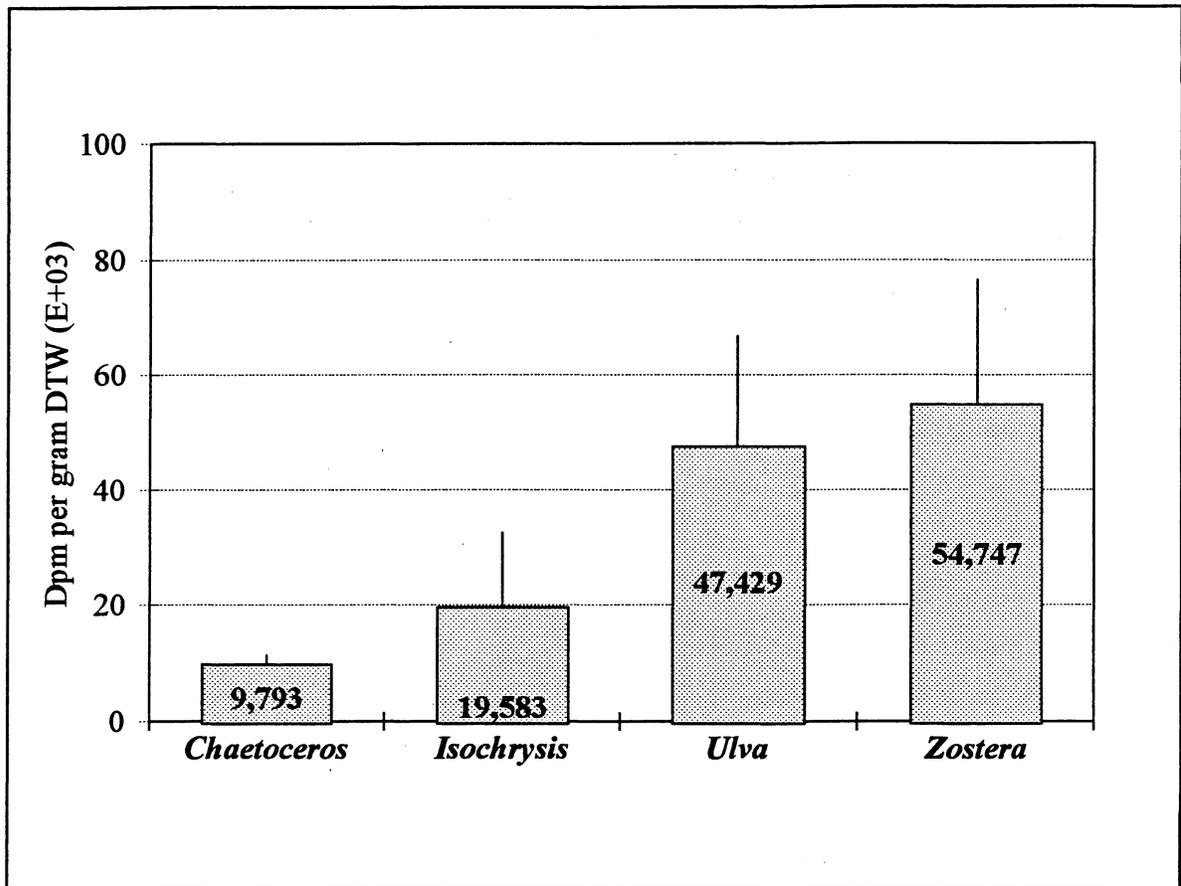


Figure 11: Respiration produced from dietary treatments.

Treatment means are enumerated based on grams dry tissue weight (DTW).

Vertical lines (|) are standard errors.

Treatments were not significantly different ($F_{\text{calc } 3, 8} = 1.45, p > 0.3000$).

Egestion

As reviewed in the introduction, bivalve digestion and food processing are complex. Food can be ingested and digested extracellularly or intracellularly. Food also can be rejected before digestion and pass into the mid-gut for subsequent elimination as feces (Purchon, 1977:229). Feces also contains products of digestion within digestive diverticula (Reid, 1982). Egestion analyzed total fecal production and represented food that was consumed and passed through the gut. Data do not differentiate between food that merely passed through the gut or food that was digested. Total fecal production (Figure 12) was not significantly different between dietary treatments ($F_{\text{calc } 3, 8} = 2.10, p > 0.1788$). This suggests that animals processed statistically similar quantities of diets.

Gut passage time

Gut passage time measured the time necessary for food to pass through clam digestive systems. A mixture of radiolabelled and unlabelled food was given for six hours, followed by twelve hours in which only unlabelled food was provided. Gut passage time was determined when negligible radiolabel was detectable in feces. Fecal production was measured in three hour increments and treatments effects were not statistically different within increments (Figure 13). Thus, all diets proceeded through animal guts in a statistically similar manner.

Peak fecal production occurred for all diets during the intervals between three and nine hours. At the end of experiments, 2% of total fecal production was detected for *Chaetoceros*, 5% for *Isochrysis*, 9% for *Ulva*, and 5% for *Zostera*. Thus, gut passage time for all diets was greater than eighteen hours. Gut passage time for all diets would approach twenty-four hours if slopes of Figure 13 are extended.

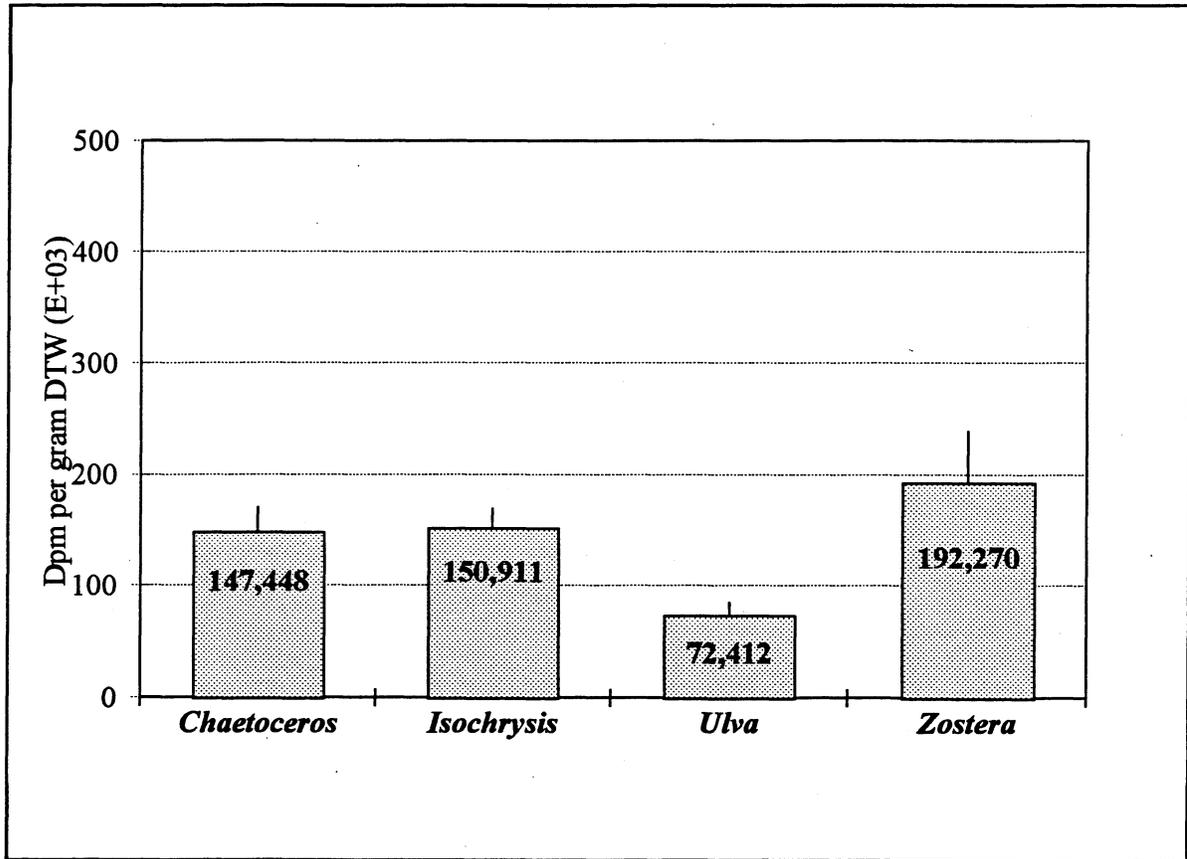


Figure 12: Egestion produced by dietary treatments.

Treatment means are enumerated based on grams dry tissue weight (DTW).

Vertical lines (|) are standard errors.

Treatments were not significantly different ($F_{\text{calc } 3, 8} = 2.10, p > 0.1788$).

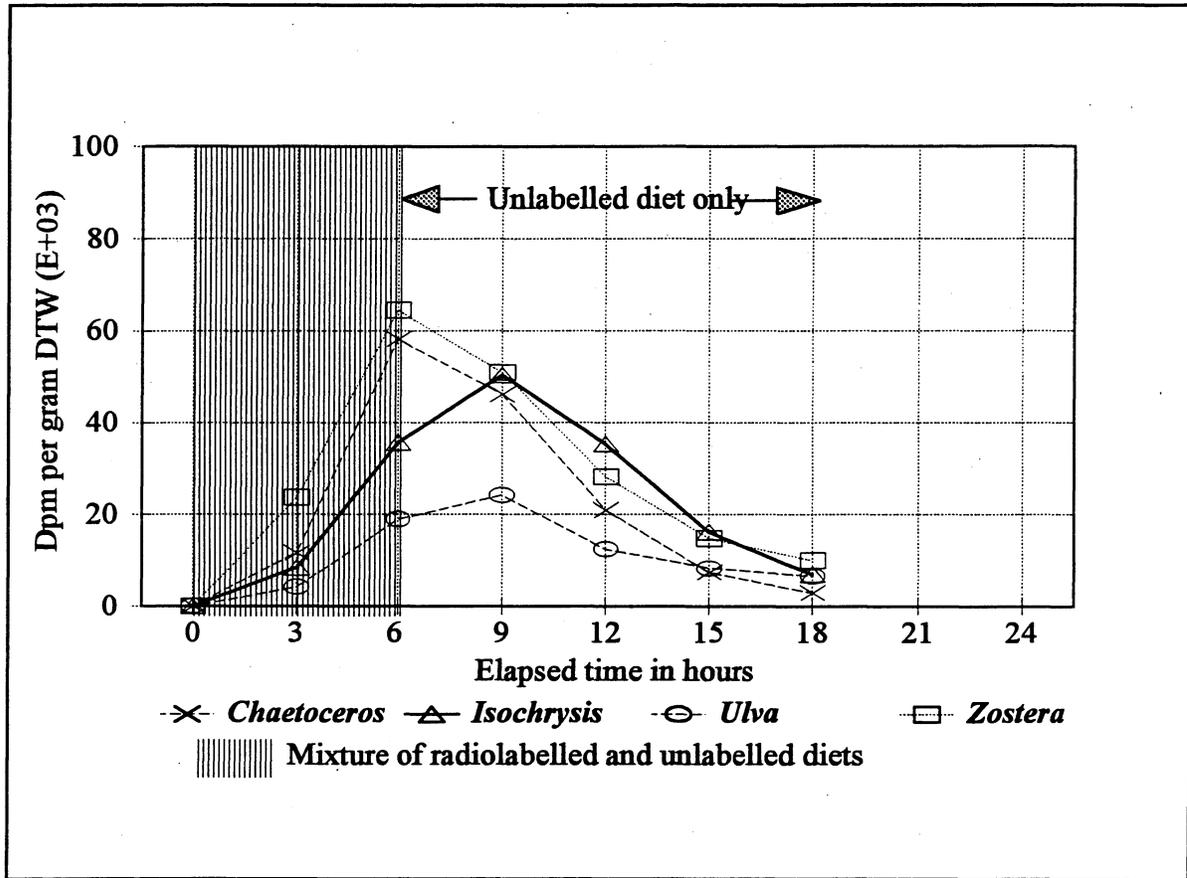


Figure 13: Mean fecal production by dietary treatments and time interval.
Treatment means are based on grams dry tissue weight (DTW).

Time interval

0 to 3 hours

3 to 6 hours

6 to 9 hours

9 to 12 hours

12 to 15 hours

15 to 18 hours

Statistical information

$F_{\text{calc } 3, 8} = 1.09, p > 0.4081$

$F_{\text{calc } 3, 8} = 0.97, p > 0.4512$

$F_{\text{calc } 3, 8} = 1.24, p > 0.3564$

$F_{\text{calc } 3, 8} = 1.78, p > 0.2278$

$F_{\text{calc } 3, 8} = 1.08, p > 0.4098$

$F_{\text{calc } 3, 8} = 2.58, p > 0.1260$

Carbon budgets

During experiments, ingested dietary treatments were allocated to several components including tissue absorption, acid wash supernatants, respiration, and egestion (Figure 14). Total ingestion varied statistically between diets composed either of phytoplankton (*Chaetoceros* or *Isochrysis*) or detrital particulates from *Ulva*. Tissue absorption varied statistically between diets composed of phytoplankton (*Chaetoceros* or *Isochrysis*) and detrital particulates (*Ulva* or *Zostera*). Although, acid wash supernatants varied statistically, a multiple comparison test could not differentiate treatment groups. No statistical differences were detected for respiration or egestion produced by animals in response to dietary treatments.

Whereas, components of ingestion enable numerical comparisons between treatment effects, carbon budgets provide relative comparisons. Carbon budgets assess contributions by dietary treatments to tissue absorption, respiration, and egestion. Data from acid wash supernatants were not included because combining unknown fractions of tissue and undigested gut material could have overestimated contributions to tissue absorption. Dietary treatments produced statistically significant differences for tissue components (Figure 15) of carbon budgets ($F_{\text{calc } 3, 8} = 6.49, p < 0.0155$). A Tukey multiple comparison test found two groups of similarity. One group consisted of both phytoplankton and the particulate, *Ulva*. The other group consisted of *Isochrysis* and both particulates. The Tukey test was unable to distinguish how to group treatment effects from *Isochrysis* and *Ulva* (Zar, 1984:190). It is possible that the null hypothesis for *Isochrysis* and *Ulva* was falsely accepted. Statistical clarity might have been provided with more runs and attendant increased sample size, both of which would have strengthened statistical power.

Based on mean percentages, 75% of *Chaetoceros* diets were absorbed into tissue compared with 23% of *Zostera* diets. Previously discussed tissue utilization found that approximately eight times more particulates were absorbed than particulates; however, carbon budget utilization described approximately three times as much absorption of *Chaetoceros* compared with detrital particulates from *Zostera*.

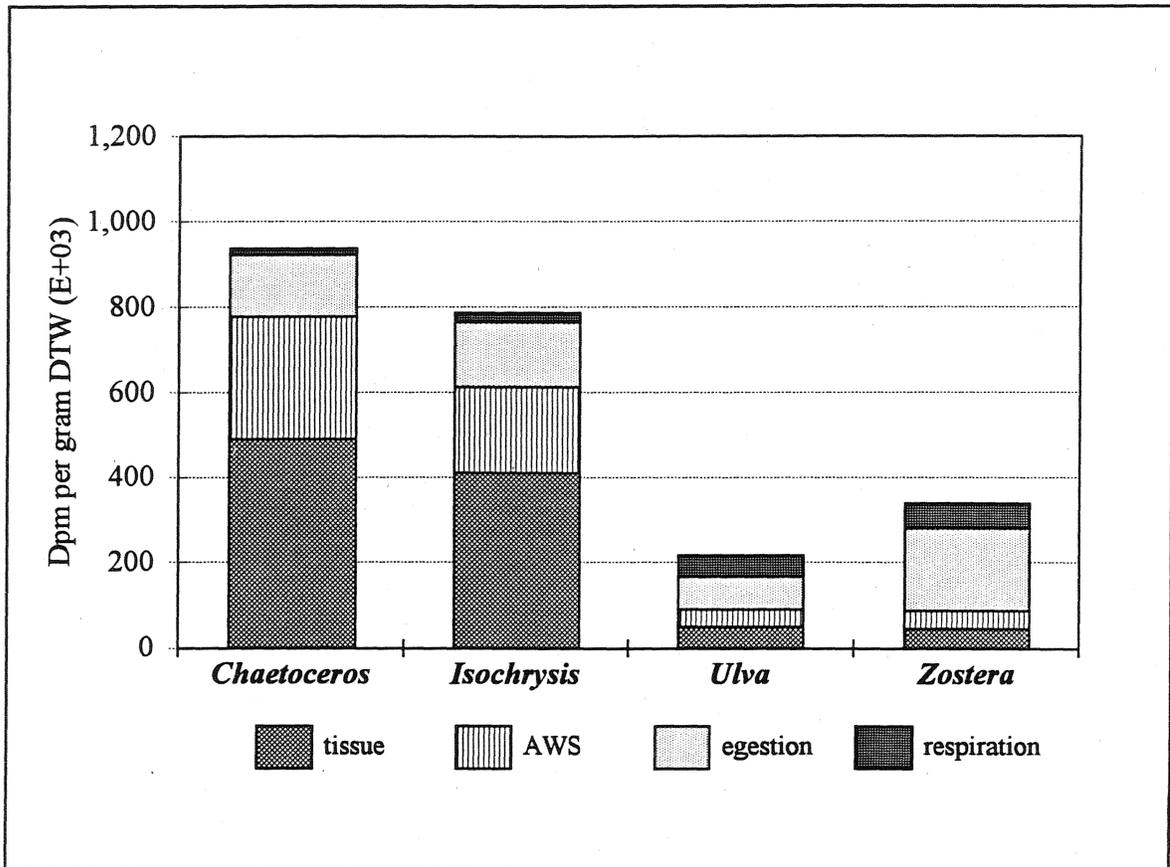


Figure 14: Components of ingestion by dietary treatment.

Components are based on treatment means using grams dry tissue weight (DTW). AWS means acid wash supernatants.

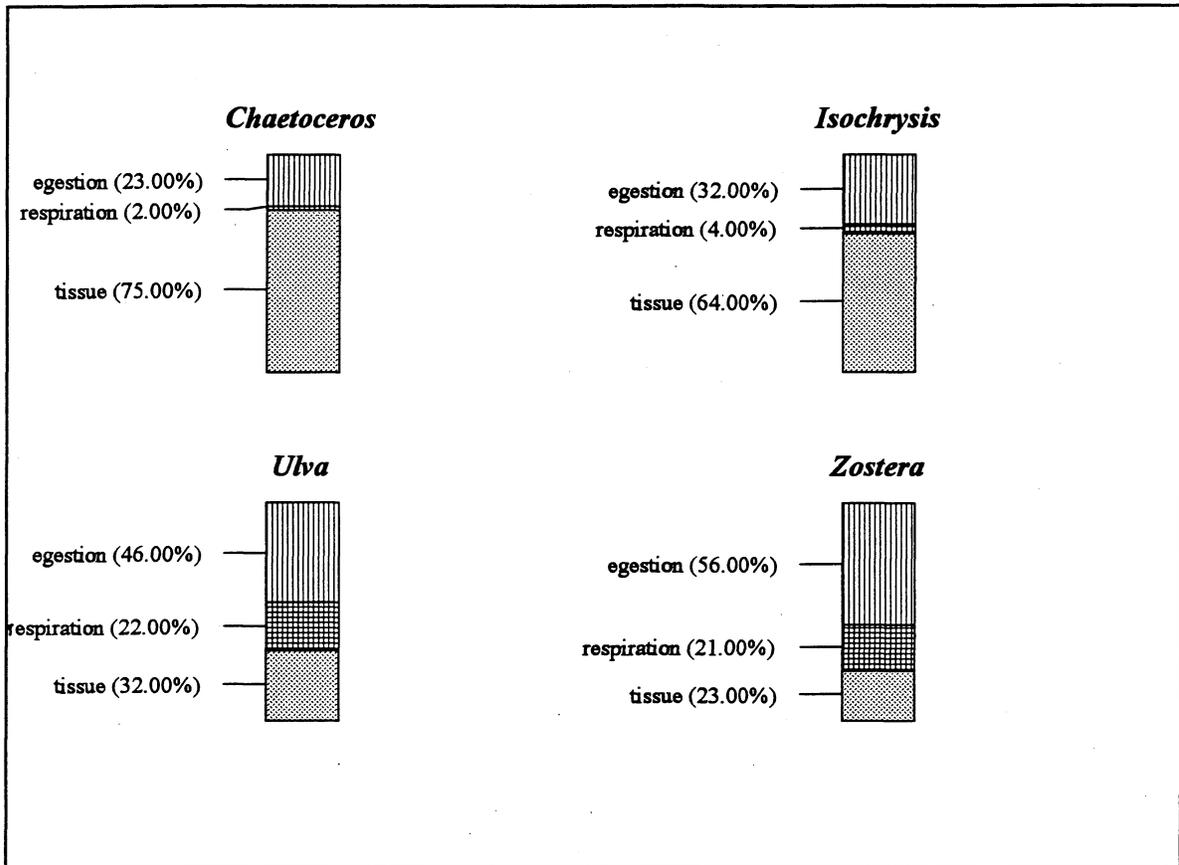


Figure 15: Carbon budgets for dietary treatments.

Enumerated data are treatment means for carbon partitions.

Tissue absorption: $F_{\text{calc } 3, 8} = 6.49, p < 0.0155$

Tukey multiple comparison: *Chaetoceros Isochrysis Ulva Zostera*

Respiration: $F_{\text{calc } 3, 8} = 4.12, p < 0.0485$

Tukey multiple comparison: unable to detect differences between treatment means.

Egestion: $F_{\text{calc } 3, 8} = 2.19, p > 0.1674$

Statistically significant differences in respiration (Figure 15) were associated with dietary treatments ($F_{\text{calc } 3, 8} = 4.12, p < 0.0485$). However, a Tukey multiple comparison test was unable to detect treatment differences through pairwise comparisons. Because a statistical difference was detected, these results differed from those discussed in the previous section on respiration. Dietary contributions to egestion (Figure 15) were not statistically significant between treatments ($F_{\text{calc } 3, 8} = 2.19, p > 0.1674$). These results were similar to those for egestion discussed previously.

Quality control factors

During each experiment, several factors were regulated and analyzed for treatment effect because statistically significant differences could have obscured conclusions concerning treatments. Total activity per treatment was calculated for each animal, by combining data for tissue absorption, acid wash supernatants, respiration, egestion, and effluent. Total activity in dpm per gram DTW (Figure 16), was statistically similar between all treatments ($F_{\text{calc } 3, 8} = 1.00, p > 0.4403$). Activity varied from 1.7×10^6 to 2.5×10^6 dpm per gram DTW, with an overall average of 2.2×10^6 dpm per gram DTW (Appendix D). Thus, all treatment groups received statistically similar quantities of radiolabelled diets.

During the sixth hour of each experiment, a sample of seawater and diet solution was taken from each feeding chamber. The purpose was to determine if specific activity was similar between treatments. Activity, expressed as dpm ml^{-1} , was statistically similar for all treatment groups ($F_{\text{calc } 3, 8} = 0.54, p > 0.6656$). Activity varied by treatment from 1,627 to 2,206 dpm ml^{-1} , with an overall average of 1,900 dpm ml^{-1} (Appendix D). Thus, treatment effects were not confounded by different doses of radiolabelled diets.

Hourly flow rates were calculated by dividing total effluent per feeding chamber by the eighteen hours of experiment duration. The purpose was to determine if all animals were exposed to statistically similar quantities of food and velocities. Hourly flow rates were statistically similar for all treatments ($F_{\text{calc } 3, 8} = 2.21, p > 0.1643$). Flow rates varied from 184 to 208 ml hr^{-1} , with an overall average of 199 ml hr^{-1} (Appendix D). Treatment effects were not affected by different flow rates.

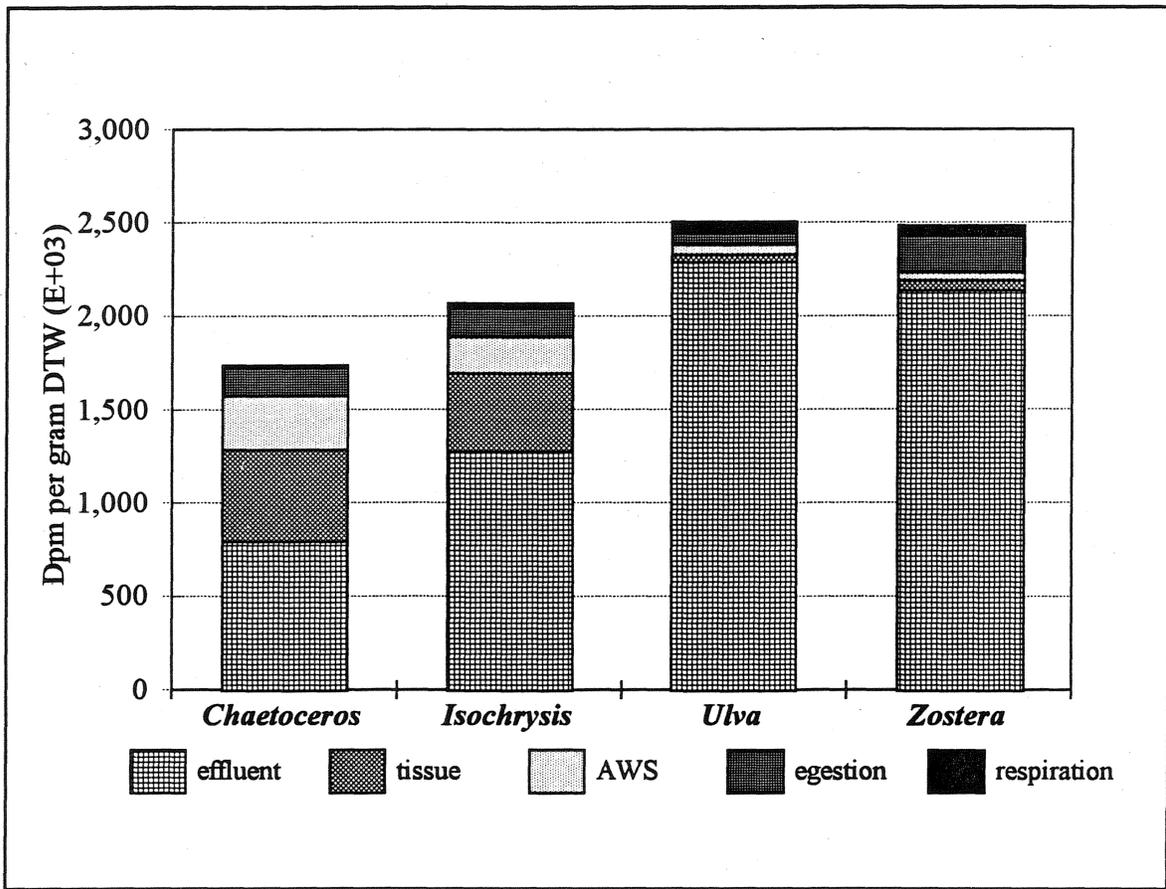


Figure 16: Total activity by dietary treatments.

Illustrated components are based on means using grams dry tissue weight (DTW).
 AWS means acid wash supernatant.

At the end of experiments, animals were sacrificed, blotted, and tissue was removed from valves. Animal wet tissue weights were statistically similar for all treatments ($F_{\text{calc } 3, 8} = 1.31, p > 0.3361$). Animal wet varied between 6.73 and 7.63 grams, with an overall average of 7.22 grams (Appendix D). For dietary work, disintegrations per minute often are equated to dry tissue weight. A regression equation, based on 94 animals, was applied to wet tissue weights of experimental animals. The resulting dry tissue weights were statistically similar between treatments ($F_{\text{calc } 3, 8} = 1.26, p > 0.3523$). Animal dry weights varied between 0.94 and 1.07 grams, with an overall average of 1.01 grams (Appendix D). Treatment effects were not affected by animal weight and attendant size.

DISCUSSION

Experiment objectives were to determine if Manila clams could absorb carbon derived from detrital particulates composed of *Ulva fenestrata* and *Zostera marina*, and to compare utilization with the phytoplankters, *Chaetoceros gracilis* and *Isochrysis galbana*. Phytoplankton were intended to exemplify nutritionally rich food sources typically available to bivalve suspension feeders that live in shallow estuaries. Detrital particulates were intended to represent suspended particles, one component of seston associated with shallow estuaries. Laboratory findings clearly demonstrated that Manila clams absorb detrital particulates; however, absorption was significantly less than with phytoplankton. This section discusses possible explanations for laboratory observations, as well as potential relevance of laboratory findings to food web relationships in eelgrass and mud flat habitats.

Laboratory findings

Absorption of detrital particulates by Manila clams must be considered with reference to ingestion, total activity per treatment, and gut passage time. Ingestion of diets was significantly different and phytoplankton was ingested more than *Ulva* particulates. Particle counts approximated 1.0×10^5 cells ml^{-1} , considered within the concentration that bivalves are able to manipulate (Foster-Smith, 1975a; Foster-Smith, 1975b; Riisgard, 1991; and Perez-Camacho *et al.*, 1994). Particle size was between 5 μm and 25 μm , also within the range that bivalves are able to manipulate (Vahl, 1972; Mohlenberg and Riisgard, 1978; Riisgard, 1988; and Sprung and Rose, 1988). Dry tissue weight and total specific activity were statistically similar between all treatments. The similarities between treatments for particle density, particle size, dry tissue weight and total specific activity did not confound experimental results for ingestion and tissue absorption.

It is possible that clams ingested different quantities of food due to selection. The paradigm for bivalve suspension feeding suggests that animals are unable to select between particles and will ingest all particles that are manipulatable (Jorgensen, 1955:402; and Chew and Ma, 1987:12). However, the review by Jorgensen (1955:402) indicated that qualitative

sorting of food by bivalves might be occurring and needed further investigation. Ward and Targett (1989) noted that bivalve research documented selection between two species of algae, between phytoplankton and inorganic particles, and between yeast and phytoplankton. Their research determined that bivalves rely on dissolved chemicals from phytoplankton as feeding cues and may reduce feeding in the presence of noxious phytoplankton (Ward and Targett, 1989). Recently, MacDonald and Ward (1994) found that sea scallops varied particle selection based on food quality of seston and increased ingestion of suspended particulate matter to obtain chlorophyll containing food. Similarly, Baldwin and Newell (1995) found that larval oysters fed diets of cultured algae and seston, altered behavior by ingesting more seston than algae, perhaps to compensate for lesser nutritional value associated with seston composition.

During experiments, clams were offered single diets and could choose to eat or not eat as a means of selection. Some clams actively fed on phytoplankton while other clams ate with less vigor when fed particulates. It is possible that clams responded to chemical cues and amended feeding behavior accordingly. This might have contributed to observed behavior with attendant differences for both ingestion and absorption.

Absorption by the Manila clam of detrital particulates from *Ulva fenestrata* and *Zostera marina* is similar to studies that address other bivalve species and detrital sources, reviewed elsewhere. Although detrital particulates were absorbed into tissue, the value of detrital particulates and phytoplankton for animal nutrition is related to food quality. Not only is food quality species dependent, but quality is influenced by other factors.

Phytoplankton biochemical composition was influenced by temperature, light intensity, and growth phase (Brown, 1991; Nelson *et al.*, 1992; and Thompson *et al.*, 1992). Although some phytoplankton increased nutritional quality with temperature, some species were adversely affected (Nelson *et al.*, 1992). Season affected the biochemical composition of *Ulva lactuca* (Abdel-Fattah and Edrees, 1973) and high lipid content occurred during winter. Growth of *U. lactuca* was enhanced with additions of dissolved inorganic carbon (Frost-Christensen and Sand-Jensen, 1990) or nitrogen (Bjornsater and Wheeler, 1990) to culture solutions. *U. fenestrata* showed positive growth in the presence of detritus from *Z. marina*,

probably due to bacterial exudates (Harrison, 1978). Amino acid composition of eelgrass detritus declined initially with age then increases with bacterial enrichment (Thayer *et al.*, 1977). Early research focused on bacterial mediation during seagrass detritus formation with attendant enrichment as the probable source of nutritive value (Harrison and Mann, 1975). However, bacterial and microalgal growth was inhibited by extracts of *Z. marina* leaves (Harrison and Chan, 1980). Although particulate detritus from seagrass experienced bacterial enrichment, the raw substrate was shown to be digestible by many animals, but of low quality, and could serve as a direct source of nutrition (Harrison, 1989).

Several laboratory and field studies have evaluated decomposition of *Z. marina*. Total nonstructural carbohydrates of leaves in anaerobic conditions were not detectable within six months (Godshalk and Wetzel, 1978). In aerobic conditions, leaves experienced an initial loss of total nonstructural carbohydrates, followed by a period in which 50% of initial carbohydrate content was regained (Godshalk and Wetzel, 1978). Montgomery and Targett (1992) found increases in complex carbohydrates and proteins with controlled laboratory leaching of *Z. marina*. By contrast, Alber and Valiela (1995) found decreased dissolved organic matter after laboratory leaching of *Z. marina* and *U. lactuca*. Similarly, Fenchel (1977) quantified decreases in carbohydrate content of *Zostera* leaves over time. Chemical content of *Z. marina* differed between roots, rhizomes, and leaves at various ages of decomposition (Seki and Yokohama, 1978; and Harrison, 1989). Drew (1980) reviewed carbohydrate composition of seagrasses and showed variation with season and locale. Carbohydrate content of *Zostera noltii* was the greatest during spring germination (DeRosa *et al.*, 1990). Velimirov (1987) found that carbon content of eelgrass leaves (*Posidonia sp.*) remained constant throughout the year, but nitrogen content varied with the seasons.

Given the paucity of information concerning variability in biochemical content, food quality for these experiments can only be relatively compared. Because data were not available for gross chemical composition associated with all dietary treatments, information on related species is provided for comparison. Table 1 indicates that phytoplankton tend to have higher percentages of lipids and proteins than *Ulva* species or eelgrass. High lipid and

Table 1: Gross chemical composition of dietary treatments. Values are expressed as percentage of dry weight.

Material	Carbohydrate	Lipid	Protein	Reference
<i>Chaetoceros calcitrans</i>	18.00%	17.3%	NA	Laing <i>et al.</i> (1990)
<i>Chaetoceros gracilis</i>	4.7%	7.2%	12%	Brown (1991)
<i>Chaetoceros gracilis</i>	16.0%	20.0%	64%	Nelson <i>et al.</i> (1992)
<i>Isochrysis galbana</i>	12.9%	23%	29%	Brown (1991)
<i>Isochrysis galbana</i>	15.6%	17.2%	60.1%	Epifanio (1982:298)
<i>Isochrysis galbana</i>	10.10%	20.60%	NA	Laing <i>et al.</i> (1990)
<i>Isochrysis galbana</i>	16.3%	33.0%	50.7%	Nelson <i>et al.</i> (1992)
<i>Ulva fasciata</i>	45%	<1%	30%	Tewari <i>et al.</i> (1990)
<i>Ulva lactuca</i>	48.03%	3.07%	8.70%	Abdel-Fattah and Edrees (1973)
<i>Ulva lactuca</i>	61.23%	1.88%	3.4%	Lowe and Lawrence (1976)
seagrass	NA	<5%	NA	Phillips (1984)
<i>Thalassia testudium</i>	63.9%	2.33%	9.11%	Lowe and Lawrence (1976)
<i>Thalassia testudium</i>	NA	NA	12-13%	Augier <i>et al.</i> (1982)
<i>Zostera capricorni</i> seeds	60%	3%	10%	Dall <i>et al.</i> (1992)
<i>Zostera marina</i>	59.7%	3.0%	33.0%	Montgomery and Targett (1992)

protein contents are identified as important for bivalves (Epifanio, 1982; Laing *et al.*, 1990; Brown, 1991; and Nelson *et al.*, 1992). By comparison, carbohydrates are the predominant fraction identified in macrophytes. Although bivalves require lipids and proteins, they also can utilize carbohydrates (Abdel-Fattah and Edrees, 1973; Lowe and Lawrence, 1976; and Montgomery and Targett, 1992). Data show that eelgrass contains more carbohydrate than *Ulva*; however, eelgrass also contains lignin and phenolic compounds (Quakenbush *et al.*, 1986), making it less labile.

Phytoplankton used in these experiments were living. Detrital particulates were prepared from freshly harvested plants that were dried, ground into particulates, and frozen to minimize bacterial enrichment. As such, gross chemical composition of detrital particulates probably approached living plants rather than aged material. Based on the foregoing considerations, the probable comparative nutritional quality of dietary treatments used in these experiments is *Isochrysis* > *Chaetoceros* > *Ulva* > *Zostera*. Detrital particulates from *Ulva* and *Zostera* are poor quality food compared with phytoplankton. Phytoplankton used in experiments have well documented nutritional value for bivalves and consequently are used in aquaculture. Thus, this research compared food with high nutritional value against detrital particulates with poorly known nutritional value.

During experiments, about one-eighth the amount of diets composed of detrital particulates were absorbed compared with diets of phytoplankton. Considering the nutritional quality of particulates, very little benefit would be provided to Manila clams fed a sustained diet of experimental particulates unless physiological compensation occurred. Examples of physiological compensation include increased gut passage time (Bayne *et al.*, 1988), additional production of crystalline style enzymes (Seiderer *et al.*, 1982), and changes in surface area of digestive tubules (Bayne *et al.*, 1987). Moreover, because ingestion of *Ulva* particulates was less than phytoplankton, no behavioral adjustments were made to compensate for lesser food value of particulates as noted with other studies (MacDonald and Ward, 1994; and Baldwin and Newell, 1995).

Assessment of absorption was based on whole animal tissue, principally because these experiments used techniques modified by other researchers who used whole body analyses

(Newell and Langdon, 1986; Kreeger *et al.*, 1988; and Kreeger *et al.*, 1990). One short-coming of whole body analysis relates to the digestive gland with its labyrinth of tubules. Bayne *et al.* (1984) found a positive correlation between gut residence time and contents of the digestive gland of three species of mussels. More ^{14}C radiolabel was consistently detected in digestive glands of mussels than in mantle or remaining tissue (Hawkins and Bayne, 1985). Because of digestive gland complexity, it is possible that particles might have been retained without digestion during the experimental time frame. Inclusion of related data would overestimate contributions to tissue absorption. In addition, the acid-alkali protocol used for experiments assessed label that absorbed into tissue as well as label that was digested but not absorbed by the end of experiments (Newell and Langdon, 1986:109).

Analysis of selected tissues could minimize vagaries associated with the digestive gland. Removal of the digestive gland would facilitate analysis of remaining tissue. However, the boundary between the digestive gland and other tissues is a gradient, and different dissections might have introduced more experimental deviation. Another technique employs biochemically stable tissues, such as muscle or mantle, with extrapolation to whole animal values (Beninger and Lucas, 1984; and Ruckelshaus, 1988:9). Alber and Valiela (1995) used adductor muscle to avoid the problem of ^{15}N being ingested but not absorbed in scallops. My research used whole body analyses because it was uncertain how accurate readings would be from small quantities of tissue analyzed with the ^{14}C ET-DPM protocol of LSC.

Data obtained from hot acid washes was differentiated data obtained from alkali treatments. Acid wash supernatants consisted of unknown fractions of unabsorbed gut material, as well as tissue. Data from hot acid supernatants were not included in tissue absorption results. In this way, estimates of tissue absorption would be more conservative and conclusions would tend to underestimate, rather than overestimate, dietary contributions.

Experiment data for gut passage time and egestion do not distinguish between fecal material that was digested or merely passed through the intestine. It would have taken about 21 to 24 hours for total gut passage of diets. Hawkins *et al.* (1990) described gut passage time to be the time required to purge 90% of total egestion. Using this estimate, gut passage time resulting from thesis experiments would approximate 18 hours. These results are

comparable with findings from other researchers. Between 15 to 18 hours were required to pass phytoplankton labelled with ^{32}P passed through guts of mussels and hard shell clams (Foster-Smith, 1975b). Stuart *et al.* (1982) found gut passage time for mussels to be approximately 24 hours when fed Rose Bengal particles. Mussels fed ^{14}C labelled phytoplankton had gut passage times of approximately 15 hours (Hawkins *et al.*, 1990). In contrast, Bayne *et al.* (1984) found gut passage times ranging from two to eight hours for South African mussels. They explained variation in gut passage time as an inverse relationship to seston food quality and a direct relationship to food content in the digestive gland. Similarly, gut passage time for mussels fed phytoplankton varied from four to six hours in June and from eight to twelve hours in March (Bayne *et al.*, 1987; and Bayne *et al.*, 1988). This was thought to be inversely related to seasonal food quality. Sorokin and Giovanardi (1995) found gut passage time to be approximately seven hours for Manila clams fed ^{14}C radiolabelled phytoplankton for fifteen minutes.

In this study, clams were fed radiolabelled diets for six hours and provided opportunity for digestive systems to become fully saturated with food. This might account for the longer gut passage time compared with findings of Sorokin and Giovanardi (1995). In addition, physiological compensation for different food quality probably was not a factor affecting absorption during experiments because there was no statistical difference for gut passage times in response to diets of phytoplankton or detrital particulates.

Carbon budgets for bivalves, obtained by other researchers, assess allocations to tissue, respiration, and egestion. Ribbed mussels fed diets of refractory *Spartina* for eighteen hours allocated between 3.0% to 10.5% to tissue, 1.0% to 5.5% to respiration, and 84.6% to 95.8% to egestion (Kreeger *et al.*, 1988; and Kreeger *et al.*, 1990). Oysters fed *Spartina* detrital complexes allocated 5% to tissue, 1% to respiration, and 94% to egestion (Crosby *et al.*, 1990). Manila clams fed various ^{14}C labelled phytoplankton allocated between 34% to 63% to tissue, 4% to 6% to respiration, and 31% to 63% to egestion (Sorokin and Giovanardi, 1995). In another study, mussels fed ^{14}C radiolabelled phytoplankton allocated 48% to feces (Hawkins and Bayne, 1985).

The tissue partition of carbon budgets was statistically different in response to dietary treatments. However, a multiple comparison test could distinguish the *Chaetoceros* treatment from diets of *Zostera* particulates. This was, in part, due to one experiment in which data from an *Isochrysis* diet were uncharacteristically low and were within the range of data obtained for the diet of *Ulva* particulates. The related batch of *Isochrysis* might not have had the same qualities as batches used in other experiments. The range of allocation to tissue was between 64% to 75% resulting from phytoplankton diets, and 23% to 32% from particulate diets. Allocations to tissue from diets composed of detrital particulates were higher than literature values for either *Spartina* or *Spartina* complexes. Fresh detrital particulates from *Ulva* and *Zostera* have more nutritional value than processed *Spartina* particles. Allocations to tissue from phytoplankton diets were similar to literature values for Manila clams.

There was a statistical difference between treatments for the respiration component of carbon budgets. Between 1.5% to 3.7% of phytoplankton diets and 21% of particulate diets were allocated to respiration. Because of large standard deviations from treatments, a multiple comparison test was unable to detect differences. Numerical values for allocations to respiration for phytoplankton diets are similar to literature values. Data for particulate diets are comparatively high. The high value might be a function of the small quantity of ingested particulate diets and the bias that enters with percentage calculations. In addition, particulate diets might have stimulated production of pseudofeces (not measured) and increased manipulative effort by clams. The increased effort associated with particle manipulation might have been expressed as an increased allocation to respiration.

No statistical difference between treatments was detected for the egestion component of carbon budgets. Between 23% to 32% of phytoplankton diets and between 46% to 56% of particulate diets were allocated to egestion. These values are comparable to findings of Hawkins and Bayne (1985), as well as Sorokin and Giovanardi (1995). However, they are less than that associated with *Spartina* diets. Similar to results for tissue absorption, it is possible that freshly prepared detrital particulates from *Ulva* and *Zostera* were nutritionally better than processed particulates from *Spartina*.

Laboratory research identified utilization of detrital particulates from *Ulva* and *Zostera* by Manila clams. However, it is important to consider relevance of these findings to the natural environment.

Potential relevance of laboratory findings to the field

Detritus from *Ulva* and *Zostera* may take on seasonal importance when phytoplankton is limited. Seasonal fluxes in seston quantity and quality are well documented. Seston quantity was found to be highest during winter months and seston quality, assessed with chlorophyll content, was highest during summer (Widdows *et al.*, 1979). Other researchers found that seston quantity was less in winter than summer; however, quality was better in winter than in summer because of increased inorganic material during summer (Berg and Newell, 1986). In addition, seston quantity varied between nearby estuaries and could be the cause of different sized oysters (Berg and Newell, 1986). Carbon and crude fiber contents of seston were higher in summer than winter; but crude fiber content varied between years in response to freshwater discharge (Kreeger *et al.*, 1988; and Langdon and Newell, 1990). In another estuary, Crosby *et al.* (1989) found crude fiber to be less concentrated than what was reported for smaller systems (Kreeger *et al.*, 1988; and Langdon and Newell, 1990), but annual variation was present.

Few studies document seasonal fluxes in detrital loads in Washington and British Columbia. In Hood Canal, dissolved organic carbon in seston, determined with stable isotope $\delta^{13}\text{C}$, was less in winter than summer probably due to freshwater storage within the marine system (Simenstad and Wissmar, 1985). In southern British Columbia, litter accumulation from macroalgae was greatest during fall (Smith and Foreman, 1984).

In Padilla Bay, suspended particulate matter in seston was greater in summer than spring for eelgrass and mud flat habitats (Ruckelshaus, 1988:47). This trend also pertained content of chlorophyll a; however, winter content was less than either spring or summer, indicating the relative absence of phytoplankton. Ruckelshaus (1988:24) determined that $\delta^{13}\text{C}$ values for *Zostera marina* were stable during decomposition processes and contributed to seston enrichment. Mussels in eelgrass habitats showed $\delta^{13}\text{C}$ enrichment (Ruckelshaus,

1988:24) and $\delta^{13}\text{C}$ enrichment of Manila clams located in mud flat and eelgrass habitats was similar to experimental mussels (Ruckelshaus, 1988:15). It was estimated that *Zostera*, in the form of particulate organic carbon, contributed 19% to summer mussel diets and 36% to winter mussel diets (Ruckelshaus, 1988:26). Mussel growth correlated better with seston quality than quantity (Ruckelshaus, 1988 30).

Manila clams used in laboratory experiments came from Padilla Bay. Prior to removal for laboratory experiments, the clams were exposed to similar conditions that existed during the 1986 field research by Ruckelshaus. $\delta^{13}\text{C}$ ratios suggesting Manila clam utilization of *Zostera* particulates (Ruckelshaus, 1988:15) combined with laboratory demonstration of tissue absorption of detrital particulates from *Zostera* underscores the conclusion that Manila clams are part of detrital food webs associated with *Zostera*. If seasonal utilization by Manila clams of *Zostera* detritus is similar to mussels of Padilla Bay, *Zostera* detritus could provide approximately one-third of winter diets.

The mechanics associated with eelgrass habitats could be a factor to enhance availability of suspended material to suspension feeders, such as Manila clams. Population density and growth of quahog clams were greater in eelgrass (*Zostera marina*) habitats than populations from nearby sand flats (Peterson *et al.*, 1984; and Irlandi and Peterson, 1991). These effects were attributed to sediment root trapping, as well as dampened current velocity that caused suspended particles to settle and increase food concentrations for the clams.

This study suggests that, in the field, Manila clams can utilize detrital particulates derived from *Ulva fenestrata* and *Zostera marina*. These macrophytes contribute to habitat structure and are important primary producers in local estuaries. In addition, detrital particulates from *Ulva* and *Zostera* might provide food sources for suspension feeding bivalves during periods of phytoplankton limitation, such as winter. Thus, the roles of *Ulva* and *Zostera* in nearshore food webs and associated energy flows are expanded in complexity. Estuarine management should consider not only the immediate contributions of *Ulva* and *Zostera* to primary production and habitat, but also the contribution to primary consumers as part of nearshore detrital food webs of mud flat and eelgrass habitats.

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Appendix A

Formula for Guillard's modified F/2 nutrient

The formula makes one liter of F/2 which is refrigerated until needed. The formula was obtained at Shannon Point Marine Center in Anacortes, Washington. F/2 nutrient is composed of primary solution, trace metals, and vitamins.

Primary solution

To one liter distilled water add:

- 75 gr. sodium nitrate
- 5 gr. sodium phosphate (monobasic, monohydrate)
- 5 gr. EDTA (ferric sodium salt)
- 2.5 gr. boric acid

Trace metals

To one liter of distilled water add:

- 98 mg. cupric sulfate (5 hydrate)
- 22 mg. zinc sulfate
- 10 mg. cobalt chloride
- 63 mg. sodium molybdate
- 1.8 gr. manganous chloride

Add 10 mls of trace metal to one liter of primary solution. Refrigerate the remainder of trace metals for future use. Autoclave the primary solution with trace metals for 15 minutes at 125°C (15 lbs psi).

Vitamin solution

To 10 ml. of autoclaved distilled water add:

- 167.5 mg thiamine hydrochloride
- 1.5 mg Vitamin B₁₂
- 1.5 mg biotin

Filter sterilize, as needed, by using a 0.45 µm Acrodisk filter attached to a luer type syringe. Add one ml to the cooled solution of primary and trace metals. Freeze the remainder of vitamin solution for future use.

Use F/2 nutrient at a rate of 2 ml per liter of seawater.

A precipitate may form when F/2 nutrient is refrigerated but this is not a problem.

Appendix B

Table B.1: Experiment date, treatment, and number of replicates

Date	<i>Chaetoceros</i> phytoplankton	<i>Isochrysis</i> phytoplankton	<i>Ulva</i> particulates	<i>Zostera</i> particulates
06-11-93		4	4	
07-18-93		4	4	
08-18-93	4			4
10-23-93			4	4
11-20-93	4			4
12-17-93	4	4		
Total	12	12	12	12

Appendix C: ANOVA tables and multiple comparisons tests

Table C.1: Partially nested one-way ANOVA by treatment for ingestion.

Calculations use log transformations of disintegrations per minute (dpm) per gram dry tissue weight (DTW). * indicates statistical significance at $\alpha = 0.05$.

Source	DF	SS	MS	F calc	p
Treatment	3	4.11364	1.37121	7.17*	0.0118
(Trt) * Run	8	1.53007	0.19126	3.99*	
Residual	36	1.72947	0.04804		
Total	47	7.37317			

Tukey (HSD) multiple comparison test:

Chaetoceros Isochrysis Zostera Ulva

Table C.2: Partially nested one-way ANOVA by treatment for tissue absorption.

Calculations use log transformations of dpm per gram DTW.

* indicates statistical significance at $\alpha = 0.05$.

Source	DF	SS	MS	F calc	p
Treatment	3	10.7748	3.59161	11.72 *	0.0027
(Trt) * Run	8	2.45071	0.30634	11.23 *	
Residual	36	0.98173	0.02727		
Total	47	14.2072			

Tukey (HSD) multiple comparison test:

Chaetoceros Isochrysis Zostera Ulva

Table C.3: Partially nested one-way ANOVA by treatment for acid wash supernatants. Calculations use log transformations of dpm per gram DTW.
 * indicates statistical significance at $\alpha = 0.05$.

Source	DF	SS	MS	F calc	p
Treatment	3	7.29149	2.43049	4.67*	0.0361
Trt * (Run)	8	4.15974	0.51996	6.38*	
Residual	36	2.93264	0.08146		
Total	47	14.3838			

Tukey (HSD) multiple comparison test:
 unable to detect differences in pairwise comparisons between treatments.

Table C.4: Partially nested one-way ANOVA by treatment for respiration.
 Calculations use log transformations of dpm per gram DTW.

Source	DF	SS	MS	F calc	p
Treatment	3	1.63581	0.54537	1.45	0.3000
(Trt) * Run	8	3.01574	0.37696	1.82	
Residual	36	7.45307	0.20703		
Total	47	12.1046			

Table C.5: Partially nested one-way ANOVA by treatment for total egestion.
 Calculations use log transformations of dpm per gram DTW.

Source	DF	SS	MS	F calc	p
Treatment	3	1.33278	0.44426	2.10	0.1788
(Trt) * Run	8	1.69373	0.21172	2.05	
Residual	36	3.72005	0.10333		
Total	47	6.74655			

Table C.6: Partially nested one-way ANOVA by treatment for egestion produced between the start and third hour of experiments. Calculations use square root transformations of dpm per gram DTW. * indicates statistical significance at $\alpha = 0.05$.

Source	DF	SS	MS	F calc	p
Treatment	3	43,718.1	14,572.7	1.09	0.4081
(Trt) * Run	8	107,200	13,395.4	4.13*	
Residual	36	116,900	3247.02		
Total	47	267,800			

Table C.7: Partially nested one-way ANOVA by treatment for egestion produced between the third and sixth hours of experiments. Calculations use square root transformations of dpm per gram DTW. * indicates statistical significance at $\alpha = 0.05$.

Source	DF	SS	MS	F calc	p
Treatment	3	71,812.2	23,937.4	0.97	0.4512
(Trt) * Run	8	196,500	24,561.1	2.42*	
Residual	36	365,500	10,153.0		
Total	47	633,800			

Table C.8: Partially nested one-way ANOVA by treatment for egestion produced between the sixth and ninth hours of experiments. Calculations use square root transformations of dpm per gram DTW.

Source	DF	SS	MS	F calc	p
Treatment	3	40,794.3	13,598.1	1.24	0.3564
(Trt) * Run	8	87,492.1	10,936.5	1.36	
Residual	36	290,500	8,068.1		
Total	47	418,700			

Table C.9: Partially nested one-way ANOVA by treatment for egestion produced between the ninth and twelfth hours of experiments. Calculations use square root transformations of dpm per gram DTW.

Source	DF	SS	MS	F calc	p
Treatment	3	35,070.8	11,690.3	1.78	0.2278
(Trt) * Run	8	52,399.0	6,549.88	1.68	
Residual	36	139,700	3,880.13		
Total	47	227,200			

Table C.10: Partially nested one-way ANOVA by treatment for egestion produced between the twelfth and fifteenth hours of experiments. Calculations use square root transformations of dpm per gram DTW.

* indicates statistical significance at $\alpha = 0.05$.

Source	DF	SS	MS	F calc	p
Treatment	3	13,206.7	4,402.24	1.08	0.4098
(Trt) * Run	8	32,509.8	4,063.73	2.68 *	
Residual	36	54,571.1	1,515.86		
Total	47	100,300			

Table C.11: Partially nested one-way ANOVA by treatment for egestion produced between the fifteenth and eighteenth hours of experiments. Calculations use square root transformations of dpm per gram DTW.

Source	DF	SS	MS	F calc	p
Treatment	3	12,070.9	4,023.62	2.58	0.1260
(Trt) * Run	8	12,463.8	1,557.98	1.96	
Residual	36	28,599.1	794.418		
Total	47	53,133.8			

Table C.12: Partially nested one-way ANOVA by treatment for the tissue partition of a carbon budget. Calculations use arcsin transformations of square root ratios. * indicates statistical significance at $\alpha = 0.05$.

Source	DF	SS	MS	F calc	p
Treatment	3	2.69880	0.89960	6.49*	0.0155
(Trt) * Run	8	1.10903	0.13863	12.33*	
Residual	36	0.40477	0.01124		
Total	47	4.21261			

Tukey (HSD) multiple comparison test:
Chaetoceros Isochrysis Ulva Zostera

Table C.13: Partially nested one-way ANOVA by treatment for the respiration partition of a carbon budget. Calculations use arcsin transformations of square root ratios. * indicates statistical significance at $\alpha = 0.05$.

Source	DF	SS	MS	F calc	p
Treatment	3	1.04079	0.34693	4.12*	0.0485
(Trt) * Run	8	0.67371	0.08421	2.29	
Residual	36	1.32118	0.03669		
Total	47	3.03568			

Tukey (HSD) multiple comparison test:
 unable to detect pairwise differences in comparisons between treatments.

Table C.14: Partially nested one-way ANOVA by treatment for the egestion partition of a carbon budget. Calculations use arcsin transformations of square root ratios. * indicates statistical significance at $\alpha = 0.05$.

Source	DF	SS	MS	F calc	p
Treatment	3	0.93632	0.31210	2.19	0.1674
(Trt) * Run	8	1.14170	0.14271	5.03*	
Residual	36	1.02233	0.02839		
Total	47	3.10036			

Table C.15: Partially nested one-way ANOVA by treatment for total dpm available to animals during experiments. Calculations use log transformations of dpm per gram DTW. * indicates statistical significance at $\alpha = 0.05$.

Source	DF	SS	MS	F calc	p
Treatment	3	0.20482	0.06827	1.00	0.4403
(Trt) * Run	8	0.54516	0.06814	5.16*	
Residual	36	0.47583	0.01321		
Total	47	1.22582			

Table C.16: Partially nested one-way ANOVA by treatment for dpm available to animals during the sixth hour of experiments.

Source	DF	SS	MS	F calc	p
Treatment	3	2.925×10^6	9.749×10^5	0.54	0.6656
(Trt) * Run	8	1.433×10^7	1.791×10^6	2.46	
Residual	36	2.619×10^7	7.275×10^5		
Total	47	4.346×10^7			

Table C.17: Partially nested one-way ANOVA by treatment for flow in ml / hour.

Source	DF	SS	MS	F calc	p
Treatment	3	4,519.6	1,506.5	2.21	0.1643
(Trt) * Run	8	5,448.7	681.08	0.98	
Residual	36	24,919	692.20		
Total	47	34,887			

Table C.18: Partially nested one-way ANOVA by treatment for animal wet tissue weight in grams.

Source	DF	SS	MS	F calc	p
Treatment	3	4.99189	1.66396	1.31	0.3361
(Trt) * Run	8	10.1488	1.26860	1.48	
Residual	36	30.8532	0.85703		
Total	47	45.9940			

Table C.19: Partially nested one-way ANOVA by treatment for animal dry tissue weight (DTW) in grams.

Source	DF	SS	MS	F calc	p
Treatment	3	0.11114	0.03704	1.26	0.3523
(Trt) * Run	8	0.23580	0.02947	1.13	
Residual	36	0.80586	0.02238		
Total	47	1.15281			

Appendix D

Table D.1: Summary of treatment means and standard deviations by factor

¹ units are disintegrations per minute (dpm) per gram dry tissue weight (DTW)

² units are percentages based on dpm per gram DTW

⁴ units are dpm per milliliter

³ units are milliliters per hour

⁵ units are grams

Factor	Treatment			
	<i>Chaetoceros</i> phytoplankton	<i>Isochrysis</i> phytoplankton	<i>Ulva</i> particulates	<i>Zostera</i> particulates
Ingestion ¹	936,954 ± 254,862	786,543 ± 315,725	217,473 ± 123,839	338,609 ± 191,115
Tissue ¹	494,611 ± 157,417	416,429 ± 235,636	51,738 ± 24,232	49,749 ± 20,048
Respiration ¹	9,793 ± 5,730	19,583 ± 44,969	47,429 ± 66,781	54,747 ± 75,344
Acid wash supernatants ¹	285,102 ± 139,937	199,830 ± 145,472	45,893 ± 26,404	41,843 ± 24,326
Egestion ¹	147,488 ± 79,591	150,911 ± 62,087	72,412 ± 41,386	192,270 ± 60,826
Egestion: 0-3 hours ¹	11,772 ± 16,798	8,525 ± 10,190	4,218 ± 10,839	23,738 ± 28,864
Egestion: 3-6 hours ¹	58,226 ± 41,759	35,606 ± 22,761	18,930 ± 18,411	64,497 ± 79,887
Egestion: 6-9 hours ¹	46,078 ± 36,131	50,416 ± 22,635	24,091 ± 13,107	50,863 ± 56,316
Egestion: 9-12 hours ¹	20,937 ± 16,361	35,377 ± 26,920	12,483 ± 9,413	28,174 ± 27,266
Egestion: 12-15 hours ¹	7,429 ± 6,360	16,300 ± 13,320	8,339 ± 5,692	14,887 ± 10,772
Egestion: 15-18 hours ¹	3,006 ± 2,779	7,035 ± 6,084	6,683 ± 2,745	10,110 ± 8,195

Factor	Treatment			
	<i>Chaetoceros</i> phytoplankton	<i>Isochrysis</i> phytoplankton	<i>Ulva</i> particulates	<i>Zostera</i> particulates
Carbon budget: tissue ²	74.93% ± 13.09%	64.24% ± 25.46%	32.43% ± 8.11%	22.73% ± 15.76%
Carbon budget: respiration ²	1.58% ± 1.08%	3.74% ± 8.28%	21.80% ± 21.29%	21.03% ± 23.69%
Carbon budget: egestion ²	23.49% ± 13.18%	32.02% ± 22.25%	45.77% ± 19.07%	56.24% ± 24.39%
Total dpm ¹	1,737,977 ± 753,217	2,067,725 ± 342,140	2,504,946 ± 817,184	2,484,689 ± 1,273,576
Activity at sixth hour ⁴	1,627 ± 821	1,690 ± 362	2,076 ± 949	2,206 ± 1,407
Flow ³	184 ± 23	208 ± 26	208 ± 25	197 ± 30
Animal wet weight ⁵	6.73 ± 0.91	7.63 ± 1.00	7.31 ± 0.74	7.21 ± 1.16
Animal dry weight ⁵	0.94 ± 0.15	1.07 ± 0.15	1.03 ± 0.12	1.01 ± 0.19

