GROWTH, REPRODUCTION AND SURVIVORSHIP RESPONSES TO FOOD
ENHANCEMENT FOR TWO SPECIES OF ESTUARINE POLYCHAETES:
STREBLOSPIO BENEDICTI AND *LAEONEREIS CULVERI*

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ABSTRACT

Previous studies evaluating effects of eutrophication in estuarine systems have produced mixed results, sometimes showing weak linkages between resource enhancement and infaunal population responses. A general lack of life history information for many benthic infaunal species, and variable links between trophic controls and population responses, indicate a need to evaluate species-specific responses to specific food resources of varying quantities. This study examined growth, reproduction and survivorship responses to varying food sources for two estuarine polychaetes, *Streblospio benedicti* and *Laeonereis culveri,* common to southeastern North Carolina. I compared responses to ten food sources fed at specific quantities: ground *Ulva*, enhanced benthic microalgae, organic mud slurry, Tetramen fish flakes (high quality food control), azoic sediment and a starvation control for both species, with the additional inclusion of *Spartina alterniflora, Phaeosphaeria spartinicola* (fungal decomposer), *Phaeosphaeria*-infused *Spartina* and an agar control for *S. benedicti*. Multiple twenty-one day feeding assays were run with 2-3 levels of food, based on N content or volume, fed to field densities of each species. Response variables included survivorship, growth, total length, reproductive condition, and total biomass. *S. benedicti* showed significant growth and reproductive responses to the benthic microalgae and mud slurry treatments, but little response to other treatments. *L. culveri* showed variable responses to several treatments. The results suggest that *S. benedicti* will respond with increased growth, survivorship and oocyte production to enhanced food in the laboratory and may be food-limited in the field. The results for *L. culveri* illustrate the opportunism utilized by this species and suggest that it may be able to grow similarly among a variety

of food sources.   The combined results for these species demonstrate the variability in responses to food enhancement and suggest that some aspect of food limitation, either availability or nutrient content, plays a role in regulating growth, abundance and reproduction in benthic communities.

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INTRODUCTION

Soft sediment communities are complex systems, affected by both physical and biological controls. The abundance and composition of infaunal species in soft substrates are influenced by the effects of resource availability and predation (Peterson 1979; Posey *et al.* 1999). Predation may control species abundances and biomass on a local level, while resource availability may limit these factors over broader spatial scales (Gutierrez *et al.* 1994; Posey *et al.* 2002). Bottom-up controls involving quantity and quality of various food resources, may influence species composition, distribution and abundance in soft substrate habitats (Dayton 1984; Wilson 1990). Space is generally not a limiting factor in soft substrates due to their three dimensional nature and variations in living strategies of many species (e.g. tube-dwelling, deep burrowing, mobile surface) (Peterson 1979; Wilson 1990), implying that food limitation may be of greater potential importance in these habitats. However, bottom-up factors, such as food limitation, may be antagonistic to top-down controls (predation) in regulating communities.

Eutrophication involves the addition of nutrients, primarily nitrogen and phosphorus, to aquatic ecosystems resulting in a proliferation of plant life (Bricker *et al.* 1999). This process has been studied extensively in freshwater pond and lake systems (Edmunson 1970; Carpenter *et al.* 1985; Elliott and de Jonge 2002). Eutrophication has received increasing attention for marine soft substrates due to its potential effect on resource availability and bottom-up control of these communities. In an upward trophic cascade, the addition of nutrients to a system can cause an increase in primary producers, such as micro- and macroalgae, theoretically triggering a corresponding increase in grazer

populations by providing increased food resources (Bricker *et al.* 1999; Elliott and de Jonge 2002).

Some field research has provided evidence of upward trophic cascades in marine environments. For example, Beukema's (1991) study of the Dutch Wadden Sea, which had been subjected to both natural and anthropogenic eutrophication over the previous decade, found that benthic infauna populations increased during the period of eutrophication, indicating a response to increased food resources. Worm *et al.* (2000) also found that increasing nutrient supply in a Baltic Sea littoral food web initially increased algae, grazer and predator densities. Similarly, Lotze *et al.* (2000) found that nutrient enrichment can override the control of herbivores on macroalgal blooms, yielding higher abundances at multiple trophic levels. Dauer *et al.* (2000) found that short-term nutrient enhancement yields increases in phytoplankton biomass and organic matter, resulting in increased benthic polychaete biomasses. In these examples, the increase in food resource quantity resulting from eutrophication precedes increases in infaunal abundances and biomass in marine and estuarine ecosystems.

However, other experiments have yielded conflicting results, indicating variable community responses to increases in food quantity. Wiltse *et al.* (1984) conducted a nutrient addition study in the Great Sippewissett Marsh in Massachusetts, finding that though microalgal food sources were increased by nutrient additions to the sediment, no increases in infaunal densities or biomass were detected compared to controls. Posey *et al.* (2002) found little evidence for nutrient effects on infauna where predators were excluded, and no evidence for an increase in size or biomass of infauna. In a comparison between four tidal creeks, faunal abundances were highest in the creek with the lowest

ambient levels of microalgal biomass and nutrient levels (Posey *et al.* 2002).

Interestingly, these findings conflict with a previous smaller-scale study showing a

significant increase in numbers and size of surface deposit feeders, including the

estuarine polychaete *Streblospio benedicti,* which showed variable responses of

significantly higher abundances, biomass and increased sizes with nutrient additions

during one year, but not in the following year with the same treatments (Posey *et al.*

1995). Increased abundances of the grazing polychaete *Laeonereis culveri* and the grazer

feeding guild have also been noted with nutrient additions (Posey *et al.* 1999).

Woodin (1999), in a review of North American literature on sedimentary habitats,

argues that field experiments indicate food limitation is a common problem in benthic

systems. Estes and Peterson (2000) suggest that food limitation is generally important to

both deposit and suspension feeders since seasonal patterns in food availability results in

periods of high fecundity, productivity and settlement.

The conflicting results between the addition of nutrients, corresponding increases

in food availability and the responses of benthic infaunal populations suggest that we are

lacking information regarding the relative importance of various food resources

commonly available to these species. Feeding assay experiments generally involving

laboratory and mesocosm experiments have become a common method of determining

what food sources provide optimal growth, reproduction and survivorship responses for

various species of infauna (Levin 1986; Levin and Creed 1986; Gremare *et al.* 1988;

Qian and Chia 1990; Taghon and Greene 1990; Tsutsumi *et al.* 1990; Taghon and Greene

1992; Gremare 1994; Levin *et al.* 1996). Levin (1996) found that enriching sewage

sludge with nitrogen (N), phosphorus (P), and silica (Si) in a mesocosm study resulted in

increased body sizes and brood enhancement in *Streblospio benedicti*. Gremare (1994) found that *Capitella* sp. brood sizes were significantly enhanced, not only by increasing food quantity but also by enriching the quality of the food (N and micronutrient content). This corresponds with an earlier study where N content was found to best describe limitations on fecundity and female size in *Capitella* sp., compared to carbon and caloric content of four food sources (Gremare *et al.* 1988).

When increased quantities of nutrients are added to ecosystems during eutrophication, a wide range of potential food sources in soft substrate habitats may respond, including microalgae, macroalgae, seagrasses and phytoplankton. Macroalgae (such as *Ulva* spp.) and seagrasses (such as *Zostera marina*) are an abundant resource for benthic infauna in some systems (Peterson and Howarth 1987; Mallin *et al.* 2000), though they also have a relatively low nitrogen content and may contain a substantial amount of refractory structural material that must be broken down before consumption (Giannotti and McGlathery 2001). Tsutsumi *et al.* (1990) found that though nutrient enrichment stimulated increases in the macroalgae *Ulva pertusa*, *Capitella* sp. polychaetes were unable to directly assimilate the organic material without specific microorganisms present to facilitate decomposition. Macroalgae may be consumed to a greater degree prior to decomposition during blooms, providing increased quantity of food but not necessarily high quality nutrition. However, macroalgae and seagrasses provide a better source of nutrition prior to decomposition than the salt marsh grass, *Spartina alterniflora*, because they are more easily assimilated (Peterson *et al.* 1985; Kneib *et al.* 1997), though macroalgae are less easily assimilated than microalgae. Benthic microalgae, an abundant food source in estuaries and tide flat systems, is

dominated by benthic diatoms (which make up about 80% of benthic microalgae) (Sigmon 1995; Cahoon 1999; Kihslinger and Woodin 2000), and can outproduce phytoplankton by factors of 10-100 fold (Mallin *et al.* 2000). Benthic microalgae are more easily assimilated than larger macrophytes. In North Carolina, the most important phytoplankton taxonomic groups, including diatoms, dinoflagellates, and cryptomonads (Mallin *et al.* 2000), offer an important food source for suspension feeding taxa (Taghon and Greene 1992).

Nearshore benthic habitats are subject to influxes of organic detritus from salt marshes, although such detritus cannot be assimilated directly by most fauna (Peterson and Howarth 1987; D'avanzo *et al.* 1991). Before decomposition, *Spartina alterniflora* detritus contains larger amounts of structural material, which is of low nutritional value, compared to macro and microalgae, and is difficult to digest. Nutritional value for salt marsh detritus can be increased by fungal and bacterial decomposers that break down structural material (Howarth and Teal 1980; Kneib *et al.* 1997; GraÇa *et al.* 2000). When combined with associated microbial fauna and fungi, salt marsh grasses can be a readily available food source for benthic fauna living in areas close to marshes (Haines and Montague 1979; Peterson *et al.* 1985).

Benthic infauna exhibit an array of feeding strategies, including suspension feeding, non-selective deposit feeding, selective deposit feeding, and carnivory (Fauchald and Jumars 1979). Many infauna are capable of exhibiting opportunistic feeding strategies and may switch between feeding behaviors depending on resource availability or flow conditions, allowing facultative use of a variety of food sources (Taghon 1981; Kihslinger and Woodin 2000). Organisms may exhibit increased opportunism, feeding

on less nutritious food resources when higher quality food sources are not available or when organisms are avoiding predation (Abrams 1982; Mitchell 1990), which may cause a reduction of overall growth and reproduction compared to times when more nutritious food sources are available (Taghon and Greene 1992; Giannotti and McGlathery 2001).

This study focuses on the responses to variable food resources by two benthic infaunal species: *Streblospio benedicti* and *Laeonereis culveri*. *Streblospio benedicti* is a spionid polychaete common to southeastern North Carolina and shallow temperate regions worldwide and is known to exhibit multiple feeding strategies (Fauchald and Jumars 1979). This species has been suggested to be a selective deposit feeder, feeding on both surface sediments (including microalgae and detritus) and on particles in the water column (Fauchald and Jumars 1979; Levin *et al.* 1996; Kihslinger and Woodin 2000). *S. benedicti* was chosen for this study due to its conflicting responses to nutrient additions in previous studies (Posey *et al.* 1995, 2002; Sarda *et al.* 1996), and because it represents a model species for many food webs worldwide. While *S. benedicti* has been intensively studied, providing a base for comparison with previous work, existing studies have not yet compared this species' physiological responses to the wide range of the food resources commonly available in the estuary (Levin 1984; Levin 1986; Levin and Creed 1986; Levin *et al.* 1987; Levin and Huggett 1990; Lindsay and Woodin 1996; Levin *et al.* 1996; Kihslinger and Woodin 2000).

Laeonereis culveri is a nereid polychaete commonly found in southeastern North Carolina estuaries as well as a dominant species in shallow estuarine waters throughout the southeast coast of the United States and South America (Mazurkiewicz 1975). It has been suggested to be a deposit feeder (Fauchald and Jumars 1979), with juveniles likely

feeding on benthic microalgae and adults feeding on a variety of food sources (e.g. benthic microalgae and detritus) (Pettibone 1971; Mazurkiewicz 1975). *L. culveri* is both a grazer and selective deposit feeder, able to switch among food sources but and yet still be selective on which particles they choose to consume (Mazurkiewicz 1975; Fauchald and Jumars 1979; Stocks and Grassle 2001). This species has shown a response to nutrient enhancement in a previous study (Posey *et al.* 1999).

The primary objective of this study was to evaluate several common food sources available to these species by comparing survivorship, reproduction and growth responses to varied types and quantities of selected food sources. I hypothesized that these species would be able to survive, grow and reproduce equally well among the variety of food sources offered, demonstrating opportunistic feeding strategies. These species are examples of dominant taxa in many food webs, and the results of this study may help explain the variable upward trophic cascades observed in many previous studies. Such information is needed to understand the linkage between increases in food quantities and varying responses in population abundances and biomasses for estuarine polychaetes.

METHODOLOGY

Overview

Five feeding assay experiments using *Streblospio benedicti* and *Laeonereis culveri* were carried out between August 2002 and June 2003. Field-collected animals were maintained at field-level densities and kept in dishes filled with 3cm sand. Food sources readily available in estuarine habitats, along with high and low nitrogen control

foods, were fed in varying quantities on alternating days for 21 days. Each food source was measured for N, P and C content. At the end of each experiment, specimens were removed from their dishes and preserved for size, biomass and reproductive condition measurements.

Collection of Target Species

Predator exclusion cages (100 x 100 x 15 cm) were placed on the intertidal mudflat of the UNCW research lease in Masonboro Sound, southeastern North Carolina, to allow the development of a high density infaunal assemblage in the absence of predators. After allowing at least two weeks to establish high densities, the top 5-7 cm of sediment (depth of oxic layer) were removed and sieved through a 500 μm screen in the field (Mazurkiewicz 1975, Levin and Creed 1986). All fauna retained in the screen were placed into plastic holding containers and held in a temperature control room at 20° C and 34 ppt salinity with aeration (Levin and Creed 1986, Levin *et al.* 1987). *Streblospio benedicti* and *Laeonereis culveri* remained in holding facilities no longer than four days prior to feeding experiments and were allowed to feed on the organic material collected with them until they were transferred into dishes containing clean sand prior to each experimental run. Specimens transferred into experimental tanks during this period had a maximum residence time with no food available for no more than four days.

Experimental Design

Twenty-two tanks (40 x 25 x 11 cm) were set up as independently recirculating, closed systems (Fig. 1). All experiments were run in a temperature control room at 20° C
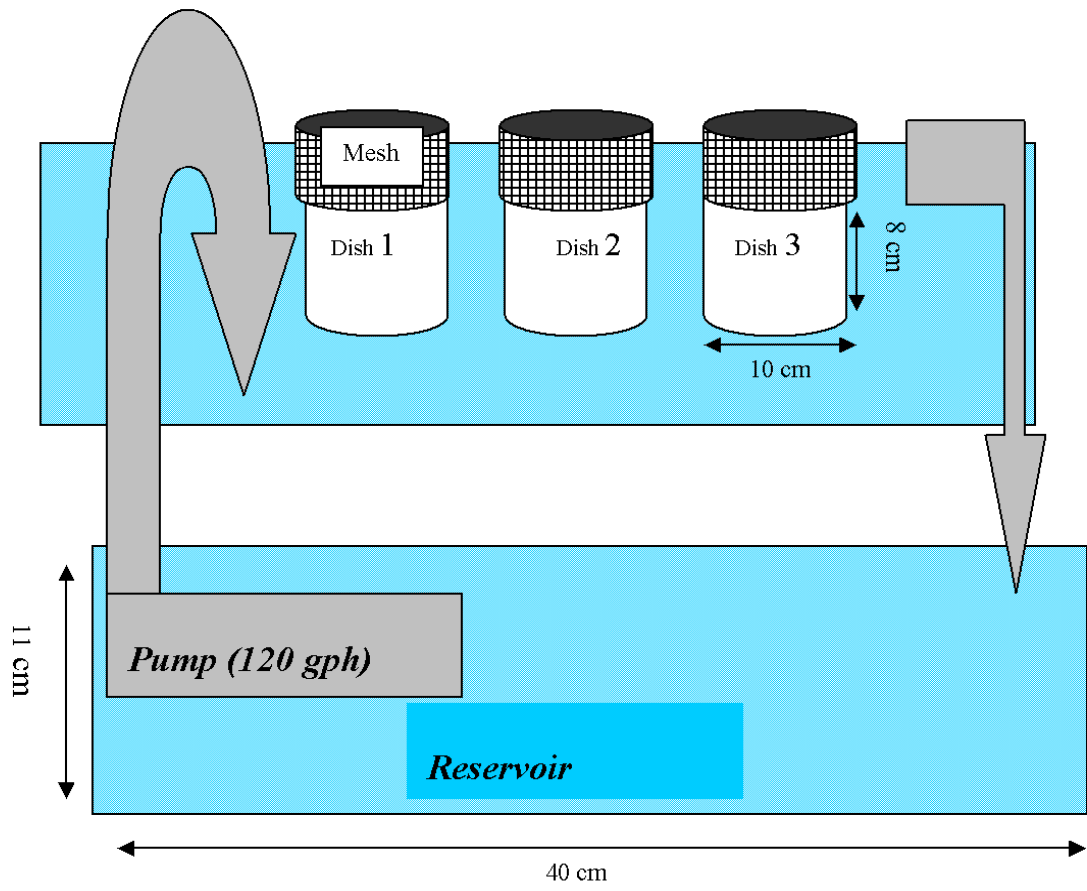
Figure 1. Recirculating system, side view

using seawater (34 ppt), filtered to 1 μm, from Masonboro Sound. Temperature and salinity settings were chosen following parameters determined to be adequate for laboratory culture by Levin and Creed (1986) and Levin *et al.* (1987). Plastic dishes (12 x 10 cm diameter) enclosed with 4 cm 125 μm mesh, extending above the water level to prevent animals from leaving the dishes. The bottom of each dish was covered with 3 cm of sterilized commercial sand that had been rinsed in seawater. Three culture dishes were placed into each recirculating system so that the entire dish, below the mesh line, was immersed. Each recirculating tank system represented a separate food treatment for one species. Though this setup created pseudoreplication with respect to food delivery, multiple dishes per tank were used to increase the sample size.

S. benedicti treatments contained 20 individuals per dish while *L. culveri* treatments contained six individuals per dish. These densities are comparable to field density measurements reported in Posey *et al.* (2002) for southeastern North Carolina. Feeding experiments began as soon as all animals were transferred into the experimental systems. Animals in the starvation treatment were placed into their containers on the day the feeding assays began so that they could feed in the collection tank until the start of the experiment. Feeding assays were run for 21 days, which has been suggested in previous literature to be an adequate amount of time to observe changes in size and reproductive condition for selected polychaetes (*Streblospio*; Levin and Creed 1987; *Capitella*; Gremare *et al.* 1988). Twenty *S. benedicti* and six *L. culveri* were randomly collected and preserved immediately prior to the beginning of each experimental to determine initial sizes and reproductive condition.

Sixteen food treatments were selected for the feeding assay experiment. The experimental food sources were chosen to represent a variety of food resources available in the estuarine benthic environment, along with non-natural sources for controls, in order to test for opportunistic feeding modes. Varying levels of foods were given in order to test for differences in growth and reproduction in response to food quantity. However, excluding starvation controls, each treatment was fed in excess, demonstrated by the presence of accessible food in the bottom of dishes between feedings. Consequently food availability was not likely to be a limiting factor.

Natural food sources included *Ulva lactuca*, a marine macroalgae chosen due to its availability as a food resource for these species in the field (Gremare et al. 1998, Tsutsumi et al. 1990), a benthic microalgae slurry, which provided a sediment treatment enhanced for the growth of both bacteria and benthic microalgae (Kihslinger and Woodin 2000; Stock and Grassle 2001), and a mud slurry, which provided a sediment treatment that was stimulated for bacterial growth but not for benthic microalgae. The two sediment treatments provided a measurement of the differences between algal-enhanced and non-enhanced sediments (Tsutsumi *et al.* 1990, Levin *et al.* 1996).

Tetramen "Best Mix" fish flakes were used as a nutritional control due to its high N and P content and its use as a high quality food source control in previous polychaete feeding studies (Gremare *et al.* 1988). An azoic sediment source was used to control for any differences between treatments with food-enhanced sediment and simple effects of sediment addition. The azoic sediment treatment, however, did contain *Spartina* detritus, and therefore likely also have served as an additional test for *Spartina* treatments. A

starvation control provided a baseline for comparison of treatments with no food additions.

Food treatments of ground, dehydrated *Spartina alterniflora* (a common estuarine marsh grass), *S. alterniflora* detritus enhanced with *Phaeosphaeria spartinicola* (a common marine ascomycete) (Padgett, personal communication), and dehydrated *P. spartinicola* only were used to assess any difference in growth and reproductive responses to marsh grass detritus, decomposers, and a simulation of marsh grass enhanced with a decomposing fungus. These treatments were run for *Streblospio benedicti* only. Previous studies have suggested that *Spartina* alone provides little nutritional value to benthic populations, but *Spartina* detritus is enhanced by the presence of decomposers (Howarth and Teal 1980). These treatments were placed in an agar base, impregnated with sand, in order to decrease buoyancy, allowing the particles to settle in the dishes. An agar-only control was also included.

All food sources were analyzed for N and P content (Table 1), and all treatments except the *Spartina* treatments (due to their agar base) were also measured for C content. Initially, I attempted to standardize all food sources for nitrogen content (3 mg N for low treatments and 6 mg N for high), which have been shown to provide increased growth for juvenile *Capitella* sp. in a previous study (Gremare *et al.* 1988). However, N content of the sediment treatments were low and required much higher volumes of food addition than the powdered *Ulva* and Tetramen treatments. To feed the amounts of sediment needed for equivalent N content of those treatments would have overwhelmed the animals (for 3 mg N; 469 ml of mud slurry, 58 ml of benthic microalgae per feeding). Therefore, the mud slurry, benthic microalgae and azoic sediment treatments were

Table 1. Total Nitrogen (TN) and Total Phosphorus (TP) content for food sources in mg/mL. N/A means that the treatment was not used during that run.

	Run 1		Run 2		Run 3		Run 4		Run 5	
	TP (mg/mL)	TN (mg/mL)	TP (mg/mL)	TN (mg/mL)	TP (mg/mL)	TN (mg/mL)	TP (mg/mL)	TN (mg/mL)	TP (mg/mL)	TN (mg/mL)
Benthic Microalgae	0.095	0.519	0.127	0.214	0.137	0.143	0.191	0.119	0.140	0.191
Mud Slurry	0.015	0.064	0.051	0.072	0.059	0.069	0.066	0.071	0.041	0.078
Azoic Sediment	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0.279	0.031
Spartina **detritus**	N/A	N/A	N/A	N/A	0.004	0.004	N/A	N/A	0.004	0.004
Spartina + *Phaeosphaeria*	N/A	N/A	N/A	N/A	0.004	0.007	N/A	N/A	0.004	0.007
Phaeosphaeria	N/A	N/A	N/A	N/A	0.006	0.023	N/A	N/A	0.006	0.023
Agar Control	N/A	N/A	N/A	N/A	0.004	0.006	N/A	N/A	0.004	0.006

Table 2. Feeding amounts for *Streblospio benedicti* and *Laeonereis culveri*. Tetramen, *Ulva*, *Spartina*, *Phaeosphaeria* and agar control treatments were fed by dry weight (g), while all sediment treatments were fed by volume (ml). N/A means the food source was not fed at that level, or was not provided for that species.

	Streblospio benedicti			*Laeonereis culveri*		
	High	Medium	Low	High	Medium	Low
Tetramen Flakes	0.006 g	N/A	0.003 g	0.018 g	N/A	0.009 g
Ulva	0.021 g	N/A	0.010 g	0.031 g	N/A	0.062 g
Benthic Microalgae	3.3 ml	1.6 ml	0.8 ml	10 ml	5 ml	2.5 ml
Organic Mud Slurry	3.3 ml	1.6 ml	0.8 ml	10 ml	5 ml	2.5 ml
Azoic Sediment	3.3 ml	N/A	N/A	10ml	N/A	N/A
Starvation control	0	0	0	0	0	0
Spartina detritus	0.021 g	N/A	N/A	N/A	N/A	N/A
Spartina + Phaeosphaeria	0.021 g	N/A	N/A	N/A	N/A	N/A
Phaeosphaeria	0.021 g	N/A	N/A	N/A	N/A	N/A
Agar Control	0.021 g	N/A	N/A	N/A	N/A	N/A

standardized by volumes used in previous *S. benedicti* feeding assays performed by Levin and Creed (1987) (Table 2). The dry weight of the sediment treatments was determined by measuring out three replicates of the respective food source at the volumes they were fed and allowing them to dry completely for 96 hours at 70° C in order to determine the average weight of sediment per volume.

Tetramen, *Ulva*, mud slurry, and benthic microalgae treatments were initially provided at low and high levels for the first experimental run. Percent N and C content for the Tetramen and *Ulva* sources were taken from previous studies (Tetramen; 7.4% N, 45.3% C; *Ulva*; 2.2 % N, 31.3% C) (Gremare *et al.* 1988). A Bran and Luebbe Auto Analyzer was used to measure the N and P content (Table 1) in each benthic microalgae and mud slurry treatment according to the persulfate digestion method (Valderrama 1981). These treatments were also measured for average chlorophyll *a* content from four samples according to the double extraction and spetrophotometry method (Whitney and Darley 1979) to test for a higher algal content in the benthic microalgae slurry (benthic microalgae; 165 mg/m^2; mud slurry; 100 mg/m^2). Average percent carbon content was determined by weighing out a predetermined volume, corresponding to the amount fed in the low treatment (Table 2), drying it for 96 hours at 70°C, and then ashing the samples at 500°C for 5 hours to determine organic content (benthic microalgae; 9.41% C; mud slurry; 9.13% C). Due to concerns over the addition of large amounts of sediment to experimental dishes in the high treatments, an intermediate level of mud slurry and benthic microalgae treatments were added after the initial run. *Spartina* treatments for *S. benedicti* were analyzed for N and P content using the same technique as the enhanced

sediment treatments and fed at weights standardized to 6 mg N only. Table 2 shows the exact food amounts that were fed to the polychaetes in each dish per feeding.

Not every food source could be used in every run due to constraints with animal collection and availability (Table 3). This resulted in an unbalanced experimental design, which gave the potential for source or timing artifacts in the statistical analysis. *A priori* we did not expect to see a difference between experimental runs since there is no evidence that the time of animal collection would have any effects on responses, the food sources were prepared the same way before each run, and runs were carried out under controlled environmental conditions.

Food Source Preparation

Tetramen "Rich Mix" fish flakes were ground into a fine powder with a mortar and pestle (Gremare *et al.* 1988) and stored in a foil-covered beaker at room temperature with desiccant to ensure that the powder did not become hydrated. Live *Ulva* sp. algae was collected from the field, rinsed to remove any sediment and frozen for 10 days at -5° C. The *Ulva* was then placed in a freeze-drier for five days to remove all liquid (Gremare *et al.* 1988). Once completely dried, the *Ulva* was ground into a fine powder using a mortar and pestle (Gremare *et al.* 1988) and stored in a foil-covered beaker at room temperature with desiccant to ensure that rehydration did not occur.

The mud slurry food source was prepared before each feeding assay by collecting the top 5 cm of sediment from three sites at the UNCW research lease, near the site of animal collection. The sediment was sieved through a 500 μm screen to remove larger

Table 3. Food sources used for experimental runs (SB = *Streblospio benedicti*, LC = *Laeonereis culveri*). X = the number of tanks used for that food source during that run, 3 dishes per tank.

	Run 1		Run 2		Run 3		Run 4		Run 5	
	SB	LC	SB	LC	SB	LC	SB	LC	SB	LC
Tetramen Low (FFL)	1X	1X	1X	1X	1X	1X				
Tetramen High (FFH)	1X	1X	1X	1X	1X	1X				
Ulva **Low (UL)**	1X	1X	1X	1X	1X	1X				
Ulva **High (UH)**	1X	1X	1X	1X	1X	1X				
Mud Slurry Low (MSL)	1X	1X	1X	1X	1X	1X				
Mud Slurry Medium (MSM)			1X	1X		1X				2X
Mud Slurry High (MSH)	1X	1X	1X	1X	1X		3X			2X
Benthic Microalgae Low (BML)	1X	1X	1X	1X	1X					
Benthic Microalgae Medium (BMM)			1X	1X	1X	1X				2X
Benthic Microalgae High (BMH)	1X	1X	1X	1X		1X	3X			2X
Spartina **Detritus (SP)**					1X				2X	
Spartina **Detritus +** *Phaeosphaeria* **(SP+F)**					1X				2X	
Phaeosphaeria **Only (F)**					1X				2X	
Agar Control (AG)					1X				2X	
Azoic Sediment Control (AZ)							3X			2X
Starvation Control (S)	1X	1X	1X	1X	1X	1X	3X			1X

macrofauna and detrital particles, then frozen at -5° C for 5-10 days to kill any remaining fauna or microalgae. After thawing, the sediment was placed in a 23 x 16 x 6 cm container with 1μm filtered seawater (34 ppt) and placed in a greenhouse (30-35° C) for five days with constant aeration to allow the stimulation of microbial growth with minimal microalgae growth (Sigmon 1995). After five days, the mud slurry was removed and placed in the 4° C temperature control room with aeration.

The benthic microalgae food source was prepared before each feeding assay and again halfway through each experiment to ensure food quality and availability. The top 1 cm of sediment was collected from three different field sites (roughly 2 m^2 per site) to maximize the diversity of microalgal species collected. Since this treatment was intended to show the effect of benthic microalgae content, it could not be frozen to kill fauna. The sediment was sieved through a 125 μm live screen to remove fauna. Excess sediment and larger particles trapped on the sieve were discarded, while fine sediment and diatoms that passed through were allowed to settle in 23 x 16 x 6 cm containers with seawater (filtered to 1 μm; 34 ppt). The containers were placed in the greenhouse, given constant aeration and fertilized with ~41 g of Osmocote slow release plant food (14-14-14) to stimulate microalgal and microbial growth (Heck *et al.* 2000). The microalgae stimulated was expected to be primarily composed of green algae and benthic diatoms (Becker 1996), though larger percentages of filamentous green algaes can be available in the late winter and early spring in natural settings (Sigmon 1995). However, since sediment collections for this food source occurred in later summer and late spring, and since no filamentous green algae were visible in any preparation, it is unlikely that filamentous species were

cultured. After seven days, the benthic microalgae culture was removed and transferred into a 4° C temperature control room under a 60-watt plant light and constant aeration.

The azoic sediment treatment was prepared by collecting the top 2 cm of sediment from the same location that the microalgal and mud slurry treatments were collected. The sediment was sieved through a 500 μm screen to remove macrofauna and large detrital particles and frozen at –4° C for two weeks to kill any remaining fauna, microalgae or bacteria. After two weeks, the sediment was thawed, dried and placed in a 4° C cold room in complete darkness in order to inhibit the growth of new material. Immediately prior to feeding, small quantities of the azoic sediment were moistened with seawater (filtered to 1μm; 34ppt) in order to be fed by wet volume, similar to microalgae and mud slurry treatments. The amount of azoic sediment fed to each dish equaled the high volume of the benthic microalgae slurry to match the high concentration of sediment added.

The *Spartina alterniflora* and *Phaeosphaeria spartinicola* treatments were prepared independently by Dr. David Padgett (Department of Biological Sciences; UNCW). Each of the *Spartina* and fungi treatments consisted of 2% phytagel, with 1.4 g of sterile 125μm sand added per 20 g of phytagel. The agar control treatment contained only phytagel and sand. The *Spartina* detritus treatment contained 0.2 g sterile dehydrated *Spartina alterniflora* leaves ground into a fine powder. The mycelium only treatment combined 0.2 g of sterile, freeze-dried *Phaeosphaeria spartinicola* with the phytagel/sand matrix. The combination marsh grass/mycelium treatment used sterile *Spartina* grass inoculated with the *Phaeosphaeria spartinicola*, which was then freeze-

dried and ground into a fine powder, with 0.2 g of the fungus-infused marsh grass used

per 20 g phytagel and 1.4g sand.

Experimental Feeding Assays

Each dish of animals was fed the appropriate food source once every 48 hours.

All experiments were conducted in a 20° C temperature control room at 34 ppt under two

60 W lamps, providing 8 hours of twilight: 16 hours dark. Salinities were monitored at

the time of feeding and adjusted with distilled water accordingly. 50% water changes for

each recirculating system were made every five days during the feeding experiments.

Before each feeding, Tetramen fish flake, *Ulva, Spartina, Spartina +*

Phaeosphaeria, Phaeosphaeria and agar control powders were weighed to the exact

feeding amount (+/- 0.0009 g). The powders were allowed to soak for one minute in

water collected from the target tank, before contents were dispersed into each

experimental dish. The mud slurry, benthic microalgae and azoic sediment sources were

measured by volume using a glass pipette and dispensed into aluminum weighing dishes.

Water from the corresponding assay tank was added to each dish in order to adjust the

temperature difference. The sediment-based food sources were then pipetted into the

culture dishes for feeding.

Growth, Survivorship and Reproductive Response Measurements

At the end of 21 days, each dish was removed and transferred into plastic storage

containers with 10% formalin and stained with rose bengal dye, with five dishes per

species sorted directly, prior to preservation, to determine survivorship. After remaining in preservative for at least 48 hours, the remaining samples were sorted and the polychaetes removed and transferred into scintillation vials containing 70% isopropyl alcohol.

Streblospio benedicti were measured for total length (prostomium to tip of last segment), length to 5th setiger (prostomium to end of 5th segment), presence of oocytes, survivorship, and average weight per worm, in order to compare growth and reproductive status between treatments. *Laeonereis culveri* were measured for total length (prostomium to tip of the last segment), length of thoracic region (prostomium to the end of the 15th setiger), width at the 15th setiger, survivorship, and average weight per worm using dry weight biomassing. Since reproductive condition for *Laeonereis culveri* could not be accurately determined by visual assessment and most individuals were likely too small to be reproductive, this variable was not measured for this species. Length measurements were performed by taking a digital image of each individual polychaete using a Zeiss dissecting microscope and SPOT digital camera. The images were analyzed using ImagePro software by drawing best-fit lines along each specimen, then calculating the pixel content of each line to determine the length and width measurements. Reproductive condition was assessed by visually determining the presence of oocytes in surviving *Streblospio benedicti*. Polychaetes were biomassed after all measurements were taken. The polychaetes from each dish were dried together in a 70°C oven for 24 hours, then weighed. Individual worms could not be weighed separately due to the very light weight per worm. The group weights were divided by the number of surviving worms to determine average weight per worm.

Statistical Analysis

   Results were analyzed using a two-way ANOVA for total length and length to 5th setiger (*S. benedicti*) and total length, length to thoracic region, and width at thoracic region (*L. culveri*) with food treatments (*S. benedicti* 16; *L. culveri* 12) and experimental run (*S. benedicti* 5; *L. culveri* 4) as the main effects (Table 4). Interactions between treatment and run were expected due to the unbalanced experimental design, however, they only occurred in two of five two-way ANOVA contrasts (Table 4). One-way ANOVAs were run for size variables individually for each run to determine if the rank ordering of treatments was consistent (Table 5). For survivorship, biomass and reproductive condition measurements, means were calculated per experimental dish, reducing the N value for these variables. For this reason, only one-way ANOVAs were run for these variables since interactions between treatment and run could not be accurately assessed. Following any significant ANOVAs, Student-Newman-Keuls tests were run to identify differences among individual treatments. Tests for heterogeneity of variances (F_{max}) were run on all data sets. Length and width data were revealed to be nonhomogeneous for variances and consequently were log_{10} transformed prior to further analysis. Survivorship and oocyte data were arcsin-square root transformed prior to statistical analysis.

Table 4. 2-way ANOVA testing effects of food treatments and experimental run on survivorship, total length, length to 5th setiger, presence of oocytes and biomass for *Streblospio benedicti* and survivorship, total length, length to thoracic region (15th setiger), and biomass from *Laeonereis culveri*. F-values are on top, followed by degrees of freedom, and P-values are in parentheses on bottom (bold values are significant, P<0.05). N/A means that analyses were not run testing these effects.

		Treatment	Run	Treatment*Run
Streblospio benedicti	Survivorship	6.13; 15df **(0.0001)**	n/a n/a	n/a n/a
	Total Length	14.24; 16df **(0.0001)**	21.16; 4df **(0.0001)**	5.72; 22df **(0.0001)**
	Length to 5th Setiger	10.70; 16df **(0.0001)**	77.52; 4df **(0.0001)**	4.24; 22df **(0.0001)**
	Oocytes	2.50; 16df **(0.0023)**	n/a n/a	n/a n/a
	Biomass	1.67; 16df (0.0576)	n/a n/a	n/a n/a
Laeonereis culveri	Survivorship	0.39; 5df (0.8516)	n/a n/a	n/a n/a
	Total Length	2.34; 12df **(0.0069)**	12.75; 3df **(0.0001)**	1.48; 16df (0.1051)
	Length to Thoracic Region	2.09; 12df **(0.0174)**	8.91; 3df **(0.0001)**	1.62; 16df (0.0627)
	Width at Thoracic Region	2.14; 12df **(0.0147)**	14.07; 3df **(0.0001)**	1.45; 16df (0.1166)
	Biomass	2.56; 12df **(0.0090)**	n/a n/a	n/a n/a

RESULTS

Streblospio benedicti

No evidence of anoxia or fouling in the dishes was observed visually during any
of the experimental runs. Survivorship of *Streblospio benedicti* differed significantly
among treatments (Table 4). Highest mean survivorship occurred with the medium and
low benthic microalgae and *Phaeosphaeria* (BMM, BML, F) treatments, as well as with
the *Phaeosphaeria*-infused *Spartina* (SP+F), and agar control (AG) treatments though
only significantly different from starvation and azoic sediment treatments (Fig. 2).
Starvation controls (S) and azoic sediment (AZ) treatments produced the lowest
survivorship with all other food sources ranked above (Fig. 2). Patterns were consistent
among different trials.

Total length for *S. benedicti* also differed significantly among food treatments
(Table 4; F=14.24; p<0.0001). The benthic microalgae food sources were consistently
associated with greater total lengths, with the high benthic microalgae treatment (BMH)
means consistently higher than any other treatment in every experimental run in which
the treatment was used (Table 5). For mean total length measurements, treatments
involving enhanced sediments consistently showed higher means across all experimental
runs (Fig. 3), with the medium benthic microalgae (BMM), high mud slurry (MSH), low
benthic microalgae (BML), medium mud slurry (MSM) and low mud slurry (MSL)
treatments, respectively, yielding the highest mean total lengths for all runs. All other
food sources produced means below the sediment treatments, in varying orders
depending on run, with the *Spartina* treatments (SP, F, SP+F, and AG), azoic sediment

(AZ), initial measurements (PM) and starvation controls (S) producing the lowest total lengths (Table 5; Fig. 3).

Measurements for the length to the 5th setiger for *S. benedicti* were used to provide a supplemental measurement of growth since fragmentation of the worms occasionally occurred (Levin 1984). Fragmentation usually occurs posterior to the fifth setiger, allowing assessment of growth in worms that have fragmented. This measurement was chosen because length to 5th setiger should provide an indicator of total length, though results from this experiment indicated a variable relationship between the two ($R^2=0.2717$) (Fig. 4). Results for the length to the 5th setiger for *S. benedicti* were more variable than those for total length, though results from the ANOVA did indicate a significant difference among food treatments (Table 4; $F=10.7$; $p<0.0001$). Benthic microalgae treatments (BMH, BMM) again produced the highest values (Fig. 5), with BMH significantly higher than any other treatment in run 1 (Table 5). *Spartina* treatments (SP, F, SP+F and AG) and starvation controls (S) produced the lowest mean lengths in each run they were used, similar to comparisons of total length. The remaining food sources were intermediate, with no clear pattern emerging between runs.

A marginally non-significant difference ($F=1.67$; $p<0.0576$) between food treatments was found for average weight per worm. MSL, MSM, BMM, and MSH treatments, respectively, produced the highest mean biomasses, with starvation and UH treatments producing the lowest (Fig. 6).

Analysis of reproductive condition showed that *S. benedicti* in the BMH treatments had significantly higher percent presence of oocytes ($F=2.50$; $p<0.0023$) than any other treatments except BMM and MSM (Fig. 7). This treatment had almost two-

fold greater percent individuals producing oocytes than any other treatment. No oocytes were present in any experimental treatments of the *Spartina*, agar control, starvation or azoic sediment controls (Fig. 7).

Laeonereis culveri

After the conclusion of the first three experimental runs, closer examination of the surviving nereid polychaetes revealed that a mixed assemblage of two species of nereids were present in the feeding assays, with both *Laeonereis culveri* and *Nereis succinea* found surviving. Live identification of these species is very difficult due to the anatomical similarities between *L. culveri* and *N. succinea*. The only visible differences between the two species are the shape of the dorsal cirri and the arrangement of the paragnaths, visible only when the proboscis has been everted, both of which are difficult to observe on live specimens. The resulting mixed assemblages illustrate the learning curve involved in live nereid identification. For the final experimental run only *L. culveri* were present. However, since a mix of species occurred in the initial runs, survivorship data for *L. culveri* could not be accurately calculated for these experiments. Raw data collected for *N. succinea* are presented in Appendix A. Survivorship for the final experimental run (containing only *L. culveri*) showed no difference among food treatments (Table 3; $F=0.39$; $p<0.8516$), though BMH and MSH treatments produced the highest mean survivorship, with AZ and BMM treatments producing the lowest (Fig. 8), a pattern similar to that found with *S. benedicti* survivorship.

There was a significant difference among food treatments for total length of *L. culveri* (Table 3; $F=2.34l$; $p<0.0069$). Low benthic microalgae treatments (BML)

produced the highest mean total lengths, followed by the low Tetramen (FFL), and high and low *Ulva* sources (UH, UL), respectively. The lowest mean total lengths resulted from the azoic sediment (AZ), starvation control (S), and medium benthic microalgae treatment (BMM) (Fig. 9). For the run containing *L. culveri* only (run 5), all food treatments (BMH, BMM, MSH, MSM, AZ) showed significantly higher total lengths ($F=5.09$; $p<0.0001$) than the starvation control and the initial measurements (Fig. 10).

There was a significant difference among treatments with respect to length to the thoracic region, defined as length to the 15th setiger (Table 3; $F=2.14$; $p<0.0174$). Length to thoracic region was measured in order to provide a surrogate measurement for worms that fragmented during preservation. The relationship between total length and length to thoracic region was significant (Fig. 11; $r^2=0.5181$). Low Tetramen treatments (FFL), high mud slurry (MSH), and high *Ulva* (UH) treatments were associated with the greatest lengths to thoracic region, with starvation controls (S) and medium benthic microalgae (BMM) treatments resulting in the lowest (Fig. 12). In the experimental run containing only *L. culveri*, all food treatments (BMH, BMM, MSH, MSM and AZ) showed significant ($F=6.92$; $p<.0001$) increases in length to thoracic region compared to starvation controls and initial measurements (Fig. 10).

Width measurements for *L. culveri* were also taken at the 15th setiger, to provide an additional estimate of body size, with a significant difference ($F=2.14$; $p<.0147$) among food treatments. The relationship between total length and width at the thoracic region was significant, though not strong (Fig. 13; $r^2=0.3962$). FFL, UH, and BML treatments produced the largest mean widths, with S and BMM treatments again producing the lowest (Fig. 14). In the experimental run containing only *L. culveri*, all

food treatments (BMH, BMM, MSH, MSM, AZ) showed a significant difference in width (F=4.87; p<.0002) compared to starvation controls and initial measurements (Fig. 10).

Average weight per worm also differed among food treatments (F=2.56; p<.0090), with high mud slurry (MSH), low Tetramen (FFL), and low benthic microalgae (BML) associated with the highest mean biomass, respectively (Fig. 15). Lowest mean biomass was associated with the azoic sediment control (AZ) and medium benthic microalgae treatments (BMM). Initial mean weight per worm was lower than all treatments, indicating that *L. culveri* gained weight in all treatments.

DISCUSSION

Results for *Streblospio benedicti* indicate that these animals responded to increases in sediment-associated food resources. Higher survivorship and growth, along with greater reproductive condition were all demonstrated for the various levels of benthic microalgae and, secondarily, mud slurry treatments. In harsh conditions, organisms may only be able to survive; however, as conditions improve, organisms respond by allocating energy to growth, and finally reproduction (Bridges and Heppell 1996). Growth responses for total length and length to 5th setiger were consistently highest for the high benthic microalgae treatments, and secondarily the medium benthic microalgae and mud slurry food sources. Reproductive response occurred mainly in high benthic microalgae treatments, and secondarily in moderate benthic microalgae and mud slurry treatments, indicating *S. benedicti* had a higher-level response to both the quality and quantity of the enhanced sediment treatments. The enhanced sediment treatments

contained N levels nearly 2-3 fold more than those found in natural settings. In a study on Masonboro Sound, Sigmon and Cahoon (1995) found a mean annual biomass of 64.6 mg chl a per m^2 for benthic microalgae, while a study conducted at the collection site for the enhanced-sediment food sources indicated the highest seasonal chl a biomass to be 71.6 mg/m^2 (Molesky 2003). The enhanced benthic microalgae treatment for this study contained a mean biomass of 164.9 mg chl a per m^2 (SE= 23.2 mg/m^2) and the mud slurry treatment contained an average of 100.4 mg chl a per m^2 (SE= 65.3 mg/m^2). The responses of $S.$ $benedicti$ to these food sources in laboratory trials indicate that this species may be food-limited in the field, and might be able to respond with increased size, biomass and fecundity with sediment enhancement.

The level of chl a in the mud slurry treatment was highly variable (35.1-165.7 mg/m^2) and may have reflected residual pigments after cells were broken down during the freezing process, and therefore may not represent live algae. Since the mud slurry was frozen in an attempt to kill all live material, the only live material introduced to this treatment should have been from the addition of 1μm filtered seawater. Since benthic diatoms and green algae are generally >10μm in size (Horne and Goldman 1994), no live algae should have been re-introduced into the mud slurry treatment. Therefore, the enhancement of this food source should primarily have been from bacterial growth. Previous research has demonstrated increases in anaerobic and aerobic bacterial decomposers in response to eutrophication in the field (Blackford 1997). The benthic microalgae treatments should primarily be composed of benthic diatoms that were highly present initially, though diatoms may require Si additions for enhancement (Becker 1996) and green algae, which respond well to N and P additions. The growth responses to the

benthic microalgae treatment are consistent with previous research indicating green algae as an important food source for these species (Mazurkiewicz 1975; Cahoon 1999).

The conflicting results from previous field studies examining responses to enriched sediment may illustrate the existence of associated negative effects on populations, such as anoxia or increased predator abundances, that are preventing expected responses in the field (Peterson 1979; Levin and Creed 1986; Wilson 1990; Posey *et al.* 1995, 1999, 2002). The early stages of eutrophication may not be associated with increased predator abundances, but the presence of predators may still be enough to limit grazer responses. Many combinations of factors, such as environmental conditions, resource limitation, predation and recruitment contribute to the structure of benthic communities (Moon and Stiling 2002), and may limit size and abundance responses to sediment enhancement in the field.

In this laboratory study, responses to sediment enhancement occurred within 21 days. In natural settings, eutrophication is often a seasonal event, and may persist for several months before decreasing (Bricker *et al.* 1999). Over time, during natural sediment enhancement, increases in primary consumer abundances may contribute to increases in predator abundances (Wiltse *et al.* 1984; Posey *et al.* 1995, 1999, 2002) and limiting abundances of primary consumers. Large algal blooms are another common response to eutrophication, which often cause reductions in dissolved oxygen as decomposition occurs (Beukema 1991; Bricker *et al.* 1999; Dauer *et al.* 2000; Elliott and de Jonge 2002). This laboratory study did not include these factors (anoxia and predation), which may help explain why the growth, survivorship and reproductive responses may not occur in field studies.

Benthic microalgae and mud slurry treatments contained less N and P content per feeding than other food sources, due to volumetric limitations of the experimental dishes, though volumes were always fed in excess. The growth, survivorship and reproductive responses of *S. benedicti* to these treatments indicates that food quality, as defined by N and P content, may not explain limitations. These results support previous studies that have shown positive reproductive responses for *S. benedicti* to organically-enhanced sediments, but not to nutrient additions alone (Levin 1986; Levin and Creed 1986; Levin *et al.* 1996). Treatments with higher levels of sediment additions consistently produced higher mean survivorship, growth and presence of oocytes throughout the experimental runs, suggesting that concentration of food may be a limiting factor. Higher additions of sediment to an equal amount of area would have increased the concentration of food per area, possibly allowing decreased foraging area and decreased time spent feeding. Increased concentrations of food sources would have allowed *S. benedicti* to reach more food per area without leaving their tubes. *S. benedicti* will not forage away from their tubes unless necessary for foraging, reducing time exposed to browsing predation (Dauer *et al.* 1981; Taghon and Greene 1992; Kihslinger and Woodin 2000). Observations made during the experiments showed that *S. benedicti* did not tend to move to different areas of the dish or extend their tubes, while *L. culveri* appeared to forage throughout the dish habitat. *L. culveri* does not utilize the same feeding behavior as *S. benedicti*, which may help to explain some of the differences in responses to the sediment treatments between these species.

Previous studies (*Capitella* sp.; Gremare *et al.* 1988; *S. benedicti*; Tsutsumi *et al.* 1990) show increased growth responses to the *Ulva* and Tetramen, which were chosen as

a high N and P sources. However, the results of this study showed low growth and reproductive responses to these food sources for *S. benedicti* in comparison to the enhanced-sediment treatments. When compared to initial measurements, Tetramen treatments did produce slightly higher mean total lengths and length to 5th setigers, though not statistically significant. Previous studies showing positive growth and reproductive responses for polychaetes to Tetramen were compared to a limited number of food sources (e.g. *Ulva* and Gerber baby cereal; Gremare *et al.* 1988; Tsutsumi *et al.* 1990), and did not look at responses in comparison to enhanced sediment treatments, which may explain why the responses to Tetramen in this study were insignificant. *Ulva* treatments were associated with decreased total lengths and length to 5th setiger in comparison to initial measurements, indicating that decomposition may be more important for this food source than previously thought. *Ulva* has also been suggested to be mildly toxic, which may have prevented it from being assimilated in large quantities. These findings are supported by Giannotti and McGlathery (2001) who found *Ulva lactuca* to be a poor food source when fed alone to *Ilyanassa obsoleta*. Since both the *Ulva* and Tetramen treatments were high in both N and P content, these results again suggest that these nutrients may not be the only limiting factor for *S. benedicti* growth in the laboratory. Other elements of nutrition, including fatty acids, trace element and other essential compounds were not measured by this study, and may help to explain the weak responses to these food sources and the positive responses to sediment-enhanced food sources that may be rich in micronutrients (Taghon 1981; Marsh and Tenore 1990; Gremare 1994). The *Ulva* and Tetramen foods were also substantially higher in C

content compared to the sediment treatment, though all treatments were fed to excess, suggesting that C itself is not a limiting factor.

Low growth, biomass, and reproductive responses were also seen in response to the *Spartina*, *Phaeosphaeria*, *Phaeosphaeria*-infused *Spartina* and agar control treatments, however, survivorship was surprisingly high for these foods. Similar responses were seen with the azoic sediment treatment, which also contained marsh grass detritus, supporting the results from these treatments. Since growth and survivorship responses were similar or greater for the agar control in comparison to the *Spartina* treatments, the results suggest that any responses may have been to nutrients present in the phytagel substance. All of the *Spartina*/fungal/agar food sources were sufficient for high survival, but the quality of these foods did not provide enough nutrition for *Streblospio benedicti* to grow or reproduce (Olivier *et al.* 1996; Kneib *et al.* 1997). Mean lengths, biomasses, and reproductive condition were all less than initial measurements taken, suggesting that *Spartina*, even when enhanced by fungal decomposers in the laboratory, is not an adequate food source for sustaining reproduction. Previous studies have found similar results for some benthic epifaunal species, showing high survivorship, but low reproductive responses to *S. alterniflora* detritus (Kneib *et al.* 1997, Graça *et al.* 2000). However, these studies also reflected a survivorship difference corresponding to the level of decomposition of leaves, with senescent leaves producing 20% mean survivorship and moderate to heavily decomposed leaves showing 56-84% survivorship for a benthic amphipod species (Kneib *et al.* 1997).

Overall responses for *S. benedicti* indicate that 21 day feeding assays are an effective test of survivorship, growth and reproductive response to various food sources.

The length of these experiments was enough to show changes in growth and reproduction compared to initial measurements. For *S. benedicti*, length to 5th setiger was not an accurate estimator of total length and should not be used as a replacement for this variable. Assessment of reproductive condition proved to be a valuable observation when examining responses to food enhancement since these measurements demonstrate a higher level response than survival or growth, suggesting that this variable should be included in future studies.

In contrast to *Streblospio benedicti*, *Laeonereis culveri* did not show overall increased growth or biomass in response to high levels of the benthic microalgae and mud slurry treatments. These results contrast with previous studies indicating benthic microalgae as an important food source for this species (Mazurkiewicz 1975; Stocks and Grassle 2001). However, significant increases in growth in response to enhanced sediment treatments did occur for run 5, which contained only *L. culveri*. The mixed assemblage of nereid species present in three of the four experimental runs for this species appeared to have an effect on growth of *L. culveri*. The results for the run containing only *L. culveri* showed significant responses for total length, length to thoracic region and width at thoracic region for all food treatments except starvation controls and initial measurements. The mixed assemblage of species caused a negative interaction between the two species, preventing the same growth responses from occurring in these runs. Previous research has shown that increased abundances of *N. succinea* in the field have corresponded with decreased *L. culveri* abundances (Cammen 1979). Also, since the number of *L. culveri* per dish for these runs would have been decreased overall, there

may not have been a large enough sample size to show statistically significant differences for these runs.

No clear difference in food type effects emerged for this species, though *L. culveri* did generally have increased growth responses to Tetramen and *Ulva* treatments (though not statistically significant). Since all treatments were fed to excess, C was never a limiting factor. Therefore, for this species, N and P quality of food sources may be more of a limiting factor since these sources contained higher levels of these nutrients than others, though not significant.

Overall, responses for *L. culveri* for all measurements were variable. No food source clearly appeared to produce significantly increased growth, biomass and survivorship. However, in comparison to initial measurements, for all food sources mean weight per worm and total length increased (except for medium levels of benthic microalgae and mud slurry, and the azoic and starvation controls). The medium benthic microalgae treatment was also the only treatment to produce lesser mean lengths to thoracic region and widths than initial measurements. This supports the idea that *L. culveri* is capable of opportunistic feeding strategies and may be able to feed on a variety of food sources, depending on availability (Mazurkiewicz 1975; Marsh and Tenore 1990). *L. culveri* and *S. benedicti* utilize different feeding modes, which may explain some of the differing responses between these two species. Unlike *S. benedicti, L. culveri* does not forage within a restricted burrow and may rapidly feed across wider areas (Mazurkiewicz 1975; Marsh and Tenore 1990). Such feeding strategies would increase the area available for foraging for this species on the scale of this experiment, making concentration of food per area a less important factor in feeding compared to *S.*

benedicti. Feeding mode may be important in explaining variable responses to food enhancement among various polychaetes.

The differences in responses between the two species of infauna in this study demonstrate the variability in responses to food enhancement. In this study, several food source quantities allowed survival, with fewer producing growth responses and only a small subset allowing for oocyte development. These results suggest that some aspect of food limitation, whether it be availability or nutrient content, plays a role in regulating growth, abundance and reproduction in benthic communities. Sediment enhancement clearly provided positive responses for the species in this study, suggesting that they should be able to respond to enrichment in natural settings. Further studies examining the responses of these species when other factors are present, such as predation or mixed assemblages of species, will help to evaluate the importance of food limitation in comparison to other structuring factors.

LITERATURE CITED

Abrams, P.A. 1982. Functional responses of optimal foragers. The American Naturalist **120**:382-390.

Becker, M.E. 1996. The effect of nutrient loading on benthic microalgae biomass and taxonomic composition. M.S. thesis University of North Carolina at Wilmington 29pp.

Beukema, J. J. 1991. Changes in composition of bottom fauna of a tidal-flat area during a period of eutrophication. Marine Biology **111**:293-301.

Blackford, J.C. 1997. An analysis of benthic biological dynamics in a North Sea ecosystem model. Journal of Sea Research **38**:213-230.

Bricker, S.B., C.G. Clement, D.E. Pirhalla, S.P. Orlando, and D.R.G. Farrow. 1999. National Estuarine Eutrophication Assessment: Effects of Nutrient Enrichment in the Nation's Estuaries. NOAA, National Ocean Service, Special Projects Office and the National Centers for Coastal Ocean Science. Silver Spring, MD: 71 pp.

Bridges, T.S. and S. Heppell. 1996. Fitness consequences of maternal effects in *Streblospio benedicti* (Annelida: Polychaeta). American Zoology **36**:132-146.

Cahoon, L.B. 1999. The role of benthic microalgae in neritic ecosystems. Oceanography and Marine Biology: an Annual Review **37**:47-86.

Cammen, L.M. 1979. The macro-infauna of a North Carolina salt marsh. American Midland Naturalist **102**:244-253.

Carpenter, S.R., J.F. Kitchell and J. R. Hodgson. 1985. Cascading trophic interactions and lake productivity. BioScience **35**:634-39.

Connor, M.S., J. M. Teal, and I. Valiela. 1982. The effect of feeding by mud snails *Ilyanassa-obsoleta* on the structure and metabolism of a laboratory benthic algal community. Journal of Experimental Marine Biology and Ecology **65**:29-46.

Dauer, D. M., C. A. Maybury and R. M. Ewing. 1981. Feeding behavior and general ecology of several spionid polychaetes from the Chesapeake Bay. Journal of Experimental Marine Biology and Ecology **54**:21-38.

Dauer, D.M., S.B. Weisberg and J.A. Ranasinghe. 2000. Relationships between benthic community condition, water quality, sediment quality, nutrient loads and land use patterns in Chesapeake Bay. Estuaries **23**:80-96.

D'avanzo, C., M. Alber, and I. Valiela. 1991. Nitrogen assimilation from amorphous detritus by two coastal consumers. Estuarine Coastal and Shelf Science **33**:203-209.

Dayton, P.K. 1984. Processes structuring some marine communities: are they general? Pages 181-197. In D. R. J. Strong, D. Simberloff, L.G. Abele and A.B. Thistle, editors. Ecological Communities: Conceptual Issues and the Evidence. Princeton University Press, Princeton New Jersey.

Edmonson, W.T. 1970. Phosphorus, nitrogen and algae in Lake Washington after diversion of sewage. Science **169**:373.

Elliot, M. and V.N. de Jonge. 2002. The management of nutrients and potential eutrophication in estuaries and other restricted water bodies. Hydrobiologica **475**:513-524.

Estes, J.A., and C.H. Peterson. 2000. Marine ecological research in seashore and

seafloor systems: accomplishments and future directions. Marine Ecology Progress

Series **195**:281-289.

Fauchald, K. and P. A. Jumars. 1979. The diet of worms: a study of polychaete feeding

guilds. Oceanography and Marine Biology: an Annual Review **17**:193-284.

Giannotti, A.L. and K.J. McGlathery. 2001. Consumption of *Ulva lactuca*

(Chlorophyta) by the omnivorous mud snail *Ilyanassa obsoleta* (Say). Journal of

Phycology **37**:209-215.

Graça, M.A., S.Y. Newell and R.T Kneib. 2000. Grazing rates of organic matter and

living fungal biomass of decaying *Spartina alterniflora* by three species of salt-marsh

invertebrates. Marine Biology **136**:281-289.

Gremare, A. 1994. What describes the fecundity of *Capitella sp.* I better: macro- or

micronutrient availability? Marine Biology **119**:367-374.

Gremare, A. A. G. Marsh, and K. R. Tenore. 1988. Short-term reproductive responses

of *Capitella sp.* I (Annelida: Polychaeta) fed on different diets. Journal of

Experimental Marine Biology and Ecology **123**:147-162.

Gutierrez A.P., N.J. Mills, S.J. Schreiber and C.K. Ellis. 1994. A physiologically based

tritophic perspective on bottom-up top-down regulation of populations. Ecology

75:2227-2242.

Haines, E.B and C.L. Montague. 1979. Food sources of estuarine invertebrates analyzed

using cabon-13/carbon-12 ratios. Ecology **60**:48-56.

Horne A.J. and C.R. Goldman. 1994. Limnology, 2nd ed. McGraw-Hill, Inc. publishers. New York, New York. 576pp.

Howarth, R.W. and J.M. Teal. 1980. Energy flow in a salt marsh ecosystem: the role of reduced inorganic sulfur compounds. American Naturalist **116**:862-872.

Kihslinger, R. L., and S. A. Woodin. 2000. Food patches and a surface deposit feeding spionid polychaete. Marine Ecology Progress Series **201**:233-239.

Kneib, R.T, S.Y. Newell and E.T. Herman. 1997. Survival, growth and reproduction of the salt-marsh amphipod *Uhlorchestia spartinophila* reared on natural diets of senescent and dead *Spartina alterniflora* leaves. Marine Biology **128**:423-431.

Levin, L. 1984. Life history and dispersal patterns in a dense infaunal polychaete assemblage: community structure and response to disturbance. Ecology **65**:1185-1200.

Levin, L. 1986. Effects of enrichment on reproduction in the opportunistic polychaetes *Streblospio benedicti* (Webster): a mesocosm study. Biological Bulletin **171**:143-160.

Levin, L., H. Caswell, T. Bridges, C. DiBacco, D. Cabrera and G. Plaia. 1996. Demographic responses of estuarine polychaetes to pollutants: life table response experiments. Ecological Applications **6**:1295-1313.

Levin, L. H. Caswell, K. D. DePatra, and E. L. Creed. 1987. Demographic consequences of larval development mode: planktotrophy vs. lecithothropy in *Streblospio benedicti*. Ecology **68**:1877-1886.

Levin, L. and E. L. Creed. 1986. Effect of temperature and food availability on reproductive responses of *Streblospio benedicti* (Polychaeta: Spionidae) with planktotrophic or lecithotrophic development. Marine Biology **92**:103-114.

Levin, L. and D. V. Huggett. 1990. Implications of alternative reproductive modes for seasonality and demography in an estuarine polychaete. Ecology **71**:2191-2208.

Lindsay, S. M. and S. A. Woodin. 1996. Quantifying sediment disturbance by browsed spionid polychaetes: implications for competitive and adult-larval interactions. Journal of Experimental Marine Biology and Ecology **196**:97-112.

Lotze, H.K., Worm, B. and U. Sommer. 2000. Propagule banks, herbivory and nutrient supply control population development and dominance patterns in macroalgal blooms. Oikos **89**:46-58.

Mallin, M., J. Burkholder, L. Cahoon and M. Posey. 2000. North and South Carolina coasts. Marine Pollution Bulletin **41**:56-75.

Marsh, A.G. and K.R. Tenore. 1990. The role of nutrition in regulating the population dynamics of opportunistic, surface deposit feeders in a mesohaline community. Limnology and Oceanography **35**:710-724.

Mazurkiewicz, M. 1975. Larval development and habits of *Laeonereis culveri* (Webster) (Polychaeta: Nereidae). Biological Bulletin **149**:186-204.

Mitchell, W.A. 1990. An optimal control theory of diet selection: the effects of resource depletion and exploitative competition. Oikos **58**:16-24.

Molesky, T. J. 2003. Interactions between oyster reefs and adjacent sandflats: effects on microphytobenthos and sediment characteristics. M.S. thesis University of North Carolina at Wilmington 64pp.

Moon, D.C. and P. Stiling. 2002. The influence of species identity and herbivore feeding mode on top-down and bottom-up effects in a salt marsh ecosystem. Oecologia **133**:243-253.

Olivier M., G. Desrosiers, A. Caron, C. Retiere and A. Caillou. 1996. Juvenile growth of *Nereis diversicolor* (O.F. Müller) feeding on a range of marine vascular and macroalgal plant sources under experimental conditions. Journal of Experimental Marine Biology and Ecology **208**:1-12.

Peterson, C.H. 1979. Predation, competitive exclusion, and diversity in the soft-sediment benthic communities of estuaries and lagoons. Pages 233-261. In R.J. Livingston editor. Ecological processes in coastal and marine systems. Plenum Publishing Corporation.

Peterson, B.J., R.W. Howarth, and R.H. Garritt. 1985. Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. Science **227**:1361-1363.

Peterson, B. J., and R. W. Howarth. 1987. Sulfur, carbon and nitrogen isotopes used to trace organic matter flow in the salt-marsh estuary of Sapelo Island, Georgia. Limnology and Oceanography **32**:1195-1213.

Pettibone, M. H. 1971. Revision of some species referred to *Leptonereis*, *Nicon*, and *Laeonereis* (Polychaeta: Nereididae). Smithsonian Institution Press. Washington DC: 53 pp.

Posey, M.H., T. Alphin, L. Cahoon, D. Lindquist, M.E. Becker. 1999. Interactive effects of nutrient additions and predation on infaunal communities. Estuaries. **22**:785-792.

Posey, M. H., T. Alphin, L. B. Cahoon, D. G. Lindquist, M. A. Mallin, and M. B. Nevers. 2002. Top-down versus bottom-up limitation in benthic infaunal communities: direct and indirect effects. Estuaries **25**:999-1014.

Posey, M.H., C. Powell, L. Cahoon, D. Lindquist. 1995. Top-down vs. bottom-up control of benthic community composition on an intertidal tideflat. Journal of Experimental Marine Biology and Ecology **185**:19-31.

Qian, P. and F. Chia. 1990. Detritus as a potential food source for polychaete larvae. Journal of Experimental Marine Biology and Ecology **143**:63-71.

Sarda, R., I.Valiela and K. Foreman. 1996. Decadal shifts in a salt marsh macroinfaunal community in response to sustained long-term experimental nutrient enrichment. Journal of Experimental Marine Biology and Ecology **205**:63-81.

Sigmon, D.E. 1995. The effects of benthic microalgae on sediment nutrient fluxes. M.S. thesis University of North Carolina at Wilmington 31pp.

Sigmon, D. E. and L. B. Cahoon. 1997. Comparative effects of benthic microalgae and phytoplankton on dissolved silica fluxes. Aquatic Microbial Ecology **13**:275-284.

Stocks, K.I., and J.F. Grassle. 2001. Effects of microalgae and food limitation on the recolonization of benthic macrofauna into *in situ* saltmarsh-pond mesocosms. Marine Ecology Progress Series **221**:93-104.

Taghon, G.L. 1981. Beyond selection: optimal ingestation rate as a function of food value. American Naturalist **118**:202-214.

Taghon, G.L. and R.R. Greene. 1990. Effects of sediment-protein concentration on feeding and growth rates of *Abarenicola pacifica* Healy et Wells (Polychaeta: Arenicolidae). Journal of Experimental Marine Biology and Ecology **136**:197-216.

Taghon, G.L. and R.R. Greene. 1992. Utilization of deposited and suspended particulate matter by benthic "interface" feeders. Limnology and Oceanography **37**:1370-1391.

Tsutsumi, H., S. Fukunaga, N. Fujita, and M. Sumida. 1990. Relationship between growth of *Capitella sp.* and organic enrichment of the sediment. Marine Ecology Progress Series **63**:157-162.

Valderrama, J.G. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Marine Chemistry **10**:109-122.

Whitney, D.E. and W.M. Darley. 1979. A method for the determination of chlorophyll *a* in samples containing degradation products. Limnology and Oceanography **24**:183-187.

Worm, B., H.K. Lotze, and U. Sommer. 2000. Coastal food web structure, carbon storage, and nitrogen retention regulated by consumer pressure and nutrient loading. Limnology and Oceanography **45**:339-349.

Wilson, W.H. 1990. Competition and predation in marine soft-sediment communities. Annual Review of Ecology and Systematics **21**:221-241.

Wiltse, W.I., K.H. Foreman, J.M. Teal, I. Valiela. 1984. Effects of predators and food resources on the macrobenthos of salt marsh creeks. Journal of Marine Research **42**:923-942.

Woodin, S.A. 1999. Shallow water benthic ecology: A North American perspective of sedimentary habitats. Australian Journal of Ecology **24**:291-301.

APPENDIX A

Appendix A-1. Raw data for total length, length to thoracic region and width at thoracic region for *Nereis succinea*. N=specimen number, BML=low benthic microalgae, BMM=medium benthic microalgae, BMH=high benthic microalgae, MSL=low mud slurry, MSM=medium mud slurry, MSH=high mud slurry, AZ=azoic sediment, FFL=low Tetramen, FFH=high Tetramen, UL=low *Ulva*, UH=high *Ulva*, S=starvation, PM=initial measurements, L=dish number, R=experimental run

	Total Length (mm)	Length to Thoracic Region (mm)	Width at Thoracic Region (mm)
N1 PM L1 R1	20.68786	2.796384	0.8627862
N2 PM L1 R1	10.31185	2.580626	0.6236344
N1 FFL L1 R1	31.2989	7.872912	1.224166
N1 FFH L2 R1	37.60463	8.939707	1.423072
N1 FFH L3 R1	52.24906	11.80634	1.81194
N2 FFH L3 R1	17.0718	5.452284	0.7643475
N1 BMH L1 R1	41.37615	10.02002	2.038455
N1 BMH L2 R1	20.03925	10.20904	1.487127
N2 BMH L2 R1	17.86853	5.905611	1.139628
N1 BMH L3 R1	44.17357	11.37118	1.928502
N2 BMH L3 R1	26.29437	7.62606	1.724749
N1 MSL L1 R1	24.30572	6.363011	1.262831
N2 MSL L1 R1	21.42251	5.780361	1.183433
N1 MSL L2 R1	25.58533	7.191459	1.461534
N1 MSL L3 R1	21.09447	5.46571	0.9635145
N2 MSL L3 R1	12.45642	4.171532	0.6480035
N1 FFH L1 R1	13.4866	5.731149	0.5641007
N1 FFH L2 R1	18.23746	9.374542	1.813178
N2 FFH L2 R1	30.58778	8.133537	1.598544
N1 FFH L3 R1	17.0806	6.594837	1.775452
N2 FFH L3 R1	29.28752	7.613322	1.153842
N4 FFH L3 R1	21.51169	8.079565	1.096344
N3 UH L2 R1	7.281647	3.418425	0.6152431
N1 UH L3 R1	32.23761	9.426339	1.849263
N2 UH L3 R1	44.89585	10.87492	2.744388
N1 BML L1 R1	14.28045	8.975855	1.641403
N1 UL L1 R1	23.5962	9.738952	1.346149
N1 UL L2 R1	31.21571	9.342242	1.461534
N1 S L2 R1	14.84133	6.007822	1.282399
N2 S L3 R1	18.46309	8.311859	1.585037
N1 MSH L2 R1	51.24269	11.91606	3.05895
N2 MSH L2 R1	21.60398	5.867274	1.499995

Appendix A-1 (continued). Raw data for total length, length to thoracic region and width at thoracic region for *Nereis succinea*. N=specimen number, BML=low benthic microalgae, BMM=medium benthic microalgae, BMH=high benthic microalgae, MSL=low mud slurry, MSM=medium mud slurry, MSH=high mud slurry, AZ=azoic sediment, FFL=low Tetramen, FFH=high Tetramen, UL=low *Ulva*, UH=high *Ulva*, S=starvation, PM=initial measurements, L=dish number, R=experimental run

	Total Length (mm)	Length to Thoracic Region (mm)	Width at Thoracic Region (mm)
N3 MSH L2 R1	7.525204	5.391229	0.7844554
N1 MSH L3 R1	30.6956	10.96588	2.71711
N1 FFH L1 R2	38.40051	8.554178	2.187773
N2 FFH L1 R2	13.86487	3.323932	1.041367
N3 UH L2 R2	31.14657	9.581106	1.868639
N1 UL L3 R2	25.82631	6.419562	1.968663
N3 S L1 R2	33.25328	9.840945	1.744711
N1 S L2 R2	7.837737	4.02774	0.8385475
N2 S L3 R2	9.833867	3.319219	0.7020399
N8 PM L1 R3	20.68022	3.090649	0.9207793
N1 BMH L2 R3	9.536123	4.709552	1.038368
N1 FFH L2 R3	17.47597	4.562866	0.7891385
N1 UL L1 R3	6.964672	5.124749	0.6481307
N3 UL L1 R3	9.713358	4.039972	0.7098214
N4 UL L1 R3	5.731098	3.385963	0.6245513
N1 S L1 R3	5.258351	2.752207	0.6382974
N6 S L1 R3	6.844034	2.138472	0.5647443
N7 S L1 R3	6.02229	3.643268	0.6518402

Appendix A-2. Average weight per worm (g) for *Nereis succinea*, averaged by experimental dish. BML=low benthic microalgae, BMM=medium benthic microalgae, BMH=high benthic microalgae, MSL=low mud slurry, MSM=medium mud slurry, MSH=high mud slurry, AZ=azoic sediment, FFL=low Tetramen, FFH=high Tetramen, UL=low *Ulva*, UH=high *Ulva*, S=starvation, PM=initial measurements, L=dish number, R=experimental run

	Mean Weight per Worm (g)
NS PM R1	0.001300
NS MSL L3 R1	0.001100
NS FFH L2 R1	0.003250
NS UH L3 R1	0.014850
NS S L2 R1	0.000400
NS BMH L2 R1	0.003467
NS UL L1 R1	0.007150
NS BML L2 R1	0.008700
NS MSL L2 R1	0.003700
NS MSH L3 R1	0.018100
NS BMH L3 R1	0.008050
NS FFL L1 R1	0.009700
NS FFL L2 R1	0.009500
NS FFH L3 R1	0.003725
NS MSL L1 R1	0.002500
NS FFL L3 R1	0.019050
NS MSH L2 R1	0.021667
NS S L3 R1	0.012400
NS UL L2 R1	0.014700
NS BMH L1 R1	0.008700
NS FFH L1 R1	0.001300
NS S L1 R2	0.012600
NS UL L3 R2	0.006200
NS UH L2 R2	0.009700
NS S L3 R2	0.000500
NS FFH L1 R2	0.012050
NS S L2 R2	0.002800
NS UL L3 R3	0.000000
NS UL L1 R3	0.000900
NS BMH L2 R3	0.002000
NS FFH L2 R3	0.001000

BIOGRAPHICAL SKETCH

Meredith Quelette Owens was born in Worthington, Ohio on June 3, 1979 to Diana and Gary Owens. In 1999, Meredith traveled abroad to spend a semester at James Cook University in Townsville, Australia studying marine biology, returning as an aquarist intern at the Texas State Aquarium in 2000. In 2001, she received her Bachelor of Science degree in Biology from Drake University after completing an undergraduate thesis on native habitat restoration under the supervision of Dr. Thomas Rosburg. After graduation, Meredith returned to Australia and New Zealand to spend the summer traveling before moving to Wilmington, North Carolina in order to pursue this master's degree. In August of 2001, Meredith began working in the Benthic Ecology lab at the University of North Carolina at Wilmington under the direction of Dr. Martin Posey. While pursuing this degree, she worked as a teaching assistant at UNCW, adjunct faculty in the Biological Sciences Department at Francis Marion University and a research assistant at the Center for Marine Science. Meredith was able to complete her thesis in the fall of 2003. She hopes to combine her education and marine biology experience to pursue a career in animal husbandry and marine education.