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Utilization of *Spartina*- and *Phragmites*-Derived Dissolved Organic Matter by Bacteria and Ribbed Mussels (*Geukensia demissa*) from Delaware Bay Salt Marshes

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Abstract *Phragmites australis* has been invading *Spartina-alterniflora*-dominated salt marshes throughout the mid-Atlantic. Although, *Phragmites* has high rates of primary production, it is not known whether this species supports lower trophic levels of a marsh food web in the same manner as *Spartina*. Using several related photochemical and biological assays, we compared patterns of organic matter flow of plant primary production through a key salt marsh metazoan, the ribbed mussel (*Geukensia demissa*), using a bacterial intermediate. Dissolved organic matter (DOM) was derived from plants collected from a Delaware Bay salt marsh and grown in the laboratory with ^{14}C-CO_2. Bacterial utilization of plant-derived DOM measured as carbon mineralization revealed that both species provided bioavailable DOM to native salt marsh bacteria. Total carbon mineralization after 19 days was higher for *Spartina* treatments (36% $^{14}CO_2 \pm 3$ SE) compared with *Phragmites* treatments (29% ±2 SE; Wilcoxon–Kruskal–Wallis rank sums test, $P<0.01$). Pre-exposing DOM to natural sunlight only enhanced or decreased bioavailability of the DOM to the bacterioplankton during initial measurements (e.g., 7 days or less) but these differences were not significant over the course of the incubations. Mixtures of ^{14}C-labeled bacterioplankton (and possibly organic flocs) from ^{14}C-DOM treatments were cleared by *G. demissa* at similar rates between *Spartina* and *Phragmites* treatments. Moreover, ^{14}C assimilation efficiencies for material ingested by mussels were high for both plant sources ranging from 74% to 90% and not significantly different between plant sources. Sunlight exposure did not affect the nutritional value of the bacterioplankton DOM assemblage for mussels. There are many possible trophic and habitat differences between *Spartina*- and *Phragmites*-dominated marshes that could affect *G. demissa* but the fate of vascular plant dissolved organic carbon in the DOM to bacterioplankton to mussel trophic pathway appears comparable between these marsh types.

Keywords Dissolved organic matter · *Spartina alterniflora* · *Phragmites australis* · *Geukensia demissa* · Trophic linkages

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Introduction

Over several decades, *Phragmites* (European strain) has been invading salt marshes along the eastern coasts of the US (Chambers et al. 1999; Weinstein and Baletto 1999; Saltontall 2002; Silliman and Bertness 2004) and replacing *Spartina*. It has been proposed that *Phragmites* invades disturbed areas (Silliman and Bertness 2004; King et al. 2007) but propagation may be limiting (Silliman and Bertness 2004). The presence and spread of *Phragmites* may represent a new and different input of DOM to an established marsh food web based on *Spartina* DOM. Considerable research has investigated whether *Phragmites*-dominated tidal marshes are capable of supporting marsh and estuarine food webs similar to *Spartina*-dominated marshes.

Most studies that have evaluated the role of *Phragmites* in food webs where *Spartina* is–was the dominant species have focused on the effects at higher trophic levels such as abundance and diversity of nekton species (Meyer et al. 2001; Able et al. 2003; Osgood et al. 2003; Raichel et al. 2003; Hunter et al. 2006), birds (Roman et al. 1984; Benoit and Askins 1999), and macroinvertebrates (Fell et al. 1998; Talley and Levin 2001; Warren et al. 2001). Wainright et al (2000); however, they utilized stable isotopes to trace the flow of nutrients from microalgae, phytoplankton, and detritus to *Fundulus heteroclitus* juveniles in *Phragmites*-dominated and *Spartina*-dominated marshes. They demonstrated that *Phragmites* marshes supported fish production in a similar manner to *Spartina* but had lower abundances of benthic microalgae potentially attributed to shading differences. However, the roles of DOM and the bacterial component were not defined. Of interest to our study, there is cursory evidence that *Phragmites* may be capable of supporting higher abundances of *Geukensia demissa* compared with *Spartina* along the seaware edges of human-influenced marshes (McClary 2004) though linkages to quality and quantity of DOM were not made.

Spartina spp. have been shown to be important sources of vascular plant-derived DOM to estuarine waters supporting bacterial metabolism (Filip and Alberts 1988; Moran and Hodson 1989, 1994; Wang et al. 2007) and overall community plankton metabolism (Turner 1978). As the DOM derived from each plant becomes part of the larger pools of DOM found in open pools and waterways, it may be modified through photochemical processes. Exposure to sunlight has positively altered the bioavailability of DOM isolated from areas dominated by *Spartina* (Miller and Moran 1997; Bushaw-Newton and Moran 1999; Moran et al. 2000). DOM derived from *Phragmites* has also been shown to fuel bacterial production in freshwater systems (Anesio et al. 1999; Denward et al. 1999) but pre-exposure to sunlight did result in decreased biomass in a few studies (Anesio et al. 1999; Farjalla et al. 2001). Assessing the role of *Phragmites* in "bottom up" processes in salt marshes requires a direct comparison of the relative roles of *Spartina* and *Phragmites* in supporting a consumer food web.

In salt marshes, *G. demissa*, the ribbed mussel, appears to use the plant matter indirectly, mediated through the microbial loop and presented to mussels as fine particles in the form of bacterioplankton and microheterotrophs (Kreeger et al. 1988, Kreeger and Newell 1996, 2000) and likely also as aggregated organic flocs (Alber and Valiela 1994, Huang et al. 2003, Pan and Wang 2004). In New England marshes, *Spartina*-derived material has been estimated to contribute up to 80% of the diet of mussels (Peterson et al. 1985) and 30–50% in mid-Atlantic marshes (Langdon and Newell 1990).

Using plants, bacteria, and mussels collected from a Delaware Bay salt marsh, we conducted several experiments to (1) evaluate the nutritional quality of the DOM produced from both plant species to native bacteria, (2) assess whether sunlight can modify the nutritional quality of each plant's DOM to native bacteria, and (3) determine if the bacteria+DOM mixture from each plant species would be used similarly by a key marsh consumer, the ribbed mussel, *G. demissa*.

Materials and Methods

Study Site

A salt marsh site at Sea Breeze, NJ, USA (39° 19' 21" N, 75° 19'11" W) was chosen out of five potential sites (Alloway, Mad Horse, Heislerville, and Goshen Landing) based on the dominance of the plant species *Spartina alterniflora* (*Spartina*), the presence of *Phragmites australis* (*Phragmites*), and the presence of the ribbed mussel, *G. demissa* (Fig. 1). Sea Breeze site was the only site dominated by *Spartina* but with a strong presence of *Phragmites* and high concentrations of mussels. Salinity was 10‰ and dissolved organic carbon, soluble reactive phosphate, and ammonium were 13 mg C per liter, 17 μg P per liter, and 356 μg N per liter, respectively.

^{14}C-Labeling and Plant Leachate Preparation

In August 2001, live plants (*Spartina* and *Phragmites*) with marsh sediment were collected in pots from Sea Breeze. In the laboratory, plants were clipped to remove aboveground biomass and grown over a 2-month period using 1.0 μm (nominal pore size) filtered marsh water.

In November 2001, four pots (two pots of each species) were selected for ^{14}C-labeling based on the appearance of viable new shoots. Plant labeling procedures were carried out using the methods of Kreeger et al. (1988). Briefly, the four pots were transferred to an air-tight growth chamber surrounded by light banks and with uniform temperature control (21°C). The growth chamber was then sealed and 5 mCi of $^{14}CO_2$ were liberated into the atmosphere of the chamber over a 5-day period (1 mCi/day). Plants were kept inside the growth chamber for 2 weeks during which time nonlabeled CO_2, water, and nutrients (f/2 culture medium) were periodically added. A second 5-day pulse of $^{14}CO_2$ was delivered to the plants between December 5 and 10, 2001, which was chased with a 10-day period of nonlabeled CO_2, water, and nutrients. At the end of this month process, the chamber was unsealed, and all aboveground, live (green) biomass was clipped and bagged separately for each species.

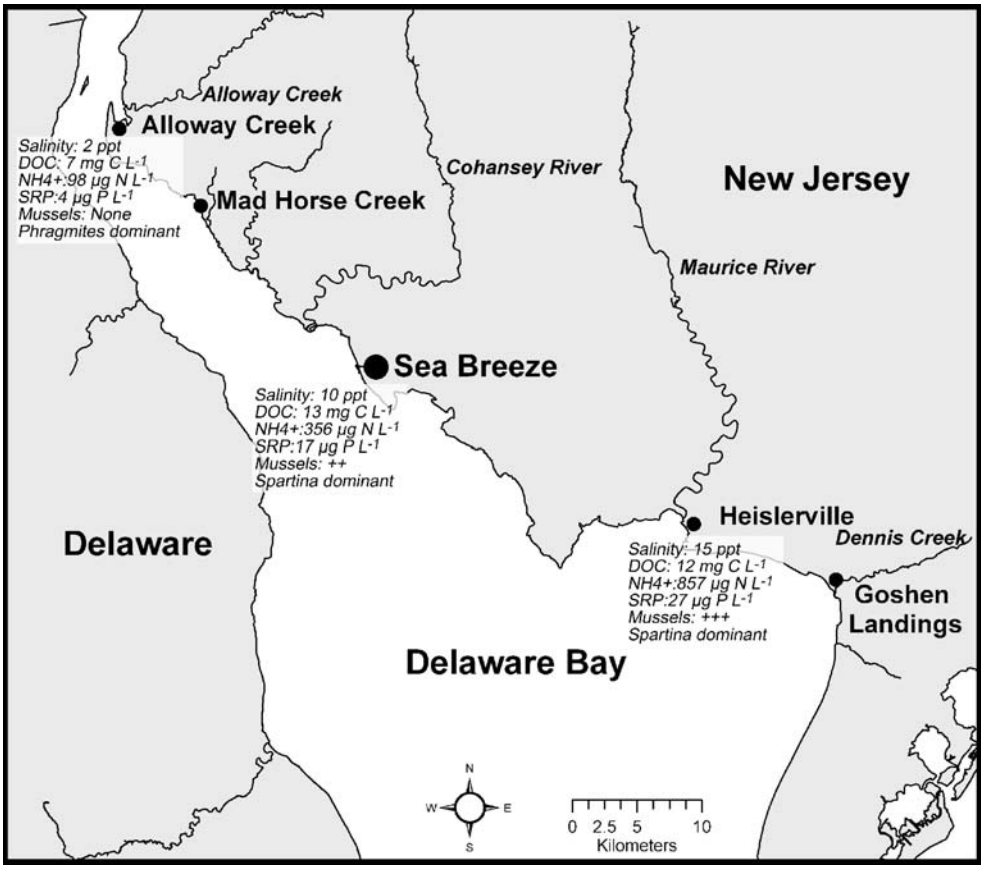

Fig. 1 Map of Delaware Bay and the salt marsh locations with key site parameters (salinity, DOC, $NH4^+$, SRP, mussel presence, and dominant plant species) *highlighted*

Due to low regrowth rates, only 1.37 g of live *Spartina* was recovered from the ^{14}C-labeling process, which is not unexpected considering that these plants may be conditioned to physiologically enter a dormant period from fall through winter. In contrast, *Phragmites* regenerated readily, and 24.45 g of live, ^{14}C-labeled plant biomass was recovered. Once dried, we recovered 0.275 g of *Spartina* and 3.1 g of *Phragmites*.

Labeled plant material was weighed and placed into muffled glassware containing either 90 ml for *Spartina* or 250 ml for *Phragmites* of filtered (0.2-µm nominal pore size) low-nutrient artificial seawater medium (Harrison et al. 1980). The flasks were placed in the dark and the plant material was allowed to leach for 3 days at room temperature (~23°C) with mixing daily. After leaching, the samples were filtered (0.2-µm nominal pore size) into muffled glass containers and stored at 4°C until use. Filtered plant material was dried and weighed and weight loss due to leaching was calculated. Subsamples were removed from each type of leachate for dissolved organic carbon (DOC) concentrations.

Sunlight Incubations and Bacterial Utilization

In May 2002, labeled *Spartina* and *Phragmites* leachates were diluted to represent natural concentrations (~25–50% of the total DOC based on measured values for the Sea Breeze site, 13 mg C per liter) using filtered low-nutrient artificial seawater (~11‰ salinity; Harrison et al. 1980). Leachate solutions were filtered through 0.2-µm filters to ensure sterile irradiations. For the irradiated (sunlight) treatments, one half of each sample was placed in a muffled 2-l round-bottom quartz flask and exposed to full sunlight for a period of 8 h (0800 to 1600) on May 15, 2002. For the dark treatments, the other half of each sample was treated identically except the flask was wrapped in aluminum foil. All flasks were placed in an ice water bath so that temperatures never exceeded 10°C, and flasks were swirled periodically during the irradiation. Subsamples for absorbance, DOC, and nutrients were removed from the flasks after incubation.

Bacterial utilization of ^{14}C-labeled *Spartina*- and *Phragmites*-derived DOM was measured through respiration based on $^{14}CO_2$ evolution and bacterial enumeration. Following irradiation, replicate 500-ml flasks (four flasks per treatment) were filled with either 100 ml of the reconstituted ^{14}C-*Spartina* or ^{14}C-*Phragmites* leachate and amended with 224 µg N per milliliter as NH_4Cl and 31 µg P milliliter as PO_4. As a control, one flask was not inoculated with bacteria (DOM only). A bacterial inoculum was made by concentrating the natural bacterioplankton community from whole water obtained from the Sea Breeze

site in May 2002 using methods outlined in Bushaw-Newton and Moran (1999). The bacterial concentrate was added to the flasks, giving initial cell densities of 5.0×10^3 cells per milliliter ($n=12$). Flasks were sealed with silicone stoppers outfitted with 0.2 N NaOH traps (2 ml in a scintillation vial) to measure $^{14}CO_2$ production. Microcosms were incubated in the dark at room temperature at 100 rpm for 19 days. Traps were changed out at 2, 7, 12, and 19 days. For bacterial number measurements, 5-ml subsamples were removed from each flask at each time point, preserved with 0.3 ml borate-buffered formalin and counted using epifluorescence microscopy after staining with 0.01% acridine orange (Hobbie et al. 1977). Bacteria were counted in ten fields per slide with between 30 and 300 bacteria per field. At the end of the bacterial incubations, the microcosms were transferred intact to begin the mussel-feeding experiments.

Mussel Utilization of DOM + Bacteria Complex

After the 19-day bacterial incubations, the microcosms containing bacteria + leachates were fed to *G. demissa*. In June 2002, representative *G. demissa* were collected from the marsh on the evening low tide prior to experimentation. After collection, they were acclimated overnight. Then, they were subjected to an 18-h ^{14}C-feeding experiment which consisted of a short 2-h pulse period, followed by a lengthy purge period. The approach and methods are briefly reviewed here; however, please see Kreeger and Newell (1996) for more details on the pulse chase design.

Throughout the experiment, mussels were fed a similar base diet consisting of natural marsh water, which was augmented with a small *w*/*w* ^{14}C-labeled supplement during the pulse. Uptake and utilization of the ^{14}C-labeled supplement were then quantified by tracing the fate of ^{14}C using established mass balance approaches (Kreeger et al. 1988; Kreeger and Newell 2001).

After the 2-h pulse when mussels were fed the ^{14}C diet (i.e., marsh water supplemented with one of four ^{14}C-labeled bacteria–DOM products), they were switched into clean marsh water containing no ^{14}C for a 4-h purge. This was followed by a 6-h purge in humidified air (to retain simulated intertidal conditions), and then a second 6-h period of submergence in unamended marsh water. Hence, mussels were fed on the labeled diet for 2 h, followed by a 16-h depuration period under simulated natural, intertidal conditions and water–food quantity and quality (100-μm sieved marsh water).

Forty-two adult mussels (shell height range=39.2–53.8 mm) were use in the experiment, each held in a 1-l beaker containing 800 ml of water. The total seston concentration in marsh water used to rear mussels averaged 14.2-mg dry weight per liter, whereas the total weight of the ^{14}C-labeled supplements was less than 1-mg dry weight per liter. Actual diet formulations for the 2-h pulse period are summarized in Table 1, which shows that all treatments received the same background diet of marsh water. However, the ^{14}C activity of supplements varied depending on the leachate treatment, with *Phragmites* products containing nearly twice the ^{14}C activity of the *Spartina* products (Table 1).

Nine mussels were individually reared on each of the four dietary treatments ($n=36$ mussels total) described in Table 1. Additional mussels were reared on control diets containing the same quantity of marsh seston but with supplements consisting of DOM-only leachates (no bacteria). The ^{14}C activities and mussel uptake data for these controls are not reported here because mussel DOM uptake is negligible and values measured in our controls were not significantly different from 0. For each of the four treatments, we also prepared controls having no mussels but with the same diet formulations, which were used to verify that live microorganisms in the mixed marsh water–bacteria–leachate diets did not appreciably respire or otherwise transform the ^{14}C diets during the pulse period.

Diet utilization was gauged by quantifying and comparing both the feeding rate and the assimilation efficiency for the various treatments (see Kreeger and Newell 2001 for calculations). The various components of the ^{14}C budget of each mussel were quantified by measuring the total ^{14}C activity generated during the 16-h purge as either $^{14}CO_2$ respiration, ^{14}C excretion, ^{14}C defecation, or ^{14}C incorporation into tissues (Kreeger and Newell 2001). Only the ^{14}C-respired and ^{14}C-incorporated fractions are considered as ^{14}C-assimilated. By summing all four of these fractions of the ^{14}C-budget, we calculated ^{14}C ingestion. All ^{14}C activities quantities were then related to the dry tissue weight of each animal following standard allometric procedures (e.g., see Kreeger and Newell 2001).

Since different ^{14}C-activities were actually delivered to mussels in the differently labeled supplements, the most meaningful biological measurements were the relative proportions of the available and ingested labeled rations

Table 1 Mean (±SE) ^{14}C activity of each bacteria–leachate stock ($n=3$ per treatment) and the amounts delivered to individual *G. demissa* ($n=9$ per treatment) for irradiated (Lt) and nonirradiated (Dk) DOM + bacteria mixtures

	Bacteria–leachate Stock	Bacteria–leachate fed to *G. demissa*[a]
	dpm ml^{-1} Lt/Dk	dpm Lt/Dk
Spartina	392 (10)/399 (3)	9,459 (549)/9,637 (502)
Phragmites	727 (10)/692 (8)	17,995 (2,113)/16,473 (1,167)

[a] In 800 ml

that were used for growth and respiration (i.e., the percentage assimilated). Therefore, we determined the proportion of available ^{14}C (in the beaker) that each mussel ingested and as described above we also calculated the fate of ingested ^{14}C in the various ^{14}C budget components as percentages of ingestion. To ensure that all mussels were behaving similarly and feeding consistently during the pulse, we also measured the clearance rates of mussels during the 2-h pulse by monitoring the disappearance of suspended ^{14}C (as per Kreeger and Newell 1996, 2001). This rate of feeding was then contrasted with the calculated ingestion rate determined from the ^{14}C budget.

Chemical Analyses

DOC concentrations were determined by high-temperature catalytic oxidation using a Shimadzu TOC-5000 carbon analyzer (Shimadzu Corp.). Ammonium and soluble reactive phosphate were determined using standard methods (US EPA 1997). Light absorbance by the leachate solutions was measured in quartz cuvettes at 350 nm using a Shimadzu 1601 UV/Vis spectrophotometer. Absorbance coefficients (a_{350}) were calculated using the formula of Miller and Zepp (1995). Fading of the DOM, loss of color due to sunlight exposure, was calculated based on a_{350} measurements. Measurements of ^{14}C parameters were made using a Perkin Elmer Wallac 1409 DSA mode liquid Scintillation Counter (Perkin Elmer Corporation). Liquid scintillation cocktail (Scintsafe, Fisher Scientific) was added to all scintillation vials for all measurements. To calculate $^{14}CO_2$ evolution for bacterial respiration measurements, disintegrations per minute (DPM) of the $^{14}CO_2$ at each time point was measured and calculated as a percent of the total DPM of the original DOM. Calculations for mussel parameters are as previously described.

Statistical Analyses

Utilization rates of DOM by bacteria were determined using linear regression models based on oxygen consumption or production of $^{14}CO_2$ through time. All datasets were tested for normal distribution according to the Shapiro–Wilks W test and equality of variances according to the Levene test (Zar 1999). Several datasets did not satisfy these criteria for standard analysis of variance; therefore, nonparametric statistics were used. Nonparametric analysis using the Wilcoxon –Kruskal–Wallis rank sums test was used to determine the effects of plant type (i.e., *Spartina* vs *Phragmites*) and sunlight exposure on bacterial and mussel utilization of DOM. In order to test more than one variable at once, parameters were combined for the statistical analyses (e.g., *Phragmites* + dark and cumulative CO_2 evolution vs *Spartina* + light and its effects on cumulative CO_2 evolution). The statistical program JMP (SAS Institute) was used in the analysis of experimental data.

Results

Leached DOM Characteristics

The weight loss from the plant biomass varied from 5% for the ^{14}C-*Spartina* to 30% for the ^{14}C-*Phragmites* DOC values ranged from 83 mg C per liter for the leachate produced from ^{14}C-*Spartina* to 185 mg C per liter for the leachate produced from ^{14}C-*Phragmites*.

There were decreases in the absorbance coefficients (a_{350}) from 23 to 16.1 m^{-1} after irradiation for the DOM derived from *Spartina*. After exposure, there was a 30% decrease in the color of the *Spartina* DOM. In contrast, exposure to sunlight increased the a_{350} for *Phragmites*-derived DOM from 13.8 to 16.1 m^{-1} resulting in a 16% increase in color for the *Phragmites*-derived DOM. DOC values were similar for each plant species before and after exposure to sunlight averaging 7.3 mg C per liter for *Spartina*-derived DOM and 5.7 mg C per liter for *Phragmites*-derived DOM (n=3 for each).

Biological Studies

The ^{14}C studies allowed us to trace the flow of organic matter from plant to bacteria to the salt marsh mussel, *G. demissa*. Production of $^{14}CO_2$ by bacteria grown on DOM solutions was measured in all treatments (Fig. 2). Over the 19-day period, bacteria utilized 29% of the DOM from sunlight-exposed *Phragmites* (*Phragmites*-Lt) to 36% in the sunlight-exposed *Spartina* DOM (*Spartina*-Lt; Fig. 2).

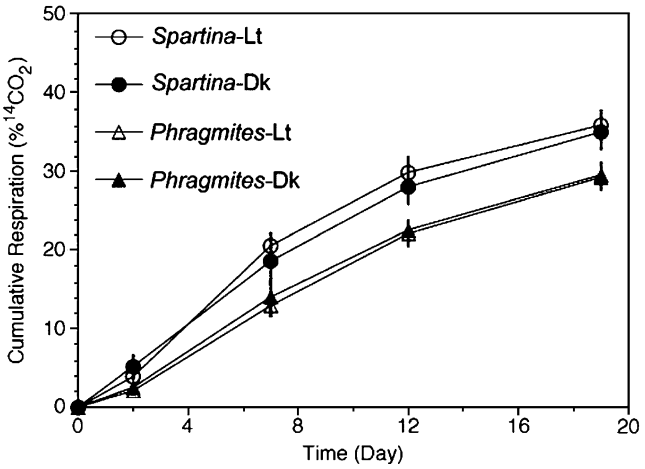

Fig. 2 Cumulative percent carbon mineralization of ^{14}C-*Spartina*- and ^{14}C-*Phragmites*-derived dissolved organic matter solutions irradiated (*Lt*) or nonirradiated (*Dk*; n=3, ±SE)

Exposure to sunlight did not enhance the bioavailability of the *Spartina* or the *Phragmites* DOM to the bacterial community when compared with the DOM not exposed to sunlight. A comparison of the overall availability of each plant species to the bacterial community demonstrated that *Spartina* DOM was mineralized more readily than *Phragmites* DOM (Wilcoxon–Kruskal–Wallis rank sums test, $P<0.01$). To account for potential recycling in the microcosms, initial rates of mineralization (percent $^{14}CO_2$ mineralized per day) were calculated based on the first 7 days (Table 2). Over the course of the first week of incubations, exposure to sunlight enhanced the mineralization rate of the *Spartina* DOM solutions (2.4% $^{14}CO_2$ mineralized per day) compared with all other DOM solutions. The nonirradiated *Spartina* DOM microcosms had the second highest rate (1.9% $^{14}CO_2$ mineralized per day) followed closely by both sets of *Phragmites* DOM microcosms (1.6% $^{14}CO_2$ mineralized per day).

During the course of the bioassays, bacterial cell numbers increased 200-fold or greater over initial levels in all DOM treatments from 1.5×10^6 bacteria per milliliter for both *Spartina*-Dk and *Phragmites*-Lt to 1.9×10^6 for *Spartina*-Lt and *Phragmites*-Dk. For both the *Spartina*-derived and *Phragmites*-derived DOM, exposure to sunlight or plant type had no effect on bacterial growth (Wilcoxon–Kruskal–Wallis rank sums test, $P>0.18$).

Mussel Utilization of DOM + Bacteria Complex

For mussels fed DOM + bacteria, clearance rates were erratic during the 2-h pulse and not significantly different between plant source types or light and dark conditions (Wilcoxon–Kruskal–Wallis rank sums test, $P>0.05$). Clearance rates for the overall diet (nonlabeled base diet plus labeled supplement) averaged 0.27 l h^{-1} g per dry tissue weight. Mussels were similarly sized (averaging 0.42-g dry tissue weight) and, not surprisingly, they fed at similar rates among treatments having identical composition of the nonlabeled majority of the diet. In quantifying uptake of the labeled dietary supplement, mussels ingested a similar proportion (Wilcoxon–Kruskal–Wallis rank sums test, $P>0.60$) of the ^{14}C supplements, denoted as the "proportion ingested" in Table 3.

In quantifying the ^{14}C budget for how the labeled diets were used by mussels, we measured both the absolute ^{14}C activities in the carbon budget as well as the relative fate of ingested ^{14}C in the budget components as percentages. The relative percent utilization is of greatest interest since absolute ^{14}C activities were skewed higher in mussels fed *Phragmites*-based diets due to the fact that the diet had twice the ^{14}C activity in the two *Phragmites* treatments compared to the *Spartina* treatments (refer to Table 1). And, since significantly greater ^{14}C was ingested (Wilcoxon–Kruskal–Wallis rank sums test, $P<0.002$), then the fate of ^{14}C activity must have been greater when totaled across the four components of the ^{14}C budget; e.g., ^{14}C-incorporated (Wilcoxon–Kruskal–Wallis rank sums test, $P<0.0001$) and ^{14}C-defecated (Wilcoxon–Kruskal–Wallis rank sums test, $P<0.0002$) were greater in mussels fed the *Phragmites* DOM + bacteria mixtures (Lt, Dk) compared to the two *Spartina* DOM + bacteria mixtures (Lt, Dk). No differences were detected in ^{14}C respiration and ^{14}C excretion between plant types and light regimes.

The relative fate of ^{14}C activities in mussels fed the different treatments was not significantly different between plant types. Of particular note, the bulk of ingested ^{14}C was actually assimilated and incorporated by mussels into their tissues, regardless of treatment (Table 3). More than half of the ingested ^{14}C was incorporated (i.e., reflecting anabolism) and between 19% and 27% was respired (i.e., reflecting catabolism). Hence, assimilation efficiencies for all four ^{14}C-labeled diets were quite high, ranging from 74 (±8 SE) and 76% (±8 SE) for ^{14}C-*Spartina*-Lt and ^{14}C-*Spartina*-Dk to 85 (±5 SE) and 90% (±5 SE) for ^{14}C-*Phragmites*-Lt and ^{14}C-*Spartina*-Dk (Table 3). Although there were some significant differences in percent utilization when comparing the four treatments directly (Table 3), these differences appeared to reflect unidentified interactions between the main effects of plant type and light environment since no significant differences in any of the percentage data were detected when comparing *Phragmites* versus *Spartina*, per se.

Table 2 Initial rates of bacterial mineralization of the DOM derived from ^{14}C-labeled plant material irradiated (Lt) and nonirradiated (Dk)

Treatment	Rate of mineralization, % $^{14}CO_2$ mineralized day^{-1}	r
	Lt/Dk	Lt/Dk
Spartina	2.4/1.9	0.9873/0.9565
Phragmites	1.6/1.6	0.9761/0.9765

Rates of mineralization were calculated based on measured production of $^{14}CO_2$ on days 0 through 7. Days 12 and 19 were excluded because production decreased and may be more reflective of recycling rather than new production ($n=3$).

Discussion

Our studies demonstrated that both plant species provide nutritional DOM to the native marsh bacteria though there were similarities and differences. Roughly one-third of the DOM from both species was mineralized in a 3-week period and cell numbers reached similar densities. Other

Table 3 Mean (±SE; $n=9$ per treatment) dry tissue weight of *G. demissa* and the fate of ^{14}C ingested by *G. demissa* under the four treatments of irradiated (Lt) and nonirradiated (Dk) leachate + bacteria mixtures

	Units	*Spartina*	*Phragmites*
		Lt/Dk	Lt/Dk
Mussel dry tissue weight	mg	0.43 (0.04)/0.46 (0.04)	0.42 (0.04)/0.38 (0.05)
Clearance rates ($P<0.003$)	L h^{-1} g^{-1} std. mussel	0.10 (0.02)/0.32 (0.06)	0.39 (0.08)/0.09 (0.02)
^{14}C ingestion ($P<0.002$)	dpm [g dtw]$^{-1}$	6,461 (821)/8,036 (904)	15,133 (2,035)/14,380 (4,346)
Proportion of dietary ^{14}C ingested	%	68 (8)/85 (9)	86 (13)/93 (33)
^{14}C excretion	dpm [g dtw]$^{-1}$	1,712 (610)/1,725 (566)	1,852 (741)/1,177 (721)
Proportion of dietary ^{14}C excreted	%	25 (8)/23 (8)	14 (5)/9 (5)
^{14}C respiration	dpm [g dtw]$^{-1}$	1,220 (350)/2,626 (1,201)	4,264 (1,864)/5,326 (4,109)
Proportion of dietary ^{14}C respired	%	19 (5)/27 (9)	22 (8)/25 (11)
^{14}C incorporation ($P<0.0001$)	dpm [g dtw]$^{-1}$	3,414 (470)/3,605 (373)	8,749 (1,072)/7,668 (1,112)
Proportion of dietary ^{14}C incorporated ($P<0.05$)	%	55 (6)/49 (7)	62 (6)/65 (8)
^{14}C defecation ($P<0.0002$)	dpm [g dtw]$^{-1}$	116 (9)/81 (64)	269 (58)/210 (42)
Proportion of dietary ^{14}C defecated ($P<0.04$)	%	2.0 (0.3)/1.1 (0.2)	1.8 (0.2)/1.6 (0.2)
^{14}C assimilation efficiency	%	74 (8)/76 (8)	85 (5)/90 (5)

When means differed significantly among treatments (Wilcoxon–Kruskal–Wallis rank sums test), the corresponding P value is reported.

studies have also found increased bacterial utilization rates after the addition of fresh *Spartina* leachate. Coffin et al. (1993) found that biological oxygen demand significantly increased almost exhausting oxygen concentrations when fresh *Spartina* DOM was added to bacterial mesocosms. Using a mass balance approach with ^{13}C, Hullar et al. (1996) found that at least 40% of the carbon derived from *S. alterniflora* was respired over a 36-h period. In both of these studies, it was hypothesized that a labile portion of the *Spartina* DOM pool was able to provide a portion of the nutritional requirements of the bacteria. Our studies demonstrate that both *Spartina* and *Phragmites* are bioavailable to the bacterial community, though *Spartina* is the preferred substrate regardless of sunlight exposure.

The Role of Photochemical Processes

We found positive and negative effects of sunlight exposure on the utilization of leached plant material by bacteria and *G. demissa*. DOM from both species was altered after exposure to natural sunlight. In several preliminary studies of freshly cut and dead *Spartina* and *Phragmites* leachate, the DOM solutions lost color and faded after exposure to solar radiation (Bushaw-Newton, unpublished data) indicating possible changes in composition with increased fading potentially indicative of increased bioavailability (Strome and Miller 1978; Moran et al. 2000; Engelhaupt et al. 2003). Bacterial utilization rates of ^{14}C-*Spartina* were increased after irradiation but not ^{14}C-*Phragmites*. These results are consistent with fading (i.e., loss of color) measurements indicating that the loss of 30–40% of the DOM color of the live *Spartina* may be the result of several different photochemical processes (i.e., oxidation and release of compounds, restructuring of the DOM matrix to increase bioavailability). Unlike previous studies (Anesio et al. 1999), our experiments did not find that DOM leached from fresh *Phragmites* negatively affected bacterial growth. Photochemical processes have the potential to restructure the DOM in ways that may decrease bioavailability (Tranvik and Kokalj 1998). Keil and Kirchman (1994) demonstrated that sunlight transformed labile protein into refractory material while Kieber et al. (1997) found that fatty acids are photo-transformed into aldehydes, which in turn may play a role in the production of marine humic substances. Several other studies also found that prior exposure of the DOM to solar radiation either caused the bacterial activities to remain the same (Bushaw-Newton and Moran 1999) or to decrease (Tranvik and Kokalj 1998; Anesio et al. 1999; Anesio and Granéli 2000; Farjalla et al. 2001) compared with bacteria grown on nonirradiated DOM. In preliminary studies, we found that leachate derived from *Spartina* and *Phragmites* material that had been sitting on the marsh surface as well as freshly cut material both positively and negatively affected bacterial growth dynamics after sunlight exposure, though the negative outcomes were short term (<3 days; Bushaw-Newton, unpublished data). Our results from those studies and this one suggest that irradiation of leachate produced from freshly cut *Spartina* and *Phragmites* was not a significant factor over the course of the incubations but did affect initial utilization of the dissolved substances by the bacteria. Further, the degree of DOM exposure to

sunlight did not appear to alter the bacteria and mussel trophic relationship with regard to carbon.

Nutritional Value of DOM + Bacteria Complex for Mussels

Our findings suggest that important marsh fauna such as *G. demissa* are not only capable of using vascular plant-derived production by microheterotrophic bacteria (as shown by Kreeger and Newell 1996, 2001) but, moreover, a substantial route of carbon flow may occur via the plant → DOC → bacteria → mussel pathway in addition to the direct plant → bacteria → mussel pathway associated with the particulate detritus complex from vascular plants. Our results also demonstrate that the type of marsh grass does not appear to be an important factor in the production of a DOC + bacteria product that is nutritious for this mussel species. DOM + bacteria mixtures produced from both *Phragmites* and *Spartina* were utilized by mussels with very high and similar efficiency. Assimilation efficiencies, for example, ranged from 74% to 90% which is similar to previously reported values of >85% for ^{14}C-labeled marsh bacteria (Kreeger and Newell 2001).

Our results provide insight to explain how *G. demissa*, the functionally dominant mussel in these salt marshes, is capable of meeting its carbon demands in *Phragmites* marshes of New Jersey. Further, both plants have extensive rhizomes that mussels can adhere to with their byssal threads. Mussel abundance is naturally patchy with clustering along creek banks (e.g., mean=149 mussels per square meter, Kreeger. D. A., unpublished data for the Delaware Estuary) and lower abundance on the high marsh (mean= 9.9 mussels per square meter). However, maximum densities reported by McClary (2004) for *Phragmites* marshes were <10 mussels per square meter which by comparison is low. This study, however, did not survey mussel abundance across the marsh plane or along transects from high to low marsh (McClary 2004). In the Delaware Estuary, *Phragmites* marshes appear to have less edge habitat per hectare than *Spartina* marshes (Kreeger, unpublished) and so they might support lower mussel densities across the marsh. Furthermore, the low densities of mussels reported by McClary (2004) in a human-altered landscape suggest that those marshes are atypical.

Another potentially important feature that may affect mussels in *Phragmites* marshes is the canopy density and its potential limitation of microphytobenthic production. *Spartina* marshes have greater light availability at the surface, which appears to fuel greater production of benthic diatoms (Wainright et al. 2000). Where they are prolific, ribbed mussels are obligate omnivores that must rely on a suite of different foods to satisfy their annual nutritional demands, and these benthic diatoms represent one of the important food sources for *G. demissa* (Kreeger and Newell 2000, 2001). Therefore, it is possible that *Phragmites* marshes might be inferior to *Spartina* marshes with regard to other components of the mussel's diverse diet.

Conclusions

We found that native bacteria from a Delaware Bay salt marsh are able to utilize DOM derived from both plant species and that the DOM derived from *Spartina* and *Phragmites* is susceptible to modification through sunlight that affects the bacteria both positively and negatively. Importantly, the ribbed mussel, *G. demissa*, is present in great abundances in these marshes, and it appears able to utilize carbon from bacteria and floc grown on DOM derived from both plant species. These studies enhance our understanding of the importance of "bottom up" processes in the functioning of a marsh system and the potential role of photochemical processes in the turnover of primary carbon.

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References

Able, K.W., S.M. Hagan, and S.A. Brown. 2003. Mechanisms of marsh habitat alteration due to Phragmites: response of young-of-the-year mummichog (*Fundulus heteroclitus*) to treatment for *Phragmites* removal. *Estuaries* 26: 484–494.

Alber, M., and I. Valiela. 1994. Assimilation of organic aggregates by marine mussels. *Marine Biology* 121: 259–265. doi:10.1007/BF00346734.

Anesio, A.M., and W. Granéli. 2000. Bacterial growth on photochemically transformed leachates from aquatic and terrestrial primary producers. *Microbial Ecology* 40: 200–208.

Anesio, A.M., C.M.T. Denward, L.J. Tranvik, and W. Granéli. 1999. Decreased bacterial growth on vascular plant detritus due to photochemical modification. *Aquatic Microbial Ecology* 17: 159–165. doi:10.3354/ame017159.

Benoit, L.K., and R.A. Askins. 1999. Impact of the spread of *Phragmites* on the distribution of birds in Connecticut tidal marshes. *Wetlands* 19: 194–208.

Bushaw-Newton, K.L., and M.A. Moran. 1999. Photochemical formation of biologically available nitrogen from dissolved humic substances in coastal marine systems. *Aquatic Microbial Ecology* 18: 285–292. doi:10.3354/ame018285.

Chambers, R.M., L.A. Meyerson, and K. Saltonstall. 1999. Expansion of *Phragmites australis* into tidal wetlands of North America. *Aquatic Botany* 64: 261–273. doi:10.1016/S0304-3770(99)00055-8.

Coffin, R.B., J.P. Connolly, and P.S. Harris. 1993. Availability of dissolved organic carbon to bacterioplankton examined by

oxygen utilization. *Marine Ecology Progress Series* 101: 9–22. doi:10.3354/meps101009.

Engelhaupt, E., T.S. Bianchi, R.G. Wetzel, and M.A. Tarr. 2003. Photochemical transformations and bacterial utilization of high-molecular-weight dissolved organic carbon in a southern Louisiana tidal stream (Bayou Trepagnier). *Biogeochemistry* 62: 39–58. doi:10.1023/A:1021176531598.

Denward, C.M.T., J. Edling, and L. Tranvik. 1999. Effects of solar radiation on bacterial and fungal density on aquatic plant detritus. *Freshwater Biology* 41: 575–582. doi:10.1046/j.1365-2427.1999.00407.x.

Farjalla, V.F., A.M. Anesio, S. Bertilsson, and W. Granéli. 2001. Photochemical reactivity of aquatic macrophyte leachates: abiotic transformations and bacterial response. *Aquatic Microbial Ecology* 24: 187–195. doi:10.3354/ame024187.

Fell, P.E., S.P. Weissbach, D.A. Jones, M.A. Fallon, J.A. Zeppier, E.K. Faison, K.A. Lennon, K.J. Newberry, and L.K. Reddington. 1998. Does invasion of oligohaline tidal marshes by reed grass, *Phragmites australis* (Cav.) Trin. ex. Steud. affect the availability of prey resources of the mummichog, *Fundulus heteroclitus* L.? *Journal of Experimental Marine Biology and Ecology* 222: 59–77. doi:10.1016/S0022-0981(97)00138-X.

Filip, Z., and J.J. Alberts. 1988. The release of humic substances from *Spartina alterniflora* (Loisel.) into sea water as influenced by salt marsh indigenous microorganisms. *Science of the Total Environment* 73: 143–157. doi:10.1016/0048-9697(88)90425-1.

Harrison, P.J., R.E. Waters, and F.J.R. Taylor. 1980. A broad spectrum artificial seawater medium for coastal and open ocean phytoplankton. *Journal of Phycology* 16: 28–35.

Hobbie, J.E., R.J. Daley, and S. Jasper. 1977. Use of nucleopore filters for counting bacteria by epifluorescence microscopy. *Applied and Environmental Microbiology* 33: 1225–1228.

Huang, S.C., D.A. Kreeger, and R.I.E. Newell. 2003. Seston available as a food resource for the ribbed mussel (*Geukensia demissa*) in a North American, mid-Atlantic salt marsh. *Estuarine Coastal and Shelf Science* 56: 561–571. doi:10.1016/S0272-7714(02)00206-8.

Hullar, M.A.J., B. Fry, B.J. Peterson, and R.T. Wright. 1996. Microbial utilization of estuarine dissolved organic carbon: a stable isotope tracer approach tested by mass balance. *Applied and Environmental Microbiology* 62: 2489–2493.

Hunter, K.L., D.A. Fox, L.M. Brown, and K.W. Able. 2006. Responses of resident marsh fishes to stages of *Phragmites australis* invasion in three Mid Atlantic estuaries. *Estuaries and Coasts* 29: 487–498.

Keil, R.G., and D.L. Kirchman. 1994. Abiotic transformations of labile protein to refractory protein in sea water. *Marine Chemistry* 45: 187–196. doi:10.1016/0304-4203(94)90002-7.

Kieber, R.J., L.H. Hydro, and P.J. Seaton. 1997. Photooxidation of triglycerides and fatty acids in seawater: implication toward the formation of marine humic substances. *Limnology and Oceanography* 42: 1454–1462.

King, R.S., W.V. Deluca, D.F. Whigham, and P.P. Marra. 2007. Threshold effects of coastal urbanization on *Phragmites australis* (common reed) abundance and foliar nitrogen in Chesapeake Bay. *Estuaries and Coasts* 30: 469–481.

Kreeger, D.A., and R.I.E. Newell. 1996. Ingestion and assimilation of carbon from cellulolytic bacteria and heterotrophic flagellates by the mussels *Geukensia demissa* and *Mytilus edulis* (Bivalvia, Mollusca). *Aquatic Microbial Ecology* 11: 205–214. doi:10.3354/ame011205.

Kreeger, D.A., and R.I.E. Newell. 2000. Trophic complexity between primary producers and invertebrate consumers in salt marshes. In *Concepts and controversies in tidal marsh ecology*, eds. M.P. Weinstein, and D.A. Kreeger, 187–220. New York: Kluwer.

Kreeger, D.A., and R.I.E. Newell. 2001. Seasonal utilization of different seston carbon sources by the ribbed mussel, *Geukensia demissa* (Dillwyn) in a mid-Atlantic salt marsh. *Journal of Experimental Biology and Ecology* 260: 71–91. doi:10.1016/S0022-0981(01)00242-8.

Kreeger, D.A., C.J. Langdon, and R.I.E. Newell. 1988. Utilization of refractory cellulosic carbon derived from *Spartina alterniflora* by the ribbed mussel *Geukensia demissa*. *Marine Ecology Progress Series* 42: 171–179. doi:10.3354/meps042171.

Langdon, C.J., and R.I.E. Newell. 1990. Utilization of detritus and bacteria as food sources by two bivalve suspension-feeders, the oyster *Crassostrea virginica* and the mussel *Geukensia demissa*. *Marine Ecology Progress Series* 58: 299–310. doi:10.3354/meps058299.

McClary, M. 2004. *Spartina alterniflora* and *Phragmites australis* as habitat for the ribbed mussel, *Geukensia demissa* in Saw Mill Creek of New Jersey's Hackensack Meadowlands. *Urban Habitats* 2: 83–90.

Meyer, D.L., J.M. Johnson, and J.W. Gill. 2001. Comparison of nekton use of *Phragmites australis* and *Spartina alterniflora* marshes in Chesapeake Bay, USA. *Marine Ecology Progress Series* 209: 71–84. doi:10.3354/meps209071.

Miller, W.L., and R.G. Zepp. 1995. Photochemical production of dissolved inorganic carbon from terrestrial organic matter: significance to the oceanic organic carbon cycle. *Geophysical Research Letters* 22: 417–420. doi:10.1029/94GL03344.

Miller, W.L., and M.A. Moran. 1997. Interaction of photochemical and microbial processes in the degradation of refractory dissolved organic matter from a coastal marine environment. *Limnology and Oceanography* 42: 1317–1324.

Moran, M.A., and R.E. Hodson. 1989. Formation and bacterial utilization of dissolved organic carbon derived from detrital lignocellulose. *Limnology and Oceanography* 34: 1034–1047.

Moran, M.A., and R.E. Hodson. 1994. Dissolved humic substances of vascular plant origin in a coastal marine environment. *Limnology and Oceanography* 39: 762–771.

Moran, M.A., W.M. Sheldon Jr., and R.G. Zepp. 2000. Carbon loss and optical property changes during long-term photochemical and biological degradation of estuarine dissolved organic matter. *Limnology and Oceanography* 45: 1254–1264.

Osgood, D.T., D. Yozzo, R. Chambers, D. Jacobson, T. Hoffman, and J. Wnek. 2003. Tidal hydrology and habitat utilization by resident nekton in *Phragmites* and non-*Phragmites* marshes. *Estuaries* 26: 522–533.

Pan, J.F., and W.X. Wang. 2004. Differential uptake of dissolved and particulate organic carbon by the marine mussel *Perna viridis*. *Limnology and Oceanography* 49: 1980–1991.

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. doi:10.1126/science.227.4692.1361.

Raichel, D.L., K.W. Able, and J.M. Hartman. 2003. The influence of *Phragmites* (common reed) on the distribution, abundance, and potential prey of a resident marsh fish in the Hackensack Meadowlands, New Jersey. *Estuaries* 26: 51–521.

Roman, C.T., W.A. Niering, and R.S. Warren. 1984. Salt marsh vegetation change in response to tidal restriction. *Environmental Management* 8: 141–150. doi:10.1007/BF01866935.

Saltontall, K. 2002. Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. *Proceedings of the National Academy of Sciences* 99: 2445–2449. doi:10.1073/pnas.032477999.

Silliman, B.R., and M.D. Bertness. 2004. Shoreline development drives invasion of *Phragmites australis* and the loss of New England salt marsh plant diversity. *Conservation Biology* 18: 1424–1434. doi:10.1111/j.1523-1739.2004.00112.x.

Strome, D.J., and M.C. Miller. 1978. Photolytic changes in dissolved humic substances. *Internationale Vereinigung fur Theoretische und Angewandte Limnologie* 20: 1248–1254.

Talley, T.S., and L.A. Levin. 2001. Modification of sediments and macrofauna by an invasive marsh plant. *Biological Invasions* 3: 51–68. doi:10.1023/A:1011453003168.

Tranvik, L., and S. Kokalj. 1998. Decreased bioavailability of dissolved organic carbon of phytoplankton origin due to interactive effects of UV radiation and humic matter. *Aquatic Microbial Ecology* 14: 301–307. doi:10.3354/ame014301.

Turner, R.E. 1978. Community plankton respiration in a salt marsh estuary and the importance of macrophyte leachates. *Limnology and Oceanography* 23: 442–451.

United States Environmental Protection Agency (US EPA). 1997. Methods for the determination of chemical substances in marine and estuarine environmental matrices, 2nd edn. EPA/600/R-97/072. Cincinnati: United States Environmental Protection Agency.

Wainright, S.C., M.W. Weinstein, K.W. Able, and C.A. Currin. 2000. Relative importance of benthic microalgae, phytoplankton, and the detritus of smooth cordgrass *Spartina alterniflora* and the common reed *Phragmites australis* to brackish-marsh food webs. *Marine Ecology Progress Series* 200: 77–91. doi:10.3354/meps200077.

Wang, X.C., L. Litz, R.F. Chen, W. Huang, P. Feng, and M.A. Altabet. 2007. Release of dissolved organic matter during oxic and anoxic decomposition of salt marsh cordgrass. *Marine Chemistry* 105: 309–321. doi:10.1016/j.marchem.2007.03.005.

Warren, R.S., P.E. Fell, J.L. Grimsby, E.L. Buch, G.C. Rilling, and R.A. Fertik. 2001. Rates, patterns, and impacts of *Phragmites australis* expansion and effects of *Phragmites* control on vegetation, macroinvertebrates, and fish within tidal wetlands of the lower Connecticut River. *Estuaries* 24: 90–107. doi:10.2307/1352816.

Weinstein, M.P., and J.H. Balletto. 1999. Does the common reed, *Phragmites australis*, affect essential fish habitat? *Estuaries* 22: 793–802. doi:10.2307/1353112.

Zar, J.H. 1999. Biostatistical analysis, 4th edn. Upper Saddle River: Prentice-Hall.