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MACROALGAL AND SEAGRASS DIETS ALTER EPIBIOTIC BACTERIAL COMMUNITIES ON THE BLUE CRAB *CALLINECTES SAPIDUS* AND THE AMERICAN LOBSTER *HOMARUS AMERICANUS*

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ABSTRACT Bacteria in the genus *Vibrio* can be opportunistic pathogens for organisms in marine and estuarine systems. Consumption of seagrass by the blue crab *Callinectes sapidus* or macroalgal kelp by the lobster *Homarus americanus* reduced epibiotic bacteria in the *Vibrio* genus. Bacterial *Vibrio* spp. densities were estimated by colony growth on thiosulfate citrate bile sucrose agar. Nonspecific epibiotic bacterial densities were estimated by colony growth on nonselective agar or by direct counting under a fluorescence microscope. Consumption of the seagrass *Ruppia maritima*, but not the aquatic plant *Stukenia pectinata* or a control diet, reduced *Vibrio* spp. densities on blue crabs. A diet containing the kelp *Saccharina latissima* reduced *Vibrio* spp. densities on lobsters compared with a control diet. Results for nonspecific bacterial densities were mixed. A reduction was observed when cultured colonies were counted but not when cells were counted directly. The results suggest that diets containing seagrass or kelp can reduce epibiotic bacterial densities of some but not all bacterial species. This reduction may involve the utilization of antibacterial compounds obtained through diet and could enhance animal health by inhibiting growth of potential pathogens.

KEY WORDS: blue crab *Callinectes sapidus*, American lobster *Homarus americanus*, seagrass, kelp, *Vibrio*, crustacean

INTRODUCTION

Bacteria in the genus *Vibrio* are ubiquitous in marine and estuarine systems. They are associated with many fishes and crustaceans and can be opportunistic pathogens (Chatterjee & Haldar 2012, Novriadi 2016). Epibiotic *Vibrio* species have long been implicated in crustacean chitinolytic shell disease (Getchell 1989, Vogan et al. 2002), although more complex microbiome studies have not observed a *Vibrio* effect (Feinman et al. 2017). Some species can also cause internal infections, usually termed vibriosis (Krantz et al. 1969, Bowser et al. 1981, Welsh & Sizemore 1985, Tall et al. 2003, Novriadi 2016).

Vibriosis and chitinolytic shell disease are common in aquaculture systems, where they can cause significant mortality (Delves-Broughton & Poupard 1976, Sindermann 1989). These problems are well described in shrimp aquaculture (Lavilla-Pitogo & de la Peña 1998, Aguirre-Guzmán et al. 2004). Occurrences of *Vibrio* infections have also been documented in crab (Jithendran et al. 2010) and *Panulirus* spp. lobster aquaculture (Goulden et al. 2012). Treatment for *Vibrio* infections typically include control through water management and sanitation, antibiotics, or the introduction of probiotic organisms (Kesarcodi-Watson et al. 2008, Cruz et al. 2012).

Growth of *Vibrio* spp. bacteria could be modulated by the presence of autotrophs, as many marine autotrophs exhibit antimicrobial activity. This has been documented for marine macroalgae, including the kelp family Laminariaceae (Hornsey & Hide 1974, Rosell & Srivastava 1987, Dubber & Harder 2008, Cox et al. 2010, Gerasimenko et al. 2010, Kadam et al. 2015). In aquatic vascular plants, antibacterial (Harrison & Chan 1980, Bernard & Pesando 1989, Devi et al. 1997, Bhosale et al. 2002), antifungal (Ballesteros et al. 1992, Jensen et al. 1998), antialgal (DellaGreca et al. 2000) and antiviral (Premnathan et al. 1992) activities have been described. Bushmann and Ailstock (2006) found antibacterial activity in three aquatic plant species from the Chesapeake Bay. All three species generally showed activity against Gram-positive and a few Gram-negative bacteria. One species, the seagrass *Ruppia maritima* L., inhibited growth of *Vibrio parahaemolyticus* (Fujino et al. 1951). The oligohaline aquatic plants *Stukenia pectinata* (L.) and *Potamogeton perfoliatus* L. were ineffective.

Marine autotrophs could impact the health of larger organisms by suppressing pathogenic bacteria. The blue crab (*Callinectes sapidus* Rathbun, 1896) and the American lobster (*Homarus americanus* Milne-Edwards, 1837) are omnivores and include both algae and vascular plants in their diets (Ennis 1973, Scarratt 1980, Elner & Campbell 1987, Rosas et al. 1994, Sainte-Marie & Chabot 2002, Meise & Stehlik 2003, Reichmuth et al. 2009). Feeding studies with adult and juvenile lobsters have shown that diets of fish alone, without algal supplement, accelerate the onset of bacterial shell disease (Prince et al. 1995, Tlusty et al. 2008). In preliminary studies, consumption of the kelp *Saccharina latissima* (L.) and the seagrass *Zostera marina* L. appeared to reduce the density of epibiotic *Vibrio* spp. in adult lobsters (Bushmann & Ailstock 2007, 2008).

The studies presented here examined how consumption of seagrasses by blue crabs and kelp by lobsters impacted the growth of epibiotic *Vibrio* spp. bacteria in closed aquaculture systems. Although not widely used in aquaculture, these crustaceans are economically important along the western Atlantic coast and are vulnerable to *Vibrio* spp. infections. The autotrophs were chosen because they are abundant in the habitats of these crustaceans and are consumed. Furthermore, the seagrasses can be grown under axenic conditions in the laboratory, allowing evaluation of plant consumption effects

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without the associated epibiotic microorganisms that exist in wild-collected specimens.

MATERIALS AND METHODS

Crab Study

Thirty mature male blue crabs (125–155 mm carapace width) were collected by trap from the Magothy River, a sub-estuary of the Chesapeake Bay. The chelae of each animal were closed with cyanoacrylate glue to prevent damage or cannibalism during the study. Crabs were then placed into a 2,250 L recirculating seawater system composed of three 750 L tanks. Water from each tank drained into a common wet/dry filter and mixed before return. Salinity was 15 ppt and temperature ranged from 20°C to 24°C.

The seagrass *Ruppia maritima* and the aquatic plant *Stukenia pectinata* were used in treatment diets. Both species were grown in axenic culture systems (Ailstock et al. 1991). Sterile explants of *R. maritima* were anchored in 70 mL jars containing Murashige minimal organic medium (Huang & Murashige 1976) solidified with 6 g/L agar and covered with 40 mL sterile acid-washed sand. These jars were placed in sterile 1-L jars containing 750 mL minimal salts solution. Sterile ambient air was bubbled into each culture jar. Explants were placed under constant light (70 $\mu mol \cdot m^{-1} \cdot s^{-1}$). Sterile explants of *S. pectinata* were placed in 150-mL culture tubes with 20 mL Murashige Shoot Multiplication Medium B (pH 5.0) (Huang & Murashige 1976) supplemented with 10 g/L sucrose. Plants in both systems were grown for 4–6 wk under constant light. All plant material was then removed from their jars or tubes, briefly washed in deionized water, dried at 40°C, and stored at –20°C until use.

The base food consisted of a 1:1 mix of freeze-dried ground krill *Euphausia superba* and marine flake food (Omega Sea Ltd.). The flake food did not contain plant or kelp material. To produce a treatment diet, seagrass was substituted for 20% w/w of base food. All diet components were mixed and ground in a food processor, packaged in 2% agar, and fed as 5% of body weight every other day. Crabs were divided into three ($n = 10$) treatment groups: a diet containing *Ruppia maritima*, a diet containing *Stukenia pectinata*, or a control diet containing only the base food.

All animals were initially assayed for epibiotic bacterial density and assayed again after 30 days of consuming their respective diets. To estimate epibiotic bacterial densities, a sterile tissue (Kimwipe; VWR International) was first held lightly against the dorsal carapace for 5 sec to remove water droplets. A sterile swab (Critical swab; VWR International) was then held perpendicular to the animal and drawn along the carapace width, measured from each lateral spine tip. The swab in this way followed the carapace width measurement. A second swab followed the track of the first. A preliminary study using juvenile lobsters determined that swabbing twice resulted in a mean removal of approximately 98% of the culturable bacteria from the swabbed area. Both swab heads were then vortexed for 30 sec in 5 mL of a sterile 3% NaCl solution. To estimate *Vibrio* spp. densities, 100 µL of this solution was spread onto four thiosulfate citrate bile sucrose (TCBS) agar plates and incubated for 24 h at 28°C. This medium is considered selective for *Vibrio* spp. (Kobayashi et al. 1963). Species in this genus produce either yellow or blue colonies, depending on their ability to ferment sucrose. After incubation, presumptive *Vibrio* spp. colonies were counted. A preliminary study determined the mean width of a swab track to be 3.6 mm ± 0.05 SE ($n = 10$). This mean width was used with the carapace length to calculate bacterial densities, expressed as colony forming units/mm^2.

Nonspecific bacterial densities were also estimated for each animal. Four 25-µL portions of the swab solution were spread onto petri plates containing nutrient agar with 3% NaCl (NA/NaCl) and incubated at 28°C for 24 h. Nonspecific bacterial densities were then calculated as with the estimates of *Vibrio* spp. This method did not allow estimation of absolute densities, as it detected only bacteria culturable on the NA/NaCl media, but it allowed comparison between the treatment groups.

Each crab was also examined for bacteria in the hemolymph. Immediately after each swab assay, 100 µL hemolymph was withdrawn from the base of the fifth periopod of all animals. Fifty µL hemolymph was then spread onto a NA/NaCl and a TCBS agar plate. The plates were incubated at 28°C for 24 h and examined for bacterial colonies.

Lobster Studies

All animals in the lobster studies were juveniles (26–41 mm carapace length) grown in the lobster rearing facility at the New England Aquarium (Boston, MA). Animals selected for each study were placed into a 1,200-L recirculating seawater system and held separately in 10 × 20 cm clear plastic containers with holes to allow water flow. All animals were housed in the same tank and thus the same water volume. System water was held at 30 ppt salinity and 18°C. Lights above the tank were set to a 14:10 light cycle.

Test animals were fed a base diet for 1 mo before treatment. This consisted of a commercial feed (Economac; Aquafauna Bio-Marine) mixed with 10% bone meal and 0.5% astaxanthin (Naturose; Aquafauna Bio-Marine). The dry material was held together with 2% agar and fed as 5% of lobster body weight every other day. The base diet did not contain plant or kelp material.

Lobster Study 1

After 1 mo of base diet, 22 animals were selected for the study. All animals were swabbed to estimate initial bacterial densities. The protocol was identical to that described for blue crabs, except the swabs followed a line from the tip of the rostrum to the posterior edge of the cephalothorax. Nonspecific and *Vibrio* spp. bacterial densities were estimated after growth on NA/NaCl and TCBS media, respectively. After the initial estimate, 11 animals were randomly selected to remain on the base diet. The other 11 animals received a treatment diet consisting of the base food with 20% w/w replaced with *Saccharina latissima*. The kelp was collected before the experiment on Mount Desert Island, ME. Collected sporophyte blades were washed with sterile water, dried at 40°C, ground into a powder, and held at –20°C until use. The treatment continued for 30 days. At the end of this period, all animals were again assayed for nonspecific and *Vibrio* spp. bacterial density.

Lobster Study 2

Twenty different juvenile lobsters were fed the base diet for 1 mo. All the animals were then challenged by the addition of *Vibrio parahaemolyticus* to the system. Lawns

of *V. parahaemolyticus* (ATCC 35177) were grown on NA/NaCl plates at 28°C for 48 h. Three plates were then washed into the tank system using a sterile swab. This produced an initial *V. parahaemolyticus* concentration in the system of approximately 22 cells/L based on plating tank seawater on TCBS agar. After 1 mo, all animals were swabbed and initial bacterial densities estimated. Only colonies showing the characteristic blue color and morphology of *V. parahaemolyticus* on TCBS agar were included in the *Vibrio* spp. estimation. Nonspecific bacterial densities were measured directly using fluorescence microscopy. After swabbing, 20 μL was removed (2×) from the 3% NaCl swab solution and placed in a sterile tube with an equal volume of Baclight Live/Dead fluorescent dye mix (Invitrogen). This contained the dyes SYSTO 9 and propidium iodide. After incubation, two 7-μL aliquots from each tube were placed on slides and examined under a Nikon Diaphot 300 inverted fluorescence microscope. Bacteria with intact cell walls (live) fluoresced green; those with damaged cell walls (dead) fluoresced red. Fluorescing green cells were counted as living bacteria. Five fields were chosen randomly and counted for each slide, resulting in 10 fields for each animal. Bacterial densities were calculated as cells/mm^2 for each animal. A comparison of the direct count and culturing methods using a *V. parahaemolyticus* serial dilution produced similar regression lines, with the direct count method producing densities higher by two orders of magnitude.

After initial bacterial density determination, the lobsters were randomly divided into two groups of 10. The first group remained on the base diet. The second group was fed a treatment diet containing *Saccharina latissima* as in the first lobster study. Densities for *Vibrio* spp. and nonspecific bacteria were estimated 30 and 60 days after the start of the treatment and base diets.

In Vitro *Studies*

The activity of *Saccharina latissima* and *Stukenia pectinata* tissue against *Vibrio parahaemolyticus* was tested *in vitro*. Five sterile culture tubes were prepared, containing 1 mL of 0.25 strength nutrient broth with 3% NaCl (NB/NaCl). Each tube received either 0.0, 0.025, 0.05, 0.075, or 0.10 g of dried, powdered *S. latissima*. A second set of five tubes were prepared in the same manner, except they received dried, powdered *S. pectinata*. A *V. parahaemolyticus* culture was prepared by inoculating 0.25 strength NB/NaCl and growing the culture overnight at 28°C. This culture was serial diluted and two 25-μL aliquots from each dilution were grown on NA/NaCl to determine the initial bacterial concentration. The tubes containing *S. latissima* or *S. pectinata* were inoculated with 10 μL of the bacterial culture and incubated overnight at 28°C. After incubation, the broth in each tube was serially diluted and two 25-μL aliquots from each dilution were grown on NA/NaCl to determine the final bacterial concentrations. Comparisons of the initial and final concentrations allowed a determination of bacterial growth.

The antibacterial activity of *Saccharina latissima* was examined in a disk diffusion assay. Polar and nonpolar fractions were prepared from 10 g of dried and powdered sporophyte blades using a published protocol (Bushmann & Ailstock 2006). The final polar methanol or nonpolar chloroform extracts represented 2 g initial dried tissue/mL solvent. These extracts were applied to sterile paper disks, dried, and applied to newly spread bacterial lawns of *Vibrio parahaemolyticus* on nutrient agar with 3% NaCl (NA/NaCl). For comparison, three medical bacteria were also tested on nutrient agar: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), and *Micrococcus luteus* (ATCC 4698). Three disks were prepared for each test and applied to a single plate. All plates were incubated for 24 h at 28°C. Sensitivity to the extract was assessed by determining any growth inhibition surrounding a disk compared with a solvent control disk. Inhibition was measured from the disk edge to the beginning of bacterial growth at four equidistant points around the disk perimeter.

The antibacterial property of lobster hemolymph was assayed. Twenty-two juvenile lobsters (carapace length 20–25 mm, not used in either of the prior diet studies) were divided into two groups of 11. These animals had been fed the lobster base diet for several months. Hemolymph (130 μL) was removed from a sinus at the base of the fifth periopod of each animal using a sterile 1-mL syringe. Syringes were preloaded with 20 μL citrate anticoagulant (Tanner et al. 2006). Hemolymph was centrifuged at 400 g for 10 min to pellet cells. Supernatant (100 μL) was removed into sterile tubes. A 20-μL aliquot of this hemolymph was plated onto TCBS and NA/NaCl plates to detect any preexisting bacterial infection. These plates were incubated overnight at 25°C. The remaining hemolymph received 10 μL sterile 30 g/L NaCl water containing a suspension of *Vibrio parahaemolyticus*. Bacterial concentration of this suspension was estimated separately by serial dilution and plating onto NA/NaCl agar. All 22 tubes were then incubated overnight at 25°C. The hemolymph was then serial diluted and all dilutions were plated 2× onto TCBS and NA/NaCl plates. These plates were incubated overnight at 25°C and counted. TCBS plate counts were used to estimate hemolymph bacterial concentration. Counts of NA/NaCl plates were compared with those from TCBS to ensure that the measured growth was from *Vibrio* spp., rather than a contaminant. After these initial measurements were taken, 10 animals continued the lobster base diet. A second set of 10 animals were fed a treatment diet with 50% w/w Economac replaced with ground, dried *Saccharina latissima*. All animals were fed daily for 2 wk. Hemolymph was then tested as before for all animals.

RESULTS

Crab Study

Diet affected the bacterial densities (cultured on nonselective NA/NaCl) on the dorsal carapace of blue crabs fed one of two seagrass treatment diets or a base diet control (Fig. 1A: ANOVA, $F = 6.38$, $P = 0.01$). All statistical tests were performed using GraphPad Prism version 6 (GraphPad Software, La Jolla, CA). Animals fed *Stukenia pectinata* showed significant increases in bacteria over a 30-day period (paired $t = 3.58$, $P = 0.01$). Bacterial densities for animals fed the control and *Ruppia maritima* diet were not significantly different before and after treatment (control: paired $t = 1.97$, $P = 0.06$; *R. maritima*: paired $t = 1.42$, $P = 0.19$).

Examination of *Vibrio* spp. densities showed significant differences between treatment groups (Fig. 1B: ANOVA, $F = 4.44$, $P = 0.01$). Animals fed the base or *Stukenia pectinata* diet showed

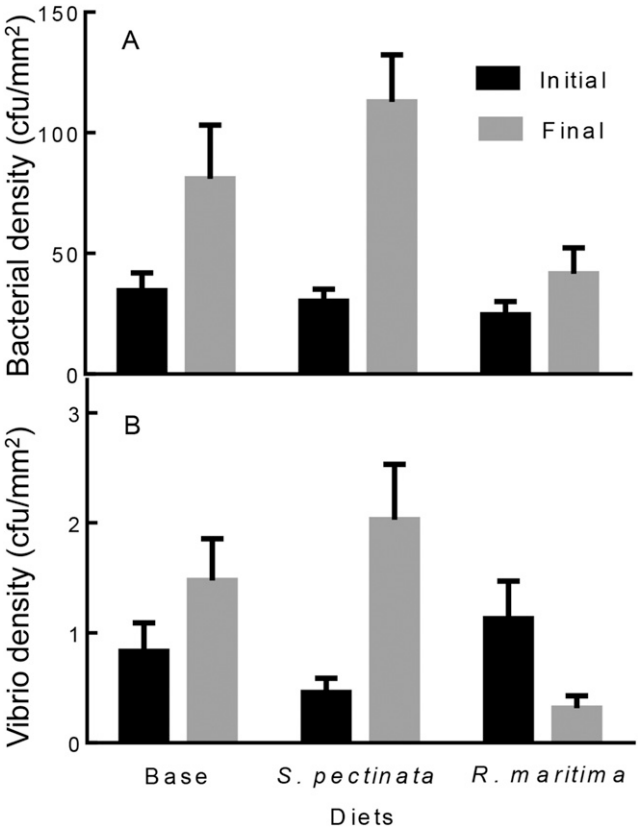

Figure 1. Epibiotic bacterial densities of blue crabs before treatment (initial) and after 30 days of a base diet, a diet containing *Stukenia pectinata*, or a diet containing *Ruppia maritima* (final). (A) Bacterial densities determined by culturing on nonselective media (NA/NaCl). (B) Presumptive *Vibrio* spp. bacterial densities determined by culturing on TCBS media.

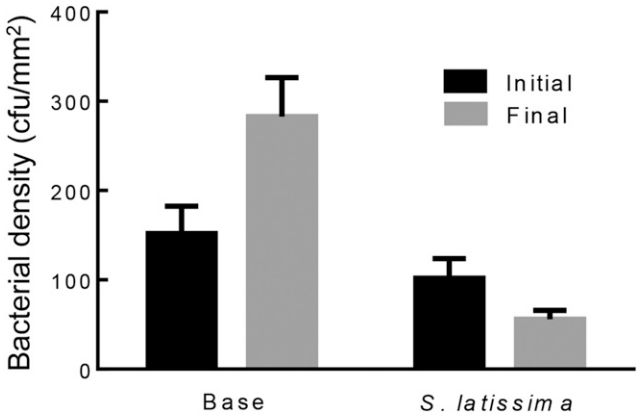

Figure 2. Epibiotic bacterial densities of juvenile lobsters before treatment (initial) and after 30 days of a base diet or a diet containing *Saccharina latissima* (final). Bacterial densities were determined by culturing on nonselective media (NA/NaCl).

significant increases over the test period (paired $t = 2.40$, $P = 0.04$; paired $t = 3.23$, $P = 0.02$ respectively), whereas *Vibrio* spp. density for the *Ruppia maritima* group declined (paired $t = 2.20$, $P = 0.04$). At the end of the test period, *Vibrio* spp. densities for both the base diet and *S. pectinata* groups were significantly higher than the *R. maritima* group ($t = 3.21$, $P = 0.01$; $t = 3.85$, $P = 0.01$).

Hemolymph collected from each animal after swabbing was incubated on nonselective NA/NaCl and TCBS agar. Bacteria were cultured from the hemolymph on NA/NaCl, but there were no significant differences between groups before or after treatments. No bacteria were detected in the hemolymph of any animal when incubated on TCBS agar.

Lobster Study 1

Juvenile lobsters fed a base diet or a treatment diet containing *Saccharina latissima* showed significant differences in carapace bacterial densities determined by growth on Na/NaCl (Fig. 2: ANOVA, $F = 10.90$, $P = 0.01$). Initial base and treatment densities were not significantly different. However, at the end of the 30-day treatment period, bacterial densities from animals fed the base diet were significantly higher than those fed the kelp diet ($t = 5.09$, $P = 0.01$). Both initial and final *Vibrio* spp. densities, estimated by growth on TCBS media, were very low. Initial mean plate counts were less than 20 CFU in 21 of 22 animals, and less than five CFU in 16 animals. These counts were considered too low for meaningful comparison (Maturin & Peeler 2001).

Lobster Study 2

After the *Vibrio parahaemolyticus* challenge, mortality was greater in the control group. There were initially 10 animals in both treatment groups. By the 30-day measurement, four animals in the base diet group had died ($n = 6$), and by the 60-day measurement nine animals had died ($n = 1$). In comparison, for the animals receiving the *Saccharina latissima* diet, one animal had died by the 30-day measurement ($n = 9$), and three animals had died by the 60-day measurement ($n = 7$). The ratios of live and dead animals were not significantly different between diet treatments on day 30, but by day 60 these ratios were different (Fisher exact test, two-tailed $P = 0.02$).

There was no statistically significant difference between treatment groups when nonspecific bacterial densities were determined by direct cell counts on a fluorescence microscope (Fig. 3A). There was a statistically significant difference between treatment groups when *Vibrio* spp. colonies were counted on TCBS media (Fig. 3B: ANOVA, $F = 5.20$, $P = 0.01$). Animals receiving the base diet showed significant increases in *Vibrio* spp. densities between the initial and 30-day measurements (paired $t = 3.16$, $P = 0.03$). Animals fed the *Saccharina latissima* diet showed no such increase. By the 30-day measurement, *Vibrio* spp. densities were significantly lower in the *S. latissima* treatment group compared with the base diet controls ($t = 2.63$, $P = 0.02$). By the 60-day measurement, *Vibrio* spp. densities for animals receiving the *S. latissima* diet had decreased significantly compared with the 30-day measurement (paired $t = 2.55$, $P = 0.04$). Comparisons with the base diet control group were not possible at 60 days, as all but one control animal had died. The remaining lobster had very high nonspecific and *Vibrio* spp. bacterial densities (169,085 cfu/mm^2 and 44.7 cfu/mm^2, respectively).

In Vitro *Studies*

Addition of 25 mg/mL *Saccharina latissima* or *Stukenia pectinata* produced a decline in the *in vitro* growth of *Vibrio*

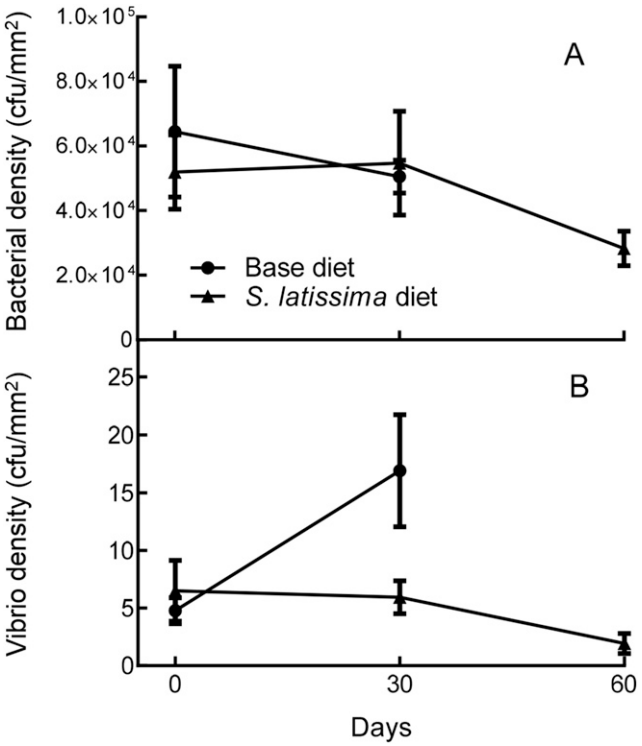

Figure 3. Epibiotic bacterial densities for juvenile lobsters presented with a *Vibrio parahaemolyticus* challenge while consuming either a base diet or a diet containing *Saccharina latissima*. Bacterial densities were estimated 0, 30, and 60 days after the challenge began. (A) Bacterial densities determined by direct counts of stained fluorescing bacteria using a fluorescence microscope. (B) Presumptive *Vibrio* spp. bacteria determined by culturing on TCBS media.

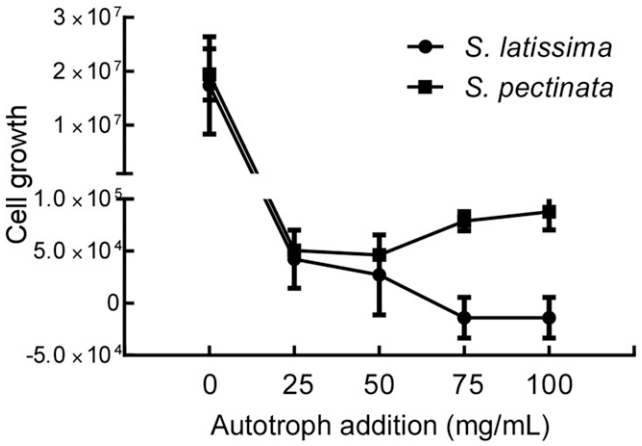

Figure 4. Cell growth of *Vibrio parahaemolyticus* with either *Stukenia pectinata* or *Saccharina latissima* added to the growth medium (NB/NaCl).

parahaemolyticus of almost three orders of magnitude (Fig. 4). At higher concentrations, *S. pectinata* did not further inhibit growth. Higher concentrations of *S. latissima* did inhibit bacterial growth, with no living cells detected in the growth media at 75 and 100 mg/mL. Linear regression slopes, using the 25–100 mg/mL data points, were significantly different between *S. latissima* and *S. pectinata* (ANCOVA: $F = 24.87$, $P = 0.01$).

Extracts of *Saccharina latissima* showed antibacterial activity against *Vibrio parahaemolyticus*, with the polar fraction producing slightly larger clearing zones (Table 1). The extracts were most effective against the two Gram-positive bacteria *Staphylococcus aureus* and *Micrococcus luteus*. For these bacteria, the nonpolar fraction produced larger clearing zones. The Gram-negative bacterium *Escherichia coli* was unaffected by the *S. latissima* extract.

Before treatment, there was no significant difference between groups in growth of *Vibrio parahaemolyticus* in lobster hemolymph (Fig. 5). After 2 wk of treatment diet, bacterial growth in hemolymph from lobsters consuming the *Saccharina latissima* diet was significantly lower compared with growth in hemolymph from lobsters consuming the base diet ($t = 2.35$, $P = 0.03$). For both diets, growth in post-treatment hemolymph was significantly higher compared with pre-treatment growth (base diet: $t = 8.05$, $P = 0.01$; *S. latissima* diet: $t = 2.71$, $P = 0.02$).

DISCUSSION

These studies demonstrate that consumption of seagrass or kelp can alter the epibiotic bacterial communities of both *Callinectes sapidus* and *Homarus americanus*. Densities of *Vibrio* spp. were reduced in blue crabs through consumption of *Ruppia maritima* and lobsters through consumption of *Saccharina latissima*. In the blue crab study, reductions were observed in both yellow and blue colonies. This suggests that multiple *Vibrio* species were sensitive to the treatment. The culturing medium, TCBS agar, allows the weak growth of some other bacterial genera, such as *Pseudomonas* and *Aeromonas*. Identification of colonies as *Vibrio* spp. must therefore be considered presumptive. However, a short 24-h incubation time and inclusion of only well-defined colonies make it likely that the colonies observed were primarily those in the *Vibrio* genus.

Changes in bacterial densities were less consistent when measured by growth on nonselective media or direct counts. In blue crabs, plate counts showed that the *Stukenia pectinata* diet was associated with a significant increase in epibiotic bacteria but the *Ruppia maritima* and base diets were not. In lobsters, *Saccharina latissima* consumption was associated with reduced bacterial densities when determined by plate counts. Direct counts of fluorescing bacteria were much higher and were unaffected by treatment. Taken together, the results suggest that the diet treatments affected a subset of the epibiotic population that included at least some *Vibrio* species. The culturing method produced an undercount of the epibiotic population and may have selected for more sensitive species.

Bacteria were collected from the dorsal carapace by swabbing. This technique could not determine absolute densities, as

TABLE 1.

Disk diffusion assay using polar and nonpolar extracts of *Saccharina latissima*.

Bacteria	Nonpolar (mm)	Polar (mm)
Vibrio parahaemolyticus	0.88 ± 0.13	1.04 ± 0.11
Staphylococcus aureus	5.83 ± 0.17	2.13 ± 0.06
Micrococcus luteus	4.67 ± 0.25	1.29 ± 0.21
Escherichia coli	0	0

Zones of inhibition were measured at four equidistant points for each disk ($n = 3$) from disk edge to the beginning of bacterial lawn. Figures represent mean inhibition zone ± SE of the mean.

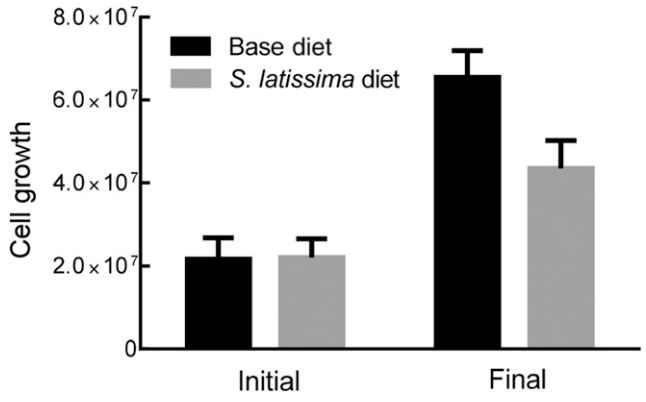

Figure 5. Cell growth of *Vibrio parahaemolyticus* in lobster hemolymph. Hemolymph was collected from lobsters fed a base diet (initial), then collected again after 2 wk of continued base diet or a diet containing *Saccharina latissima* (final).

it undoubtedly picked up bacteria in water clinging to the carapace after blotting and many *Vibrio* species do not culture (Colwell et al. 1985). However, as all animals were swabbed identically and held either in the same tank or in the same system, it was possible to compare bacterial densities between treatments. Another source of variation was the width of the track produced by a swab head (3.26 mm ± 0.05 SEM). Although this likely contributed to the variability in calculated bacterial densities, the variability in swab track width was insufficient to account for the difference in bacterial densities observed between treatment groups.

It is possible that bacterial densities were reduced through action of antibacterial compounds present in the autotrophs used in the treatment diets. The *in vitro* and disk diffusion studies showed *Saccharina latissima* activity against *Vibrio parahaemolyticus*. Similar results have been reported for *Ruppia maritima* (Bushmann & Ailstock 2006). At least for *R. maritima*, any treatment effects must have originated in the plant rather than an epiphyte, as only axenic plants were used. The ineffectiveness of *S. pectinata* suggests that the observed bacterial reductions were not a general nutritional effect of adding plants to the diet. Utilization of plant-derived compounds is well known in insects (rev. in Duffey 1980) but has not been described in marine arthropods. Marine crustaceans are capable of utilizing plant compounds, as they acquire the carotenoid astaxanthin through diet (D'Abramo et al. 1983, Tlusty & Hyland 2005) and transport this compound to the cuticle where it is complexed with proteins to produce red, yellow, or blue color patterns.

If crabs or lobsters acquire antimicrobial compounds through diet, those compounds should be at least transiently present in the hemolymph. Hemolymph from lobsters fed a *Saccharina latissima* diet showed reduced growth of *Vibrio parahaemolyticus* compared with hemolymph from animals fed a base diet. Post-treatment growth was elevated for both diets compared with pre-treatment. This was probably caused by a known mechanical failure in the incubator such that all post-treatment hemolymph was incubated 4°C higher than pre-treatment hemolymph. This prevented a comparison of pre- and post-treatment growth. However, there was no significant difference between groups in pre-treatment animals. After treatment, bacterial growth in *S. latissima* diet hemolymph was significantly less than growth in hemolymph from animals fed a base diet.

It is possible that treatment differences were influenced by antibacterial compounds directly contacting epibiotic bacteria during feeding, although the studies were designed to limit this effect. Blue crab treatment groups were in separate tanks but all tanks were part of a single closed recirculating system. Lobsters were housed in individual containers but all containers were held within the same tank. However, blue crabs and lobsters are messy eaters, with bits of food often caught in the gill current. It is possible that a transient buildup of antibacterial compounds around the animals during feeding affected epibiotic bacterial densities.

These results may have application in closed aquaculture systems. Reduction in *Vibrio* spp. densities may lessen the likelihood of associated diseases such as chitinolytic shell disease and vibriosis and therefore improve health and survivorship for the consuming organism. This control method has several advantages. Autotrophs are a natural dietary component and likely provide nutritional benefit in addition to any bacterial inhibition. They can be cultured in an aquaculture facility, eliminating the need for field collection and removing the risk of introducing pathogens or contaminants. Replacing a portion of the diet with macroalgae or plant material may reduce costs. Syslo and Hughes (1981) showed that up to 50% of animal protein could be replaced with plant material in lobster diets without affecting growth.

The kelp and seagrass species used in these studies are consumed by blue crabs and lobsters in natural ecosystems. The bacterial reductions observed in the laboratory are, therefore, likely to occur in wild populations. The importance of autotrophs as producers in coastal food webs is well established. The studies presented here suggest that some macroalgae and marine plants may have additional impacts in coastal ecosystems. A recent field study found that seagrass meadows contain lower densities of some bacterial pathogens, compared with un-vegetated areas (Lamb et al. 2017). Benthic autotrophs, particularly seagrasses, have declined in many areas (Orth & Moore 1984, Short & Burdick 1996). Their loss may have further impact if they play a role in controlling bacteria and bacterial disease in organisms associated with seagrass and kelp habitats.

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