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# Kelp forest size alters microbial community structure and function on Vancouver Island, Canada

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**Abstract.** Bacteria are ubiquitous and important components of marine ecosystems, yet the interaction between bacteria and higher trophic levels remain poorly understood. The trophic cascade involving sea otters, urchins, and kelp in the North Pacific is a classic case of altered ecosystem states; however, its impacts on microbial communities are unknown. We investigated the response of microbial communities to variation in kelp abundance between regions with and without sea otter populations along the west coast of Vancouver Island, British Columbia, Canada. We compared bacterial community structure and function between regions with large and small kelp forests, including an subset of the bacterial community that produces alginate lyase, which allows direct utilization of kelp carbon. The abundance and activity of alginate-lyase-producing bacteria were 3.2 and 1.4 times higher, respectively, in the region with large kelp forests, and declined rapidly with increasing distance from kelp. Total bacterial abundance was 2.7 times greater, and bacteria grew faster and experienced more zooplankton grazing and viral-mediated mortality in the presence of large kelp forests. These patterns suggest that larger kelp forests produce more detritus and dissolved organic matter, which stimulate microbial activity. Our results indicate that variation in kelp forest size alters the community structure and productivity of microbes and contributes to the growing evidence that top predators interact with microbes and ecosystem processes through a cascade of indirect effects.

**Key words:** *alginate lyase; bacteria; kelp; Macrocyctis pyrifera; microbes; North Pacific; sea otters; trophic cascades; viruses.*

## INTRODUCTION

Bacteria are the most numerically abundant organisms in oceans, where they play essential roles in species interactions and ecosystem processes. Between  $10^5$  and  $10^9$  bacterial cells are found in every milliliter of seawater (Azam et al. 1983, Suttle 2005), and their biomass exceeds that of all marine zooplankton and fish combined (Pomeroy et al. 2007). Bacteria cycle energy and nutrients through ecosystems via trophic interactions, decomposition of detritus, and re-mineralization of organic matter. They are also readily consumed by micrograzers such as protozoans or infected by viruses (Pomeroy 1974, Azam et al. 1983, Hennes et al. 1995, Suttle 2007), and thus play important roles in trophic transfer, recycling of nutrients, and the export of detritus to the sediments (Fuhrman 1999, Pomeroy et al. 2007). Despite their central role in marine ecosys-

tems, the interactions of microbes with higher trophic levels and their environment remain poorly understood.

Human activities have transformed marine ecosystems through alterations of nutrient cycles, thermal structure and stratification, acidification, the introduction of nonnative species, and the extirpation of exploited species, particularly large apex predators (Halpern et al. 2012). The removal of top predators is among the most pervasive perturbations to marine ecosystems (Estes et al. 2011). Hunting, fishing, and other human activities have caused top predatory fishes and marine mammals to become locally extinct (Pauly et al. 1998, Baum and Worm 2009, Field et al. 2009). The removal of these predators can drastically alter energy and nutrient flow through ecosystems as top-down effects cascade through lower trophic levels (Paine 1980, Frank et al. 2005, Wilmsers et al. 2012).

A classic example of ecosystem alterations associated with the loss of a top predator is the trophic cascade involving sea otters, urchins, and kelp in the North Pacific (Estes and Palmisano 1974). The fur trade in the 1800s effectively removed sea otters (*Enhydra lutris*) from Alaska to Mexico. Sea otters voraciously consume invertebrates such as sea urchins, bivalves, snails, mussels, and crabs (Estes et al. 1982). In the absence of sea otters, grazing urchins are released from predation and their abundance and consumption rates increase dramatically, creating urchin barrens where

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kelp (order Laminariales) are sparse or absent (Estes and Duggins 1995, Watson and Estes 2011). Kelp forests are among the most productive ecosystems in the world (Mann 1973, Steneck and Sala 2005) and play major roles in creating habitat and supporting secondary production through the consumption of living and dead kelp tissues (Duggins et al. 1989). Nutrient cycling and ecosystem productivity are both affected by sea otter removal (Schmitz et al. 2010, Wilmers et al. 2012). However, the response of microbial communities to variation in kelp abundance driven by sea otters, and the role of microbes in determining the fate of kelp productivity, remain unknown.

To examine the potential effect of sea otters on bacteria via alternations in kelp forest size, we took advantage of the variation in the presence of sea otters along the west coast of Vancouver Island, British Columbia, Canada. Between 1969 and 1972, 89 sea otters were reintroduced into Checleset Bay (50.03° N, 127.10° W) at the northern end of Vancouver Island (Bigg and MacAskie 1978). In the past 40 years, the sea otter population has grown by 30-fold from 89 to 2673 (2001 estimate from Nichol et al. 2005), spreading >100 km south to Clayoquot Sound (49.10° N, 125.82° W; Watson and Estes 2011). Although sea otters were historically present along the entire west coast of Vancouver Island, there are currently no sea otters in the sounds at the southern end of the island, including Barkley Sound (48.90° N, 125.27° W). The recovery of sea otters has dramatically increased the biomass of perennial brown algae. Biomass of *Pterygophora californica* and *Laminaria setchelli* increased by 10- to 20-fold with the recovery of sea otters in several sounds on Vancouver Island (Watson and Estes 2011). *Macrocystis pyrifera* kelp forests are 18.8 times larger in terms of area and 3.7 times deeper in the region with sea otters than in the region without sea otters (Markel 2011). Watson and Estes (2011) showed in surveys over more than 20 years that this increase in kelp forest size and prevalence occurred simultaneously with the return of sea otters and the decline of sea urchin populations, similar to the pattern observed in the Aleutian Islands, Alaska, USA (Estes and Duggins 1995). The variation in kelp abundance between our study regions is therefore likely due to the trophic cascade associated with sea otters.

We used the patchy distribution of sea otters as a space-for-time substitution (Pickett 1989) to compare bacterial community structure and function between two regions, one with sea otters and large kelp forests and one without otters and only small kelp forests. We investigated a subset of the bacterial community that uses alginate as a carbon source. Alginate is a major component of kelp (34–45% of dry mass) and is found in cell walls (Preiss and Ashwell 1962, Wainwright 1981). Some bacteria produce alginate lyase, an enzyme that degrades alginate (Wong et al. 2000). We measured the abundance and activity of alginate-producing-

bacteria because they directly use kelp-derived alginate as a carbon source. We also measured the abundance, growth, and losses (to microzooplankton predators or viruses) of the entire pelagic bacterial community. We compared bacterial community structure and function in two regions, one with otters and abundant kelp (Kyuquot Sound), and another without otters and with little kelp (Barkley Sound), along a gradient of distance from kelp forests to ask two questions: (1) Are the abundance and activity of alginate-lyase-producing bacteria different between the two regions? (2) Are there differences in total bacterial abundance, growth rates, and the rate at which bacteria are removed by microzooplankton grazing or viral infections in the two regions?

We sampled at three distances along transects offshore from kelp forests to determine how microbial community structure and function change with region and proximity to kelp.

## METHODS

### Sampling

Samples were collected from July to September 2010 in two regions along the west coast of Vancouver Island, British Columbia, Canada (Appendix: Fig. A1): Kyuquot Sound (50.07° N, 127.22° W; see Plate 1), where sea otters have been present since 1990 (Watson and Estes 2011), and Barkley Sound (48.90° N, 125.27° W), where otters remain absent. These two areas will be referred to as regions with large and small kelp forests, respectively, since Watson and Estes (2011) and Markel (2011) showed that kelps are more abundant and urchins are less abundant in Kyuquot compared to Barkley Sound. In each region, water samples were collected along three transects at distances 0, 250, and 500 m offshore of *Macrocystis pyrifera* kelp forests. Transects were spaced <10 km apart. Two offshore samples (2000 or 10 000 m offshore from any kelp forest) were also collected in each region. At each sampling location, a 20-L polyethylene carboy (Fisher Scientific, Ottawa, Ontario, Canada) was rinsed three times with seawater and then filled with subsurface (0.5 m) water. All three samples were collected within 2 h of each other whenever possible (74% of the time). Samples were kept in a cool, dark place on the boat until being returned to a laboratory for processing.

### Abundance of alginate-lyase-producing bacteria

The abundance of alginate-lyase-producing bacteria was determined from bacterial colonies growing on plates containing the kelp-derived carbon source alginate. Three dilution series were made from each seawater sample by diluting the seawater with sterilized artificial seawater containing 3.5% NaCl, 0.5% (NH<sub>4</sub>)SO<sub>4</sub>, 0.2% K<sub>2</sub>PO<sub>4</sub>, 0.1% MgSO<sub>4</sub>·7H<sub>2</sub>O, and 0.0001 FeSO<sub>4</sub>·7H<sub>2</sub>O, with a pH of 7.2 (Uchida and Nakayama 1993; all chemicals from Sigma-Aldrich, Oakville, Ontario, Canada). Two hundred µL of each

dilution (1:1 and 1:10) within a series were streaked onto petri dishes containing ~10 mL of the artificial seawater solidified with 1.5% agar and 0.3% sodium alginate (Sigma-Aldrich). Plates were inverted and incubated at room temperature (20°C) for two weeks. Control plates were streaked with the artificial seawater used to make the dilutions and incubated in the same way. After incubation, the plate in each series that contained 30–300 colonies was used to determine the abundance of bacteria using alginate as a carbon source by counting the number of colonies on the plate and converting that number into colony-forming units (CFU)/mL of seawater. Only non-indented colonies were counted because indented colonies indicate that the bacteria are using agar (instead of alginate) as a carbon source.

#### *Alginate lyase activity*

To quantify the ability of bacteria to degrade kelp, the activity of alginate lyase was determined from seawater samples collected in each region. A byproduct produced in the alginate degradation pathway absorbs UV light (Ostgaard 1993). The activity of bacterially produced alginate lyase was determined by measuring the changes in absorption at a wavelength of 235 nm in the seawater incubated with alginate. From each sample, three 7.5-mL subsamples of seawater were mixed in 10-mL glass culture tubes with 2.5 mL of artificial seawater (Uchida and Nakayama 1993) containing 1% sodium alginate (Sigma-Aldrich). These tubes were covered with parafilm and gently inverted twice to ensure complete mixing. Control tubes contained 7.5 mL of artificial seawater mixed with 2.5 mL of artificial seawater containing alginate. Additional control tubes containing 7.5 mL of seawater mixed with 2.5 mL of artificial seawater containing no alginate were also occasionally included to ensure there was no activity in the artificial seawater itself. In all cases, the tubes were incubated for 48 h at room temperature. Prior to incubation ( $T_0$ ), 1 mL was removed from each tube and absorption at 235 nm was measured using a spectrophotometer (Cary 50 scan UV-Vis spectrophotometer, Agilent, Mississauga, Ontario, Canada). Absorption was also determined after 24 and 48 h of incubation ( $T_{24}$  and  $T_{48}$ ) for each tube. The absorption of each subsample or control tube was plotted against time (see Appendix: Fig. A2) and the slope coefficients for absorption between  $T_0$  and  $T_{24}$  as well as between  $T_{24}$  and  $T_{48}$  were calculated. The slope coefficients are a measure of enzyme activity and indicate the rate at which alginate is degraded into the intermediate product via the bacterial enzyme alginate lyase.

#### *Total bacterial abundance*

The total abundance of pelagic bacteria in each seawater sample was determined using flow cytometry following Brussaard et al. (2010). This technique determines abundance from fluorescently labeled bacterial DNA. From each sample, two 1-mL seawater

samples were fixed with 25% glutaldehyde (Sigma-Aldrich) to a final concentration of 1%, mixed by inversion, incubated at 4°C for 30 min, and then flash frozen in liquid nitrogen. These fixed samples were kept frozen (–80°C) until processed. To determine bacterial abundance, the samples were thawed, diluted (1:5, 1:10, 1:50, or 1:100) with Tris-EDTA buffer (pH 8.0, Sigma-Aldrich) and stained with SYBR Green I (final concentration  $0.5 \times 10^{-4}$  of the commercial stock; Invitrogen, Burlington, Ontario, Canada) for 15 min at room temperature. The diluted and stained samples were then analyzed on a Becton-Dickinson (Mississauga, Ontario, Canada) FACSCalibur flow cytometer by exposing them to green fluorescence for 2 min at a flow rate of ~66  $\mu\text{L}/\text{min}$  in order to ensure an event rate of 100–1000 events/s. A known concentration of yellow-green 0.92  $\mu\text{m}$  beads (Fluoresbrite Microparticles, Polysciences, Warrington, Pennsylvania, USA) was added to each sample as an internal standard. Bacterial abundance was calculated using the freeware CYTOWIN (*available online*).<sup>3</sup> Briefly, counts within a defined box on a green fluorescence vs. side-scatter cytogram were calculated in order to exclude the internal standard and background noise. These counts, along with duration, flow rate, and dilution were used to calculate bacterial abundance (cells/mL).

#### *Bacterial grazing and growth rates*

Microzooplankton grazing rates on bacteria and bacterial growth rates were determined using the Landry and Hassett (1982) approach of creating a dilution series in order to lessen grazing on bacteria by reducing their encounter rate with microzooplankton. The increase in bacterial abundance over time is determined and the apparent growth rate of bacteria in each dilution is regressed against the fraction of seawater present to determine both bacterial growth and grazing rates. Grazing rates on bacteria were reduced by diluting seawater with 0.22  $\mu\text{m}$  filtered (Durapore; Millipore, Billerica, Massachusetts, USA) grazer-free water. For each sample, three dilution series were created containing 0.33, 0.66, and 1.0 or 0, 0.25, 0.50, 0.75, and 1.0 fractions of seawater. Dilutions (final volume of 50 or 100 mL) were incubated at room temperature (20°C) for 48 h. Initial ( $T_0$ ) and final ( $T_{48}$ ) bacterial abundance (BA) was determined from each dilution using flow cytometry according to the methods described for bacterial abundance. Additional seawater samples were collected over the course of the experiment to determine in situ bacterial abundance.

For each dilution within the series, the bacterial growth rate,  $\mu_k$ , was determined as

$$\mu_k = \ln(\text{BA}_{T_{48}}/\text{BA}_{T_0})/t$$

<sup>3</sup> <http://www.sb-roscoff.fr/Phyto>

TABLE 1. Terms used in the Landry and Hassett (1982) dilution approach to determining microzooplankton grazing rates on bacteria and bacterial growth rates.

Term	Descriptive equation	Units
Apparent growth rate ( $k$ )	$\ln(BA_{T_{48}}/BA_{T_0})/t$	$d^{-1}$
Grazer-mediated mortality ( $m$ )	(slope of regression)(-1)	$d^{-1}$
Regression growth rate ( $\mu_k$ )	y-intercept of regression	$d^{-1}$
In situ growth rate ( $\mu_i$ )	$\ln(N_2/N_1)/t$	$d^{-1}$
Corrected growth rate ( $\mu_c$ )	$\mu_k - \mu_i$	$d^{-1}$
In situ abundance of bacteria ( $\langle B \rangle$ )	bacterial counts	cells/mL
Potential production	$(\mu_c)(\langle B \rangle)$	cells·mL $^{-1}$ ·d $^{-1}$
Production grazed	$(m)(\langle B \rangle)$	cells·mL $^{-1}$ ·d $^{-1}$
Percentage of bacterial turnover due to grazing	$[(\text{production grazed})/(\text{potential production} + \langle B \rangle)] \times 100$	percentage per day

*Note:* Throughout the table,  $BA_{T_{48}}$  refers to final bacterial abundance,  $BA_{T_0}$  to initial bacterial abundance (where the subscripts indicate 48 and 0 h of incubation, respectively),  $t$  to time,  $N_1$  to in situ bacterial abundance at time 1, and  $N_2$  to in situ bacterial abundance at time 2.

where  $BA_{T_{48}}$  and  $BA_{T_0}$  are bacterial abundances in the dilution at  $T_{48}$  and  $T_0$ , and  $t = 2$  d. The bacterial growth rates estimated from the dilutions are hereafter referred to as apparent growth rates, following Landry and Hassett (1982) to clearly distinguish them from both the in situ ( $\mu_i$ ) and corrected ( $\mu_c$ ) growth rates. To determine microzooplankton grazing rates and bacterial growth rates, the apparent bacterial growth rate calculated from each dilution within a series was regressed against the fraction of seawater present in the dilution (Appendix: Fig. A3). The slope coefficient of this regression represents the microzooplankton-mediated bacterial mortality rate ( $m$ ;  $d^{-1}$ ) while the y-intercept ( $\mu_k$ ;  $d^{-1}$ ) represents the bacterial growth rate in each sample. Following Landry and Hassett (1982) and Landry et al. (1995), only regressions with slopes significantly different from zero ( $P < 0.05$ ) were used to estimate these rates.

Corrected bacterial growth rates ( $\mu_c$ ) were calculated by adding or subtracting the difference between in situ bacteria growth ( $\mu_i$ ) and the average apparent growth rate in the whole-water dilutions (1.0 fraction of seawater dilution) from the regression growth rate ( $\mu_k$ ). Corrected growth rates compensate for any possible bottle effect. The potential bacterial production (cells·mL $^{-1}$ ·d $^{-1}$ ) in each sample was then determined by multiplying the corrected bacterial growth rate ( $\mu_c$ ) and the in situ bacterial abundance (see Table 1).

#### Total viral abundance

The total abundance of pelagic viruses in seawater was determined using flow cytometry with fluorescently labeled viral DNA, using methods similar to that used for determining bacterial abundance (Brussaard et al. 2010). Samples were collected and processed as described for total bacterial abundance, except that the fixed samples were diluted to 1:10, 1:100, or 1:1000 with Tris-EDTA buffer and then incubated with SYBR Green I (Invitrogen) for 10 min at 80°C and then for 3 min at room temperature. The samples were then analyzed on the flow cytometer, where they were

exposed to green fluorescence for 1 min at a flow rate of  $\sim 32$   $\mu\text{L}/\text{min}$ . A known concentration of yellow-green 0.92- $\mu\text{m}$  beads (Fluoresbrite Microparticles) was added to each sample as an internal standard. Viral abundance was calculated using CYTOWIN. Counts within a defined box on a green fluorescence vs. side-scatter cytogram were calculated in order to exclude the internal standard and background noise. These counts, along with duration, flow rate, and dilution were used to calculate viral abundance (viruses/mL).

#### Data analysis

In order to test for differences between bacterial communities present in regions with large and small kelp forests, the abundance of alginate-lyase-producing bacteria as well as total bacterial abundance, bacterial grazing and growth rates, and total viral abundance were compared using a linear mixed-effect model (LME), where region (Kyuquot vs. Barkley Sound, or large vs. small kelp forests) and distance from the kelp bed (0, 250, or 500 m) were factorial fixed independent factors. Transect (1, 2, or 3) was treated as a random factor in the model. The abundances of alginate-lyase-producing bacteria, total bacteria, and viruses were  $\log_e$ -transformed prior to analysis to normalize variance. All analyses were performed using SYSTAT 12 (Systat, Chicago, Illinois, USA) and results were graphed in SigmaPlot 12 (Systat).

Because alginate lyase activity was measured over two time periods (24 and 48 h), repeated-measures ANOVA (RM ANOVA) was used to test for differences in alginate lyase activity between regions and distances from kelp. In this case, region (large and small kelp forests) and distance (0, 250, 500 m) were fixed independent factors, while transect (1, 2, or 3) and time (0, 24, and 48 h) were random factors in the model. Changes in absorption were measured over two time periods ( $T_0$  to  $T_{24}$  and  $T_{24}$  to  $T_{48}$ ). The RM ANOVA also tested for significant interactions between region and sampling distance. This analysis was performed in R



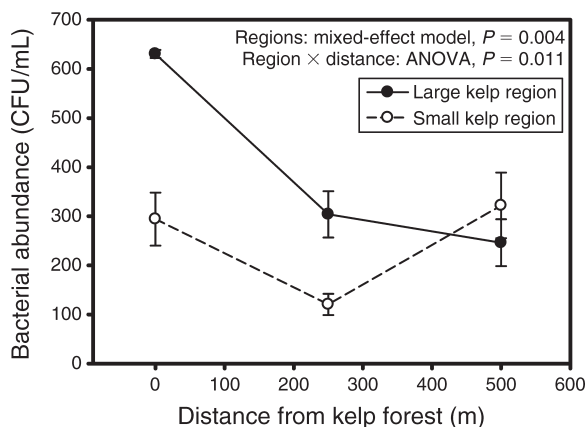


FIG. 1. Abundance of alginate-lyase-producing bacteria (mean  $\pm$  SE) in relation to distance from *Macrocystis pyrifera* kelp forests off Vancouver Island, British Columbia, Canada, for large (solid symbols and solid line) and small (open symbols and dashed line) kelp forest regions; symbols and lines are the same in all figures. Bacterial abundance was determined from the number of colony-forming units (CFU) growing on plates of artificial seawater solidified with alginate.

version 2.15.3 (R Development Core Team 2013) and results were plotted in SigmaPlot 12.

## RESULTS

### Abundance of alginate-lyase-producing bacteria

The abundance of bacteria using kelp-derived alginate as a carbon source was higher in the region with large kelp forests than the region with small kelp forests (Fig. 1; linear mixed-effect model, effect of region:  $F_{1,56} = 8.943$ ,  $P = 0.004$ ). There was no significant main effect of sampling distance (linear mixed-effect model;  $F_{2,56} = 2.060$ ,  $P = 0.137$ ); however, there was an interaction between region and distance (ANOVA;  $F_{2,55} = 4.931$ ,  $P = 0.011$ ). The abundance of alginate-lyase-producing bacteria was highest ( $630.556 \pm 8.268$  CFU/mL; all means presented  $\pm$  SE) at the 0-m sampling locations in the large kelp forest region and lowest ( $120.556 \pm 21.626$  CFU/mL) at the 250-m location in the small kelp forest region.

### Alginate lyase activity

The activity of the enzyme alginate lyase was significantly higher in the region with large kelp forests. In all cases, the absorption at 235 nm increased with time (Appendix: Fig. A2) but leveled off by 72 h (not shown). In order to compare regions, the absorption vs. time slope coefficients for each replicate were calculated for 0–24 h and 24–48 h and treated as repeated measures. Both average slope coefficients were significantly higher in the large kelp forest region than in the small kelp forest region (RM ANOVA;  $t = 3.333$ ,  $df = 105$ ,  $P = 0.0012$ ; Fig. 2). There was also a significant interaction between region and sampling distance (RM ANOVA;  $t = -2.790$ ,  $df = 105$ ,  $P = 0.0063$ ). Average

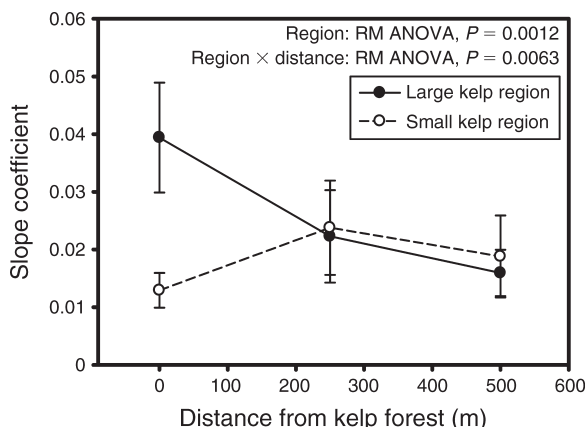


FIG. 2. Alginate lyase activity assay slope coefficients (mean  $\pm$  SE) at each sampling location by distance from the large and small kelp forest regions. Average regional slope coefficients were calculated from both the 0–24 and 24–48 h repeated-measures ANOVA (RM ANOVA).

alginate lyase activity was higher in the large kelp forest region at the 0-m sampling distance, but the two regions showed similar activity at the other sampling distances (250 and 500 m). The average offshore alginate lyase activity (expressed as slope coefficients on an hourly basis) was  $0.0039/\text{h} \pm 0.0001/\text{h}$  after 24 h and  $0.016/\text{h} \pm 0.0023/\text{h}$  after 48 h.

### Total bacterial abundance

The abundance of pelagic bacteria was 2.65 times higher in the region with large kelp forests (Fig. 3, main effect of region,  $F_{1,33} = 63.57$ ,  $P < 0.0001$ ). Sampling distance did not have a significant main effect (linear mixed-effect model;  $F_{2,33} = 0.134$ ,  $P = 0.875$ ) and there was no interaction between region and distance (ANOVA;  $F_{2,33} = 0.010$ ,  $P = 0.990$ ). The average abundance of pelagic bacteria at the offshore stations was  $3.77 \times 10^6$  bacteria/mL.

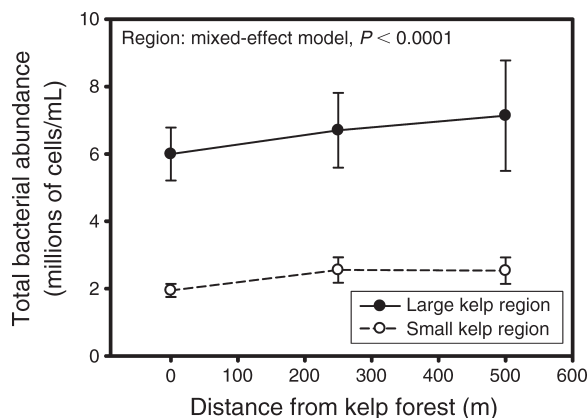


FIG. 3. The abundance of pelagic bacterial cells in the large and small kelp forest regions along a distance gradient. Abundance was determined using flow cytometry.

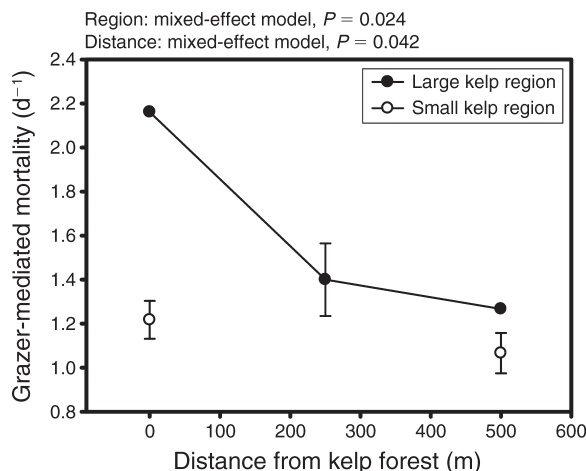


FIG. 4. Grazer-mediated bacterial mortality rates in the large and small kelp forest regions along a distance gradient. Grazing rates on bacteria were determined using the Landry and Hassett (1982) dilution approach.

#### Bacteria grazing and growth

Grazer-mediated bacterial mortality and bacterial growth rates were higher in the region with large kelp forests. Significant regressions of apparent growth rate vs. fraction of seawater (i.e., Appendix: Fig. A3) were observed in 64% of the experiments, and these experiments were used to calculate bacterial mortality and growth rates (nonsignificant regressions occurred in one experiment at both the 0-m and 500-m locations in the large kelp forest region and all the 250-m locations in the small kelp forest region). Average grazer-mediated bacterial mortality rates were higher in the region with large kelp forests than in the region with small kelp forests (linear mixed-effect model;  $F_{1,3} = 17.986$ ,  $P = 0.024$ ; Fig. 4). In each region, rates were greatest in seawater collected immediately adjacent to kelp forests (the 0-m sampling locations, means in the large and small kelp forest regions were  $2.162$  and  $1.3031$   $\text{d}^{-1}$ , respectively). As a result, there was a significant effect of sampling distance (linear mixed-effect model;  $F_{2,3} = 10.941$ ,  $P = 0.042$ ), but no interaction between region and distance (ANOVA;  $F_{2,2} = 2.007$ ,  $P = 0.333$ ). Grazer-mediated mortality at the offshore location was  $1.02$   $\text{d}^{-1}$ .

Bacterial growth rates were significantly higher in the region with large kelp forests; Fig. 5). Bacteria in the large kelp forest region grew at  $1.43 \pm 0.19$   $\text{d}^{-1}$ , while in the small kelp region bacterial growth rates were  $0.65 \pm 0.20$   $\text{d}^{-1}$  (linear mixed-effect model;  $F_{1,3} = 21.589$ ,  $P = 0.019$ ). Potential bacteria production was  $7.42 \times 10^6$   $\text{cells} \cdot \text{mL}^{-1} \cdot \text{d}^{-1}$  in the large kelp forest region and  $3.18 \times 10^6$   $\text{cells} \cdot \text{mL}^{-1} \cdot \text{d}^{-1}$  in the small kelp forest region. Sampling distance did not have a significant main effect (linear mixed-effect model;  $F_{2,3} = 3.565$ ,  $P = 0.161$ ), nor was there an interaction between region and distance (ANOVA;  $F_{2,2} = 0.032$ ,  $P = 0.969$ ). The growth rate of bacteria at the offshore location was  $1.50$   $\text{d}^{-1}$ .

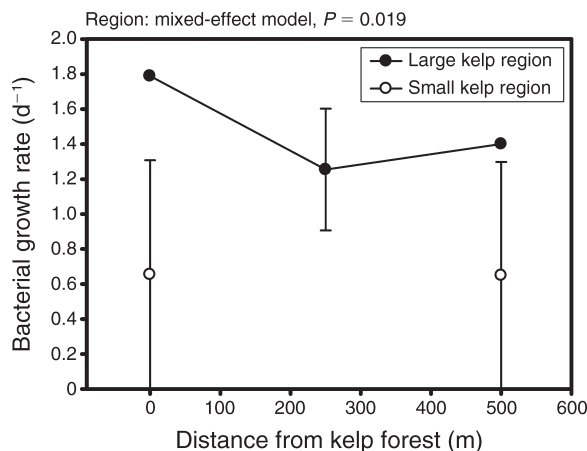


FIG. 5. Bacterial growth rates along a distance gradient in the large and small kelp forest regions as determined using the dilution approach (Landry and Hassett 1982).

#### Total viral abundance

The abundance of pelagic viruses was 2.89 times higher in the region with large kelp forests; Fig. 6;  $F_{1,40} = 22.558$ ,  $P < 0.0001$ ). Sampling distance did not have a significant effect (linear mixed-effect model;  $F_{2,40} = 0.332$ ,  $P = 0.719$ ) and there was no interaction between region and distance (ANOVA;  $F_{2,39} = 0.071$ ,  $P = 0.932$ ). The average abundance of pelagic bacteria at the offshore locations was  $3.44 \times 10^7$  viruses/mL.

#### DISCUSSION

Our results indicate that the expansion of kelp forests along the west coast of Vancouver Island driven by sea otter recovery is associated with substantial variation in microbial community structure, abundance, and function. In Kyuquot Sound, the region with large kelp forests, bacteria were more abundant, produced more alginate lyase, grew faster, and were subject to more mortality associated with microzooplankton grazing

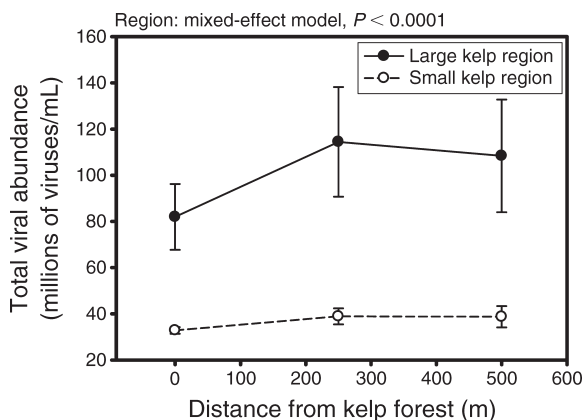


FIG. 6. The abundance of viruses in the large and small kelp forest regions along a distance gradient. Abundance was determined using flow cytometry.



PLATE 1. Typical sampling location in the abundant kelp forest region where sea otters have been present since 1990, Kyuquot Sound, British Columbia. Photo credit: J. L. Clasen.

and/or viral infection than in Barkley Sound, which has much smaller kelp forests. The effects relating to alginate-lyase-producing bacteria were more pronounced in the immediate vicinity of the larger kelp forests in Kyuquot Sound, and diminished rapidly over distances of 250–500 m offshore, indicating that bacteria using alginate as a substrate were more abundant and active in the region with otters and large kelp forests. All of these patterns are consistent with the hypothesis that larger kelp forests produce more detritus and dissolved organic matter, which stimulate microbial activity. Our results suggest that the sea otter–urchin–kelp trophic cascade indirectly alters the community structure and productivity of marine microbes.

#### *Alginate-lyase-producing bacteria*

The elevated abundance and activity of alginate-lyase-producing bacteria in Kyuquot Sound suggests that extensive kelp forests indirectly stimulate the productivity of the bacterial community. Alginate lyase is an extracellular enzyme that cleaves alginate and initiates a degradation pathway that ultimately results in small monomers, which are assimilated by bacteria (Kim et al. 2011). By examining alginate-lyase-producing bacteria, we targeted a subset of the total bacterial community that directly uses the alginate in kelp as a carbon source. As expected, both the abundance (Fig. 1) and activity (Fig. 2) of alginate-lyase-producing bacteria were higher in the region with larger kelp forests. The greatly elevated kelp biomass in Kyuquot relative to Barkley Sound (Markel 2011, Watson and Estes 2011) likely

produces more kelp detritus, including alginate, which sustains more alginate-lyase-producing bacteria.

Although, we expected the regional differences in alginate-lyase-producing bacteria to extend across the entire transect, the higher abundance and activity of alginate-lyase-producing bacteria in the large kelp forest region were largely driven by local processes occurring in the immediate vicinity of kelp forests. The abundance of alginate-lyase-producing bacteria was highest immediately adjacent to kelp forests in the large kelp forest region; however, the two regions had comparable abundances of alginate-lyase-producing bacteria at a 500-m distance from kelp (Fig. 1). Likewise, although alginate lyase enzyme activity differed significantly between the two regions, the difference was largely driven by the activity measured at the 0-m sampling distance (Fig. 2). Kelp-derived detritus has often been detected at distances of 10s to 100s of km offshore of kelp forests (Kaehler et al. 2006, Krumhansl and Scheibling 2012, Ramshaw 2012). However, our results suggest that the influence of kelp forests on the abundance and productivity of alginate-lyase-producing bacteria is spatially confined to the area immediately surrounding the kelp forest. More spatially resolved sampling is required to identify the scale of influence of kelp on bacterial communities.

Alginate lyase may be produced by other organisms including gastropods, marine algae including some brown algae, *Chlorella* viruses, and many marine and soil bacteria (Wong et al. 2000, Kim et al. 2011). Since we sampled alginate lyase activity in coastal pelagic



water, bacteria were likely the predominant source of alginate lyase in our samples. Furthermore, we measured the abundance of alginate lyase bacteria using a culture-dependent technique which, like all culture-dependent methods, underestimates true abundances (Staley and Konopka 1985). Culture-independent methods like quantitative PCR would be a more powerful way to measure the abundance of alginate-lyase-producing bacteria. The colonies that grew on our plates likely represent just a few bacterial types capable of growing on the artificial seawater solidified with alginate. The culturing data can be used to compare relative abundance among sites but almost certainly underrepresent the true abundances.

#### *Total bacterial communities*

Total pelagic bacterial abundance, bacterial growth rates, grazer-mediated bacterial mortality, and viral abundance, which is likely to be related to virally mediated bacterial mortality, were higher in the region with large kelp forests than in the region with small kelp forests. Unlike the alginate-lyase-producing bacteria, there was no interaction between region and distance for any of the measures of the broader bacterial community, suggesting that the differences observed in total bacterial communities extend across the regional transects. This implies either that the influence of detritus produced by kelp forests through compounds other than alginate extends farther offshore from kelp, or that other environmental differences favored greater bacterial abundance and activity in Kyuquot relative to Barkley Sound.

Greater total bacterial abundance and growth rates in the large kelp region suggest that more organic substrate is available in this region to sustain bacterial growth. Kelps are a major source of particulate organic carbon as they are degraded by physical and biological processes (Bustamante and Branch 1996, Krumhansl and Scheibling 2012). Most kelp biomass is not directly consumed by metazoan herbivores (Duggins et al. 1989). Instead, 66–82% of annual kelp productivity enters the detrital food web as particulate organic matter (Stuart et al. 1981, Krumhansl and Scheibling 2012). The region with large kelp forests contained more kelp-derived particulate organic carbon (Ramshaw 2012). Therefore, more kelp carbon (including alginate, agar, and laminaran) is available for bacterial enzyme degradation, which increases the amount of carbon substrate available for bacterial utilization (Bengtsson et al. 2011). Grazing on bacteria influences ecosystem productivity and represents an important link between bacteria and higher trophic levels. The microbial loop allows kelp carbon and nutrients to enter the grazer food web (Azam et al. 1983). We found higher microzooplankton grazing rates in the large kelp forest region than in the region with small kelp forests (Fig. 4), which may stimulate the microbial loop and support greater secondary production. The higher availability of kelp-

derived organic carbon likely explains both higher total bacterial abundances and growth rates in the large kelp forest region.

Viral abundance was significantly higher in the region with large kelp forests (Fig. 6). Viral-mediated bacterial mortality influences both nutrient cycling and bacterial diversity. Viruses infect and lyse 20–40% of bacteria cells daily (Suttle and Chan 1993, Suttle 2005), quickly recycling bacterial nutrients back into the particulate and dissolved organic matter pools, where they can be degraded or assimilated by other bacteria (Suttle 2007). Since infection rates likely increase with viral abundance, our results suggests that bacterial mortality due to viruses was higher in Kyuquot Sound than Barkley Sound. Higher rates of viral infections indicate a more dynamic microbial community, which may include enhanced viral-mediated nutrient cycling and genetic exchange. Both mechanisms could contribute to the diverse microbial communities observed near or on kelp forests (Bengtsson et al. 2010, 2012, Michelou et al. 2013).

#### *Caveats and alternative explanations*

The differences between bacteria in the large and small kelp forest regions are consistent with expectations based on the documented effects of the sea otter–urchin–kelp trophic cascade. However, 170 km of coastline separates the two regions in this study, and the differences observed could be driven by oceanographic processes and abiotic environmental differences (see Horner-Devine et al. 2004, Hanson et al. 2013). However, several lines of evidence suggest that these alternative mechanisms may not explain the patterns we observed.

Temperature regulates bacterial metabolism, which affects bacterial growth rates and ultimately abundance (del Giorgio and Cole 1998). Higher abundances and growth are typically found in warmer waters (Wetzel 2001, Sarmiento et al. 2010, Vezzulli et al. 2013). Over a 10-year period (1999–2009), sea surface temperatures (SST) were consistently 1°C warmer in Barkley Sound, the small kelp forest region, than in Kyuquot (Singh et al. 2013). SST was 0.616°C warmer in Barkley Sound than Kyuquot at the times that we sampled. However, we found that bacterial abundance, growth rate, and activity were higher in the colder region with larger kelp forests. Therefore, temperature differences cannot explain the bacterial differences observed between the two regions.

The physical oceanography along the west coast of Vancouver Island is affected by wind, bottom topography, rocky reefs, deep fjords, and large peninsulas. The Vancouver Island Coastal Current (VICC) is the dominant current shoreward of the 100-m depth contour and generally flows poleward all year long. However, in the summer, when strong northwest winds create favorable upwelling conditions, the VICC can reverse and flow southward (Thomson 1981, Thomson

et al. 1989). Upwelling brings nutrient-rich water to the surface and can stimulate productivity. The 1990–2010 mean annual upwelling indices were stronger in the small kelp forest region than the large kelp forest region (Singh et al. 2013). However, the mean daily upwelling index over our sampling period was  $48.17 \pm 6.23 \text{ m}^3 \cdot \text{s}^{-1} \cdot 100 \text{ m}^{-1}$  of coastline in Kyuquot Sound, the large kelp forest region, and  $22.23 \pm 2.62 \text{ m}^3 \cdot \text{s}^{-1} \cdot 100 \text{ m}^{-1}$  in Barkley Sound, the small kelp forest region, suggesting that the large kelp forest region was experiencing stronger upwelling than the other region. Therefore, the large kelp forest region was receiving inorganic-nutrient-rich water, which could contribute to the higher bacterial abundance, growth, and activity in this region. However, coastal bacteria are typically limited by organic carbon substrates rather than inorganic nutrients such as N and P (del Giorgio and Cole 1998, Kirchman et al. 2000). Therefore, upwelling of inorganic nutrients cannot explain the differences we observed between regions. In addition, our results relating to alginate-lyase-producing bacteria suggest that the portion of the bacterial community that relies directly on kelp-derived carbon was more abundant and active in the region with large kelp forests than small kelp forests, suggesting that greater kelp abundance is at least partly responsible for the variation in microbial communities we observed.

Our study represents a comparison between two regions that differ in the presence of sea otters and the extent of kelp forest cover. Because sea otter populations are expanding from the north on Vancouver Island, it is impossible to separate the presence of otters from geography. The intermediate region where sea otters have only been present for about five years was not sampled due to its extremely different coastline. As a result, we focused on the endpoints of the sea otter/kelp gradient along the west coast of Vancouver Island. Regional comparisons are essential for understanding how communities are shaped by processes that occur at scales too large for experimental manipulation. These types of studies have been used to examine ecosystem responses to climate change (Wernberg et al. 2012) and top-down vs. bottom-up controls in the intertidal zone (Menge et al. 2002). Our design allowed for a comparison between the microbial communities in two regions along a gradient of kelp forest size. Watson and Estes' (2011) long-term study of the west coast of Vancouver Island found that sea urchin populations declined and kelp forest area expanded dramatically in Kyuquot Sound, our large kelp forest region, with the repatriation of sea otters. Furthermore, Estes and Duggins (1995) report similar variation in kelp abundance (biomass) caused by changes in sea otter populations in the Aleutian Islands. The difference in kelp forest area between our regions is therefore very likely a consequence of the trophic cascade initiated by the recovery of sea otters. Any environmental factor that influenced kelp forest size (e.g., wave energy; Reed et al.

2011) might elicit a similar response by the microbial community. Ecologically important parameters such as current velocity, nutrient (dissolved organic carbon) concentrations, fish recruitment, and abundance of kelp propagules are also affected by kelp forest size (Graham 2004, Rosman et al. 2007, Deza and Anderson 2010, Ramshaw 2012). The patterns observed in this study are consistent with the expected effect of increased kelp forest detritus from larger kelp forests stimulating the microbial loop and enhancing the abundance of bacteria, including those that rely on alginate.

Human activities directly and indirectly affect bacterial communities and microbially mediated ecosystem processes. For example, temperature, nutrient availability, and acidification all affect the abundance and composition of marine bacterial communities (see Allison and Martiny 2008, Krause et al. 2011, Nogales et al. 2011, Vezzulli et al. 2013). Our study suggests that removal of a vertebrate top predator can have substantial indirect effects on bacteria by altering the abundance of kelp, a foundational and highly productive marine macroalga. Our results contribute to the growing evidence that human activities exert profound influences on microbial communities (Allison and Treseder 2011, Allison et al. 2013). Perturbations in the abundance of sea otters likely structure microbial communities through a chain of cascading trophic interactions that affect the production of detritus.

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#### SUPPLEMENTAL MATERIAL

##### Ecological Archives

The Appendix is available online: <http://dx.doi.org/10.1890/13-2147.1.sm>