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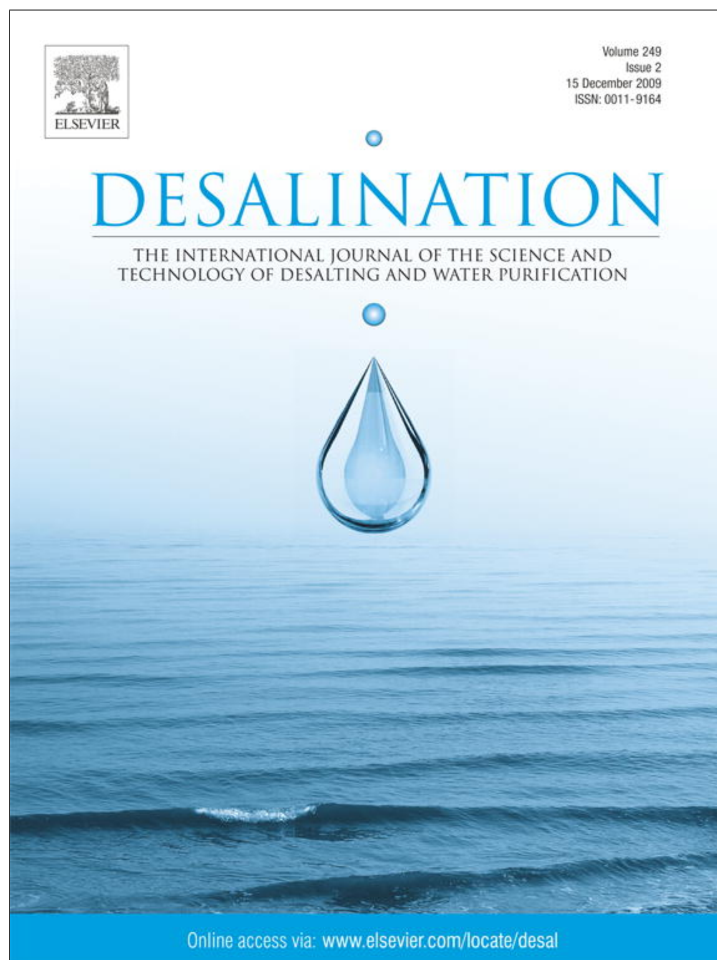

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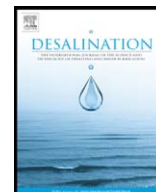

Desalination technology waste streams: Effect of pH and salinity on metabolism of marine microbial assemblages

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ABSTRACT

Effluents from desalination technologies may influence natural bacterial assemblages due to changes in salinity, pH, dissolved organic carbon concentration (DOC), DOC quality and cellular hydrostatic pressure. Salinity, pH, and pressure change effects on heterotrophic bacterial production (as measured by leucine incorporation) were examined in experiments with surface water from the Delaware Bay, Atlantic Ocean and Pacific Ocean. Bacterial production decreased by 57–67% when salinity of Atlantic Ocean and Delaware Bay surface water samples were increased from ambient to 60 PSU. Decreasing ambient seawater pH from 8.0 to below 5.0 with CO_2 gas reduced production by 96–100%. Decreasing seawater pH by 1.5 units at 33 PSU caused equivalent inhibition to increasing salinity by 27 PSU (pH 8.0). Bacterial production in Pacific Ocean surface water pressurized for 72 h was decreased 40% by increasing salinity, when measured 1 h after decompression. However, production increased 43% with increasing salinity when measured 24 h after decompression. One explanation for these divergent effects between the 1- and 24-h sampling at ambient pH may be community adaptation. Strains amongst the natural assemblage that survived the pressurization by saturating their membrane phospholipids would likely be better adapted to compete for available nutrients under elevated salinity.

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1. Introduction

The importance of examining the environmental impact of desalination technologies has garnered attention during the past decade (see review by [1]). Wastewater discharged from desalination plants can have qualities that are significantly different from those of the aquatic ecosystem into which it is discharged. This can lead to adverse ecological effects on the macrobiota adjacent to the desalination plant [2]. Brine represents the most significant component of the wastewater with billions of liters produced daily worldwide [3]. The focus of most environmental studies has been on the local macrobiota where the significant effects are observed within 200 m of the discharge pipe [1], though with natural ecological variation and current monitoring techniques, effects can be difficult to quantify [4].

Hydrate formation-based technologies have been proposed as a desalination technology for transforming seawater into potable water [5]. Under low temperature and high pressure, hydrocarbon gases dissolved in seawater form clathrate crystals that exclude salts and dissolved organic matter [6,7]. If separated from the brine prior to melting, the clathrate crystals can melt into potable water and purgeable hydrocarbon gas. Physical separation of the clathrate ice crystals from the aqueous brine prior to melting has been a technological challenge in the development of this technology [5,8]. In addition to brine production that is typical in desalination technologies, the hydrate-based strategy may also release effluent with reduced pH and harboring a bacterial assemblage affected by depressurization.

Brine input to coastal waters can alter the abundance and composition of macrobiota near the outfall, but it can also alter carbon and nitrogen cycling through the ecosystem [9] and change the microbial flora associated with these processes [10]. Such discharge into coastal surface waters can affect the natural bacterial assemblage in primarily two ways: metabolic rate and assemblage composition. The most commonly reported measure of bacterial metabolism for natural assemblages is heterotrophic bacterial production (leucine incorporation method) [11–13]. This study focuses on the effect of salinity and pH on bacterial production rate of natural marine assemblages maintained at one atmosphere and depressurized from 600 m below sea surface.

2. Material and methods

2.1. Water sample collection

Samples were collected in 250 mL Nalgene bottles previously acid-washed and rinsed with MilliQ grade water. Three experiments

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Nomenclature

Symbols

C	carbon
d	day
DOC	dissolved organic carbon
h	hour
kg	kilogram
L	liter
min	minutes
mg	milligram
PSU	practical salinity units
µg	microgram

were conducted using coastal surface water samples collected from (1) Delaware Bay (Lewes, DE) on 7 December 2006, (2) Atlantic Ocean (Indian River, DE) on 14 July 2007, and (3) Pacific Ocean (Honolulu, HI) on 13 August 2007. For experiment (1), Delaware Bay surface water was collected from the Lewes–Cape Henlopen pier. For experiment (2), Atlantic Ocean surface seawater was collected from Indian River Inlet, DE on an incoming tide. For experiment (3), Pacific Ocean surface seawater was collected from a pier off Honolulu.

2.2. Salinity

To increase salinity, NaCl (17.55 mg mL^{-1}), KCl (1.33 mg mL^{-1}), $MgSO_4$ (12.38 mg mL^{-1}), and $CaCl_2$ (1.11 mg mL^{-1}) (Sigma Chemical, St. Louis, MO) were added to subsamples of the water. The salts were baked prior to use to remove any organic carbon. Because it was not possible to make a sufficiently concentrated stock solution, dry salts were added to 200 mL surface water subsamples for final salinities of 29, 33, 39, 47, 56, and 60 Practical Salinity Units (PSU) for experiment (1); 33, 41, 50, 60, and 68 PSU for experiment (2); and 30, 40, 50, 60, and 70 PSU for experiment (3). Salinity was determined with a refractometer. The highest salinity concentrations examined are typical of desalination effluent [4].

2.3. pH

CO_2 gas was bubbled into the water samples for up to 45 min and pH changes were monitored with a pH meter during the sample preparation. The final pH of treatments were 8.0 (no bubbling), 7.55, 6.50, 5.53, and 4.88 for experiment (1). For experiment (2), the salinity was adjusted prior to bubbling with CO_2, so each salinity treatment has pH adjusted separately to 8.03 (no bubbling), 6.60, 4.91 for 33 PSU; 8.05, 6.78, and 4.94 for 41 PSU; 7.99, 6.32, and 4.92 for 50 PSU; 7.98, 6.49, and 4.95 for 60; and, 7.91, 6.63, and 4.97 for 68 PSU. For experiment (3), the pH treatments were 8.0 (no bubbling) and pH 4.9. The effect of the salt addition on final pH of the solution was not measured.

2.4. Heterotrophic bacterial production

The leucine incorporation method [11–13] was used to measure bacterial production. A 1.0 mL water sample from each treatment was added to 2 mL microcentrifuge tubes (three experimental and one control) which were pre-charged with [^{3}H-4,5]-L-leucine (154 mCi mmol^{-1}). Samples were incubated for 2 h at *in situ* temperatures and subsequently processed by the method of Smith and Azam [13] but centrifuging for 7 min instead of 10 min. A constant isotope dilution factor of 1000 was used for all samples. This was estimated from actual measurements of sediment dissolved free amino acids [14] and saturation experiment estimates [15]. Leucine incorporation rate was converted to bacterial carbon using factors determined by Simon and Azam [16].

2.5. Pressure chamber experiments

The pressurization and subsequent depressurization experiments were designed to mimic the effects of a hydrate-based desalination on the natural bacterial assemblage in the effluent waste stream. Fifty milliliters of Pacific Ocean surface water was bubbled with CO_2 for 45 min to lower the pH from 8.0 to 4.9. This sample (pH 4.9) and another 50 mL sample that was not bubbled (pH 8.0) were pressurized in separate chambers and incubated for 72 h at 25 °C and *in situ* pressure corresponding to a 600 m depth in a laboratory pressure facility at the University of Hawaii [17]. The chambers were depressurized to ambient laboratory pressure (1 atm). Within 10 min of depressurization, 1 mL aliquots were placed in 2 mL microcentrifuge tubes with sea salts added to increase the salinity to experimental conditions. After 1 h of depressurization, one set of tubes representing each pH (4 and 8) and salinity (30, 40, 50, 60, and 70 PSU) were processed for bacterial production. The second set was processed 24 h after depressurization.

3. Results and discussion

The heterotrophic bacterial production rate of surface water samples from the Delaware Bay mouth (December 2006) decreased linearly ($r^2 = 0.95$) with increasing salinity from 29.5 to 60 PSU at the ambient pH 8.0 (Fig. 1). This represents a 57% decrease in production from 0.420 (±0.020) µg C L^{-1}d^{-1} at ambient salinity (29.5 PSU) to 0.180 (±0.010) µg C L^{-1}d^{-1} (60 PSU). This is very similar to the 67% decrease in production with salinity increase from 33 to 68 PSU in the Atlantic Ocean surface water sample (July 2007) (Fig. 2) despite the large difference in production of the respective unamended samples (0.42±0.02 verses 17.9±1.9 µg C L^{-1}d^{-1}). This difference between unamended Delaware Bay and Atlantic Ocean water is very likely due to seasonal difference in their collection times (December verses July, respectively).

The pH effect was also very similar between these two sampling events. The heterotrophic bacterial production rate in Delaware Bay surface water decreased 96% when pH was lowered from its initial value of 8.00 to 4.88 with CO_2 bubbling at 29.5 PSU (Fig. 3). Likewise, Atlantic Ocean surface water bacterial production decreased 100% when pH was lowered from its initial value of 8.00 to 4.99 at salinities ranging from 33 to 68 PSU (Fig. 4). In the Atlantic Ocean sample, the combined salinity and pH effects were examined. Increasing salinity decreased bacterial production at pH 8.0 from 17.9±1.9 µg C L^{-1}d^{-1} at 33 PSU to 5.94±0.4 µg C L^{-1}d^{-1} at 68 PSU. The inhibitory effect of 1.5 pH units (from pH 8.0 to 6.5) at 33 PSU was approximately equal

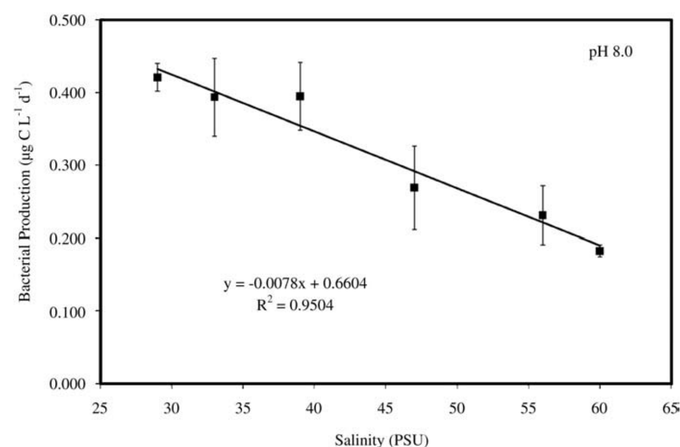

Fig. 1. Rate of heterotrophic bacterial production (µg C L^{-1}d^{-1}) of surface water taken from the mouth of the Delaware Bay (7 December 2006) decreased 57% with increasing salinity from its initial unamended value of 29.5 to 60 PSU at pH 8.0.

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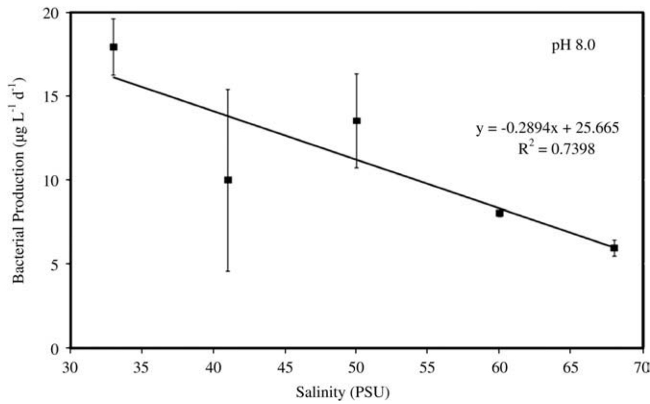

Fig. 2. Rate of heterotrophic bacterial production (μg C $L^{-1} d^{-1}$) of Atlantic Ocean surface water (14 July 2007) decreased 67% with increasing salinity from its initial unamended value of 33 to 68.

to that from increasing the salinity 27 PSU (from 33 to 60 PSU) at pH 8.0 (pH 8.0, 60 PSU = 8.02 ± 0.20 μg C $L^{-1} d^{-1}$; pH 6.5, 33 PSU = 6.67 ± 1.82 μg C $L^{-1} d^{-1}$) (Fig. 4).

Finally, Pacific Ocean surface water (August 2007) was incubated in a pressure chamber (equivalent to a depth of 600 m below sea surface) for 72 h at 25 °C. Prior to incubation, the sample was split into two aliquots with one bubbled with CO_2 for 45 min to reduce the pH to 4.9 while the other was maintained at ambient pH 8.0. After incubation, both samples were depressurized and salinity of subsamples was adjusted from the ambient 30 PSU to 40, 50, 60 and 70 PSU. Heterotrophic bacterial production was measured on a subset of these samples 1 h and 24 h post depressurization. Bacterial production rate ?tlsb-.02pt?> decreased 40% with increasing salinity from its initial unamended value of 30 to 70 PSU at pH 8.0 when measured 1 h after depressurization (Fig. 5). However, at 24-h post depressurization, bacterial production increased 43% as salinity increased from 30 to 70 PSU. This relationship with salinity was linear for both the 1-h (T0) and 24-h (T1) samplings though the regression was stronger with the 1-h sample set ($R^2 = 0.9798$) than with the 24-h ($R^2 = 0.8434$) (Fig. 5).

Bacterial production showed little relationship with increasing salinity from its initial unamended value of 30 to 70 PSU at pH 4.9 when measured either 1-h or 24-h post depressurization (Fig. 6). However, production averaged among all salinities for a given time point increased four-fold from 1-h to 24-h post depressurization (Fig. 6). As was seen with the Delaware Bay and Atlantic Ocean experiments, decreasing the pH from 8.0 to 4.9 inhibited bacterial production 99.2% even after 24 h at 33 PSU (20.22 ± 3.8 verses 0.159 ± 0.004 μg C $L^{-1} d^{-1}$)

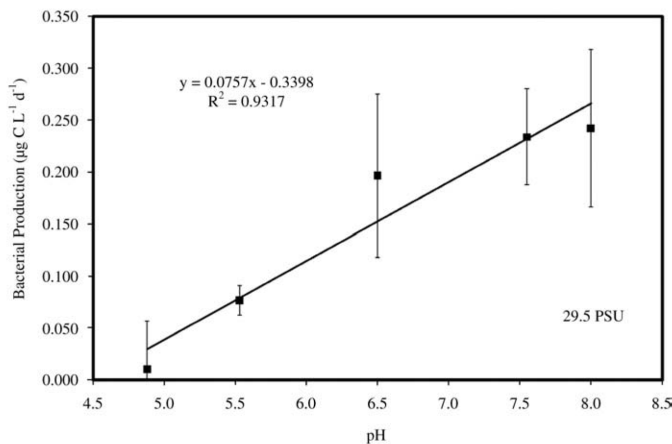

Fig. 3. Rate of heterotrophic bacterial production (μg C $L^{-1} d^{-1}$) of surface water taken from the mouth of the Delaware Bay (7 December 2006) decreased 96% when pH was lowered from its initial value of 8.00 to 4.88 with bubbling by CO_2 at 29.5 PSU salinity.

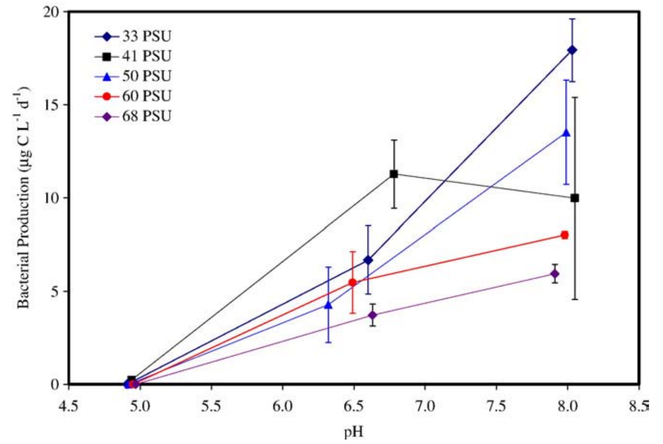

Fig. 4. Rate of heterotrophic bacterial production (μg C $L^{-1} d^{-1}$) of Atlantic Ocean surface water (14 July 2007) decreased 100% when pH was lowered from its initial value of 8.00 to 4.99 with bubbling by CO_2 at salinities ranging from 33 to 68 PSU.

(Figs. 5, 6). The inhibitory effect of low pH on bacterial production was similar to that reported by Coffin et al. [17] for natural assemblages from deep Pacific Ocean water.

One explanation for the divergent effect of salinity on production between the 1-h and 24-h sample sets at ambient pH may be community adaptation. Although there are few reports examining the effects of desalination wastewater on the natural microbial assemblage, high local fluctuations in salinity that would inevitably result from the hydrate formation may be comparable to those seen in sea ice formation. Kaartokallio et al. [18] found that open ocean assemblages decreased genotype diversity and bacterial production (leucine incorporation rate) in response to salinity stress relative to sea ice assemblages. Salinity response of a particular assemblage appears to be related to the composition of membrane lipids [19]. Increases in saturation of membrane phospholipids will decrease the fluidity and make the cell more resistant to bursting under osmotic shock or increased temperature. Using a deep-sea bacterial strain, Kaye and Baross [20] found that high pressure increased the degree of lipid saturation making it less susceptible to elevated salinity. Strains amongst the natural assemblage that survived the pressurization by saturating their membrane phospholipids would be better adapted to compete for available nutrients under elevated salinity.

The decrease in bacterial production with salinity after one h of depressurization may be the result of the metabolic costs of

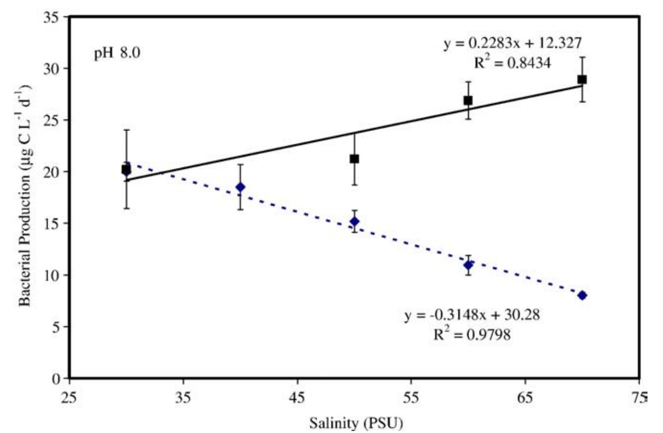

Fig. 5. Pacific Ocean surface water (13 August 2007) was incubated in a pressure chamber (equivalent to 600 m) for 72 h at 25 °C. Rate of heterotrophic bacterial production (μg C $L^{-1} d^{-1}$) decreased 40% with increasing salinity from its initial unamended value of 30 to 70 PSU at pH 8.0 when measured 1 h after decompression (blue diamonds). However, 24 h post decompression, bacterial production increased 43% as salinity increased from 30 to 70 PSU (black squares).

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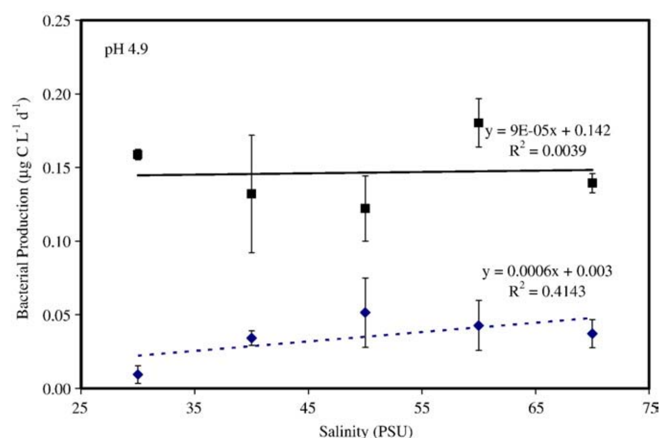

Fig. 6. Pacific Ocean surface water (13 August 2007) was incubated in a pressure chamber (equivalent to 600 m) for 72 h at 25 °C. Rate of heterotrophic bacterial production (μg C L^{-1} d^{-1}) showed little relationship with increasing salinity from its initial unamended value of 30 to 70 PSU at pH 4.9 when measured either 1 h after decompression (blue diamonds) or 24 h post decompression (black squares). However, bacterial production averaged among all salinities for a given time point increased four-fold from 1 to 24 h post decompression.

osmoregulation. Cells newly exposed to elevated salinity would have to divert some portion of their energy to osmoregulation (uptake of solutes compatible with cellular function) and respiring organic matter to CO_2 rather than the creation of new bacterial biomass [21]. This reduction in the bacterial growth efficiency (BGE) due to environmental stress could lower the bacterial production values (new protein synthesis) at the 1-h sampling [22]. Once established within the new salinity regimes, the respective assemblages may increase production of new bacterial biomass using nutrients and organic matter unused during the lag period or possibly newly released from leaking or lysed bacteria, phytoplankton, and protozoan grazers. Assemblage adaptation occurs rapidly through change in gene expression (phenotypic adaptation) by individual cells and over longer time scales via natural selection and differential growth rates under the new conditions (genotypic adaptation)[23].

Previous experiments assessed the effect of hydrate formation-based technologies on water quality and growth rate of the natural bacterial assemblage [24]. Coffin et al. [24] measured heterotrophic bacterial production, dissolved organic carbon concentration and stable isotope values, and fluorescence spectra of the organic matter that partitions into the hydrate and wastewater streams. They found that effluent streams from a mesocosm scale representation of this process inhibited bacterial growth based on the difference in production between the source water and process water samples. Though Coffin et al. [24] and the work described in this paper looked specifically at the impact of hydrate-based technologies, the results from the impact of brine discharge are more generally applicable to coastal desalination plants. Design considerations may help ameliorate the adverse and poorly understood effects on coastal ecosystems [25].

4. Conclusions

Bacterial production (measured by rate of ^{3}H-leucine incorporation) decreased from 57–67% when salinity of Atlantic Ocean and Delaware Bay surface water was increased from ambient (27 to 33) to 60 PSU. Decreasing the pH of ambient seawater from 8.0 to below 5.0 by bubbling with CO_2 gas reduced bacterial production from 96–100% on these same source waters. The inhibitory effect of decreasing ambient seawater pH by 1.5 units (from pH 8.0 to 6.5) at 33 PSU was approximately equal to that from increasing the salinity 27 PSU (from 33 to 60 PSU) at pH 8.0. Pacific Ocean surface water that was pressurized for 72 h at 25 °C showed a 40% decrease in bacterial production with increasing salinity when measured one h after decompression. However, replicate samples increased production 43% with increase in salinity when measured 24 h after decompression. One explanation for the divergent effects of salinity on production between the 1-h and 24-h sampling at ambient pH may be community adaptation. Strains amongst the natural assemblage that survived the pressurization by saturating their membrane phospholipids would be better adapted to compete for available nutrients under elevated salinity.

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