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Characteristics of Bacterial Communities in the Gulf of Alaska

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Abstract. Taxonomic diversity, physiological tolerance ranges, and nutrient utilization capabilities were determined for bacterial communities in Gulf of Alaska surface waters and sediments. Taxonomic diversity was assessed using Shannon Weaver (H') and equitability (J') indices. Physiological tolerance and nutritional versatility indices were developed to further assess the state of "informational heterogeneity" within the bacterial communities. The Gulf of Alaska bacterial communities were characteristically diverse; the bacterial populations in these marine ecosystems generally were eurytolerant and nutritionally versatile. The maintenance of a high degree of informational heterogeneity was found to be characteristic of these bacterial communities. It appears to be of adaptive advantage to maintain diverse populations with physiological tolerances whose ranges exceed those experienced within the natural habitat, and for the bacterial communities to possess a high degree of nutritional versatility within these marine ecosystems.

Introduction

The present study represents a continuing effort to characterize microbial communities in potential offshore oil and gas lease areas of the Gulf of Alaska. In a previous paper we described the taxonomic relationships and features of bacterial populations in the Gulf of Alaska [4]. In the current paper we extend the description to characteristics of the bacterial communities in this region, including within Cook Inlet.

Community parameters have been applied to bacterial species only rarely. Diversity indices frequently are used to describe community structure for higher organisms [1, 5, 11, 16–24, 28], but relatively few papers concerning bacteria have used such indices [2, 6, 7, 12, 13]. The concept of a species is difficult, if not impossible, to define for bacteria [10], and the concept of a species diversity index, in the true sense, probably is not applicable to bacteria. However, it is possible to utilize the same calculations to describe taxonomic diversity within the bacterial community, albeit with somewhat less definition, utilizing arbitrarily defined taxonomic groupings, such as clusters or phena,

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generated from numerical taxonomic analyses [7]. we have used such data to calculate the taxonomic diversity of bacterial communities in Gulf of Alaska surface waters and sediments. In this study we use the Shannon Weaver index to calculate diversity (H') [19, 23, 24] and equitability (J') [20, 22].

The term *diversity* is used by ecologists to describe the heterogeneity of the biological complex of populations within a community occupying a habitat. Microbiologists often use the term diversity to qualitatively describe limited features of microbial populations, such as morphological shapes [26]. Such observations can be extended to a quantitative estimate of diversity within microbial assemblages [6]. A restricted number of metabolic tests can also be used to determine groupings of similar populations and to estimate community diversity [3, 13]. The use of a small number of features may create the appearance of low diversity as an artifact of the tests chosen, which can result in the formation of "false" clusters or groups. In our study we relied on a large number of tests to establish group similarity boundaries, which emphasized heterogeneity. The inclusion of a large number of features of bacterial populations permits a more extensive analysis of different aspects of metabolic capabilities, structure, and diversity of bacterial communities in various habitats [12].

In addition to the "conventional approach" to calculating diversity within a community, which relies on taxonomy and definition of species, we have developed indices of physiological "diversty" (tolerance) and nutritional "diversity" (substrate utilization). These indices should prove useful to microbiologists since they convert information on individuals or populations to community parameters. A physiological "tolerance" index gives information on the tolerance of a community to deviations from ambient conditions. The index is scaled such that a score of 1 is equivalent to the maximal physiological diversity (eurytolerance), and a score of 0 represents a total lack of tolerance to environmental fluctuations (stenotolerance). In this study, we have calculated individual tolerance indices for variations in temperature, salinity, and pH, and a combined physiological tolerance index that includes all three parameters. The nutritional "diversity" index measures the range of substrates that can be utilized by members of the bacterial community without weighting given to whether substrates are used by many or only a limited number of sample populations. We have calculated separate indices for carbohydrates, alcohols, carboxylic acids, amino acids, and hydrocarbons, and a total substrate utilization index. These indices have been scaled so that a score near 0 represents a community of nutritionally fastidious organisms and an index near 1 represents a community whose member populations can utilize a wide range of substrates. A community with one nutritionally versatile population has a high community nutritional diversity index, as does a community with complimentary populations, each of which utilizes limited numbers of substrates.

The informational contents of the taxonomic, physiological, and nutritional "diversity" indices are different. Each is a community parameter descriptor that can be used to assess the status of a community and the likelihood of maintaining community stability, i.e., tolerating a deviation from status quo conditions in the form of environmental fluctuations or perturbations. They do not appear to be redundant indices; it is possible to have a community with high nutritional or physiological diversity that has low taxonomic diversity, as well as other combinations of high and low values for the separate indices.

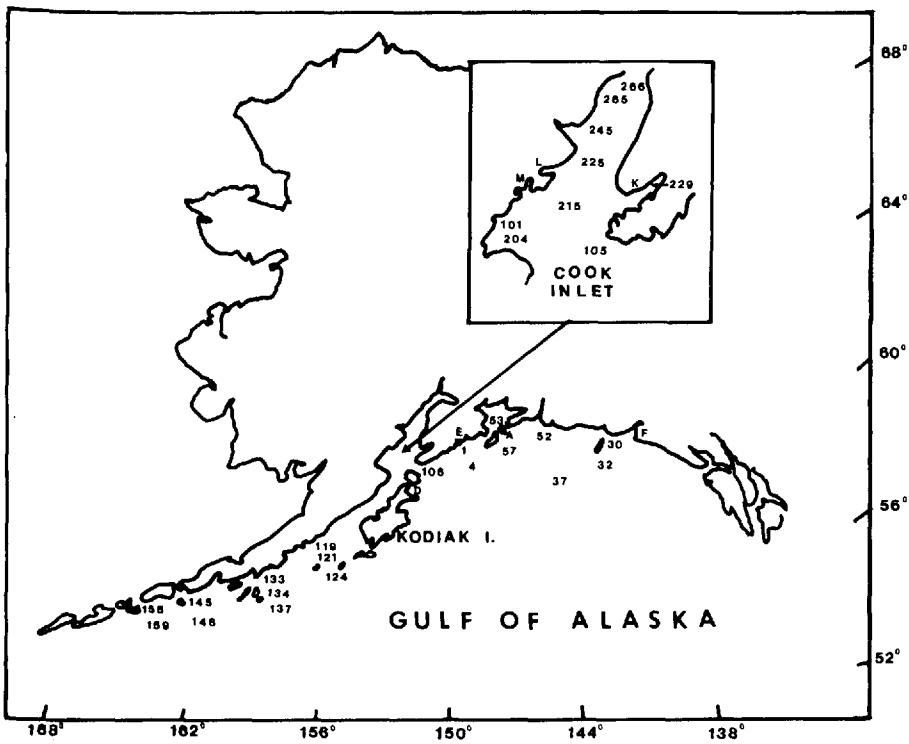

Fig. 1. Map showing sampling locations in the Gulf of Alaska and Cook Inlet. Offshore stations are indicated by number designations; intertidal stations are indicated by letter designations.

Methods

Sampling and Characterization of Bacterial Populations

Samples were collected in the Northwest Gulf of Alaska, between Unimak Pass and the mouth of Cook Inlet, during October 1975; in the Northeast Gulf of Alaska, from Kodiak Island to Yakutat Bay, during March 1976; and within Cook Inlet during October 1976. Sampling locations are shown in Fig. 1. Temperature and salinity measurements were made using a Plessey Environmental Systems salinity-temperature meter or with a Beckman RS7A salinity meter and reversing thermometers. Surface water samples were collected with a sterile Niskin butterfly sampler (General Oceanics); sediment samples were obtained using a Van Veen or Smith MacIntyre grab sampler. Intertidal sediment and water samples were collected directly with sterile wide mouth containers.

Procedures were initiated within 1 hour of collection for enumeration and selection of representative populations of the bacterial community. Serial dilutions of samples were made in Rila marine salts solution (Rila Products), plated on marine agar 2216 (Difco), and incubated at 5°C for 3 weeks. All materials had been cooled to 5° before plating. Platings were performed in triplicate. Following incubation, colonies that developed were counted with the aid of a Quebec colony counter and the mean viable count was recorded. All discrete colonies were numbered sequentially using a stereo microscope (30×) so that even relatively slow growing bacteria were included in the pool of organisms available for selection for further study. Using random number tables,

approximately 20–60 bacterial colonies from each sample were selected for isolation. These were considered as representative of the major populations of the bacterial community since they were obtained from countable plates of greatest dilution. The selected organisms were subcultured on marine agar plates to ensure purity and viability. Some strains lost viability and had to be eliminated from the study. A total of 1033 representative isolates were maintained through the study.

Approximately 300 phenotypic characteristics were determined for each isolate. Phenotypic characteristics examined included morphological examination of both cells and colonies, physiological growth ranges including tolerance to temperature, salt, and pH, biochemical tests including determination of a variety of enzymatic activities, and nutritional characteristics including the abilities to utilize a large number of biochemically diverse substrates. Details of the test procedures have been described previously [8].

Data were coded and processed for computer analysis [8]. Data on individual isolates were sorted by sampling station and the feature frequencies of each phenotypic characteristic were calculated for each water and sediment sample using the computer program FREAK [27]. Data were also subjected to cluster analysis to determine taxonomic groupings (phenotypic clusters) using the Jaccard coefficient (S_j) and single-linkage clustering (GTP2 program, courtesy R. R. Colwell) [25]. Details of taxonomic analyses for Northeast and Northwest Gulf of Alaska isolates have been reported [4]; results of Cook Inlet taxonomic analyses will be reported separately. Taxonomic groups were defined at approximately the 75% similarity level. The resultant data from the feature frequency and cluster analyses were used to calculate community "diversity" parameters as described below.

Physiological Tolerance Indices

Indices were developed to describe the capacity of the bacterial community to tolerate (maintain ability to grow) deviations from ambient conditions of temperature, salinity, and pH. Ambient conditions were considered as 5°C, 3% NaCl, and pH 8, which approximate both environmental and isolation conditions. Feature frequencies for the ability to grow at 10, 15, 20, 25, and 37°C; 0, 0.5, 5, 7.5, and 10% NaCl; and pH 5, 6, 7, 9, and 10 were used for calculating physiological tolerance indices. The physiological tolerance index for temperature (P_T) was calculated according to the formula:

$$P_T = \frac{G_{10} + G_{15} + G_{20} + G_{25} + G_{37}}{5}$$

where G_x = the proportion of the populations (represented by the isolates) within the community which are capable of growth at temperature x. According to this calculation, a community composed entirely of true psychrophiles (organisms that cannot grow at 20°C or above [14]) would have a P_T of ≤ 0.4. Similarly, a community in which all member populations could grow over the entire range of temperature from 0 to 37°C would have a P_T of 1.

The physiological tolerance index for salinity (P_S) was calculated as:

$$P_S = \frac{G_0 + G_{0.5} + G_5 + G_{7.5} + G_{10}}{5}$$

where G_x = the proportion of the populations within the community capable of growth at NaCl concentration of x percent. A "true marine bacterium" cannot have a $P_S = 1$ since by definition marine bacteria require NaCl and cannot grow at 0% NaCl [30, 31].

The physiological tolerance index for pH (P_H) was calculated as:

$$P_H = \frac{G_5 + G_6 + G_7 + G_9 + G_{10}}{5}$$

where G_x = the proportion of the populations within the community capable of growth at pH value x.

Nutritional Utilization Indices

Indices were calculated to assess the nutritional versatility of bacterial communities. Separate indices were calculated for carbohydrates (N_c), alcohols (N_a), carboxylic acids (N_{ca}), amino acids (N_{aa}), and hydrocarbons (N_h). Each nutritional utilization index was calculated by summing the number of substrates that could be utilized by any member population and dividing by the total number of substrates within that class. A combined nutritional utilization index (N_T) was calculated for all substrates regardless of compositional class. The substrates employed in determining these indices were carbohydrates—arabinose, ribose, xylose, rhamnose, fructose, galactose, glucose, mannose, sorbose, salicin, cellobiose, lactose, maltose, sucrose, trehalose, raffinose; alcohols—1-butanol, ethanol, 1-propanol, 2-propanol, 1, 2-propanediol, glycerol, arabitol, dulcitol, mannitol, sorbitol, *m*-inositol, phenol, phenylethanol; carboxylic acids—acetic, butyric, caproic, caprylic, lauric, propionic, valeric, glutaric, malonic, succinic, oleic, fumaric, itaconic, glyceric, β-hydroxybutyric, lactic, tartaric, citric, 2-ketogluconic, pyruvic, α-ketoglutaric, benzoic, *m*-hydroxybenzoic, *p*-hydroxybenzoic, *o*-hydroxybenzoic, ascorbic, galacturonic, gluconic, stearic; amino acids—alanine, γ-amino butyric, arginine, asparagine, aspartate, cystine, cysteine, glycine, leucine, isoleucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine; hydrocarbons—*n*-hexadecane, *n*-pentadecane, 2-methylnaphthalene, 1-methylnaphthalene, phenyldecane, pristane, pentadecylcyclohexane.

A total of 16 carbohydrates, 13 alcohols, 29 carboxylic acids, 20 amino acids and 7 hydrocarbons (= 85 total substrates) were employes. An $N_x = 1$, for any substrate class x, indicates that all substrates included in that class can be utilized by some member population(s) of the bacterial community. An N_x near 0 indicates a lack of versatility of substrate utilization by the sampled (dominant) bacterial populations of the community. It should be noted that N_x is sensitive to rare populations not selected by the plating and isolation procedures employed in this study.

Taxonomic Diversity

The number of taxonomic groups and the number of individuals within each group, determined by the cluster analyses, were used to calculate the Shannon diversity index, H' [19, 23, 24]. The formula $H' = C/N(N \log_{10} N - \Sigma n_i \log_{10} n_i)$ was used, where $C = 3.3219$, N = total numbers of individuals, and n_i = total numbers of individuals in the ith taxonomic grouping [9]. An H' value near 0 represents a community with low diversity; H' values near 4 represent rather high diversity. Equitability (J') was calculated according to the formula $J' = H'/H_{\max}$ where $H_{\max}$ = the maximal value of H' for a given sample size [20, 22]; it assumes that each cluster can be single membered representing taxonomically distinct populations, i.e., $H_{\max} = C \log_{10} N$. A J' value of 1 shows an even distribution; when J' is near 0 there is an uneven distribution of individuals within the taxa of the community.

Statistical Analyses

Analyses of variance were performed using the SPSS computer programs [15] to determine the levels of statistical significance of differences between grouped data. The Duncan mean comparison test was used to determine if the means of individual groups were significantly different from each other, e.g., was there a significant difference between the taxonomic diversity indices (H') between water and sediment communities. An α value < 0.05 was considered necessary for establishing a significant difference.

Results

Sample Characteristics

The abiotic parameters of temperature, salinity, and depth and the viable bacterial counts and numbers of isolates from each water and sediment sample are presented in Table 1.

Table 1. Abiotic parameters, viable counts, and numbers of selected isolates from Gulf of Alaska and Cook Inlet samples

Station	Water				Sediment				
	Temp. °C	Salinity $^{o}/_{oo}$	Viable count	No. of Isolates	Temp. °C	Salinity $^{o}/_{oo}$	Depth m	Viable count	No. of Isolates
156	6.4	31.7	2.5×10^2	14	—	—	—	—	—
159	7.5	31.9	2.2×10^2	15	—	—	—	—	—
145	8.0	31.4	1.3×10^2	16	—	—	—	—	—
134	—	—	—	—	—	—	154	1.4×10^6	22
137	—	—	—	—	7.7	31.9	99	6.2×10^5	19
121	—	—	—	—	4.7	33.5	230	1.1×10^6	17
101	—	—	—	—	9.3	30.8	91	1.0×10^4	21
204	5.5	28.0	1.6×10^2	19	9.1	28.2	34	2.3×10^6	20
215	9.5	27.0	1.0×10^1	20	10.0	27.0	73	8.7×10^5	19
225	—	—	—	—	10.0	26.0	75	2.9×10^4	19
245	9.0	26.0	1.1×10^2	19	—	—	—	—	—
265	9.0	19.5	6.1×10^2	17	—	—	—	—	—
266	8.5	21.0	2.2×10^2	20	—	—	—	—	—
229	8.5	24.0	2.0×10^2	20	9.3	26.0	28	6.0×10^6	20
105	9.0	25.5	3.0×10^1	17	7.5	28.0	120	1.5×10^5	20
1	3.0	31.7	3.3×10^1	30	4.3	32.4	190	5.2×10^5	23
4	3.5	32.2	2.1×10^1	19	4.5	32.8	183	1.1×10^5	29
53	3.0	31.8	5.9×10^1	24	4.9	32.5	303	3.0×10^6	22
57	4.0	32.1	1.0×10^1	14	4.3	32.3	71	4.2×10^5	28
52	3.2	31.8	7.2×10^1	27	3.7	31.9	46	1.7×10^5	29
37	—	—	—	—	2.1	34.6	2282	3.7×10^3	26
30	3.8	31.7	3.3×10^1	17	4.2	31.9	40	5.8×10^3	31
32	—	—	—	—	5.8	33.2	175	1.1×10^5	28
D	2.0	31.4	2.2×10^4	31	2.0	31.4	0	1.8×10^5	29
M	6.5	22.0	1.0×10^4	20	6.5	22.0	0	1.7×10^7	20
L	6.0	17.0	2.9×10^4	20	6.0	17.0	0	8.4×10^5	20
K	12.0	23.0	1.1×10^5	20	—	—	—	—	—
E	3.0	31.1	1.1×10^3	26	—	—	—	—	—
A	5.0	31.1	7.7×10^2	22	5.0	31.1	0	4.0×10^6	17
F	3.0	30.8	3.8×10^2	28	—	—	—	—	—

The temperature ranges were as follows: for surface water 2.0–5.0°C during March and 5.5–12.0°C during October; and for bottom water (sediment) 2.0–5.8°C during March and 6.0–10.0°C during October. The temperatures during late winter thus in both water and sediment were almost 5°C lower than during early fall. The range of salinities was 17.0–33.5$^{o}/_{oo}$ for all samples; the low salinity values within Cook Inlet (17–24$^{o}/_{oo}$) are probably indicative of a high terrestrial freshwater input. Comparison of temperature and salinity data, which determine water density, for surface and bottom waters at most Cook Inlet sites indicates a potentially unstable water column, which should result in a high degree of turnover and mixing. In contrast, although there was not apparent thermocline, indicative of a high degree of stratification, the water densities indicate a stable water column existed at most sites outside Cook Inlet.

Bacterial Population Characteristics

There was no significant differences in viable counts between March and October samples despite warmer fall temperatures (Table 1). In offshore surface waters viable counts ranged from 1.0×10^1 to 6.1×10^2/ml; intertidal water samples had significantly higher viable counts, 3.8×10^2 to 1.1×10^5/ml, viable counts in sediments ranged from 3.7×10^3 to 1.7×10^7/g dry wt.

Selected morphological, physiological, and biochemical characteristics of representative dominant bacterial populations in each sample are shown in Table 2. Gram-negative rods predominated in all samples. Approximately one-half of the populations, represented by the isolates, were pigmented, predominantly with yellow, orange, and brown pigments. Slightly less than half of the isolates were motile. As expected, higher percentages of motile bacteria were found in water samples (49%) than in sediment samples (38%).

The majority of the bacterial populations grew at temperatures of 5–20°C, but true psychrophiles, incapable of growth at 20°C, only were found in 33 of the 45 samples. The majority of isolates at most stations required NaCl for growth. Three intertidal beach stations (D, M, and L) and one water station [52] showed anomalously low proportions of NaCl-requiring bacterial populations. This observation likely indicates the occurrence of bacterial populations of terrestrial origin, but which are capable of growth in the marine environment in these regions. At many other stations all of the dominant bacterial populations required NaCl.

The ability to reduce NO_3^- to NO_2^- was characteristic of many of the bacterial populations present at all but two stations. Nitrate reducers predominated in most sediment samples and were only slightly less abundant in water samples. The ability to reduce nitrate can permit organisms to respire under anaerobic conditions, which are commonly found in marine sediments. Nitrite reduction was a common characteristic of bacterial populations at most stations, but the number of nitrite reducing populations was always far less than that of the corresponding nitrate reducers.

The ability to produce extracellular enzymes was tested using gelatin and starch, for protease and amylase enzymes, respectively. Isolates capable of hydrolyzing either gelatin (protein) or starch (polysaccharide) were found at all stations tested, in similar numbers. In this region saccharolytic bacterial populations appear to occur as frequently as proteolytic populations.

Physiological Tolerance Indices

The physiological tolerance indices for growth over ranges of temperature (P_T), pH (P_H), and salinity (P_S) are shown in Table 3. Considerable variations in P_T are apparent at different stations. There was no significant difference, though, in P_T values between communities sampled in March and October. There was a significant difference ($\alpha < 0.01$) in P_S values for communities in surface waters east and west of Kodiak Island: the mean P_S for western stations was 0.11; the mean P_{S1} for eastern stations was 0.61. P_S values also were significantly higher in intertidal samples than in offshore samples. In most cases the temperature tolerance index (P_T) is distinctly larger than the indices for either pH range or NaCl concentration. This indicates that the majority of the

Table 2. Selected features of populations in Gulf of Alaska and Cook Inlet water and sediment showing % positive

Station	Gram neg.	Rods	Motile	Spores	Pig-mented	Psy-chro-philic	NaCl re-quired	Nitrate reduced	Nitrite reduced	Gelatin hy-drol.	Starch hy-drol.
					Water						
156	100	93	43	0	82	25	100	63	13	—	70
159	100	100	27	0	72	0	100	0	0	—	75
145	87	88	38	6	31	0	82	58	0	—	22
137	92	67	92	0	25	25	100	70	10	—	33
101	100	100	78	0	0	0	100	88	0	—	87
204	100	95	42	0	5	21	100	—	—	16	37
215	100	90	95	0	50	0	90	—	—	75	40
245	100	94	78	0	10	10	95	—	—	68	37
265	100	81	25	0	53	0	53	—	—	41	41
266	100	84	26	0	45	5	90	—	—	45	45
229	100	90	30	0	25	0	80	—	—	84	85
105	100	94	50	0	47	12	88	—	—	63	50
1	97	83	37	0	20	27	80	82	7	46	52
4	100	89	11	0	74	21	84	43	0	31	38
53	100	79	13	0	62	21	50	37	0	29	35
57	100	93	100	0	0	0	100	17	7	86	8
52	63	7	0	0	100	0	4	93	0	7	11
30	100	47	24	0	21	22	47	59	6	24	6
D	100	100	26	0	68	0	39	29	7	68	29
M	100	100	65	0	20	5	35	—	—	85	30
L	100	74	70	0	10	10	85	—	—	50	45
K	100	100	20	0	5	0	100	—	—	100	90
E	100	85	58	0	35	15	69	25	23	65	5
A	95	86	55	0	41	5	91	57	19	64	50
					Sediment						
134	95	100	27	0	19	50	100	100	17	0	42
137	100	100	26	0	65	7	100	71	0	—	70
121	100	94	29	0	25	43	94	100	6	—	19
101	100	95	52	0	43	20	100	76	0	—	67
204	100	95	20	0	10	5	100	—	—	20	85
215	100	95	47	0	16	16	100	—	—	11	32
225	100	100	72	0	16	10	100	—	—	68	53
229	100	90	30	0	45	15	95	—	—	40	60
105	100	85	90	0	30	5	95	—	—	60	65
1	96	91	48	0	65	27	82	65	0	39	44
4	83	79	34	0	76	7	45	81	18	48	28
53	100	91	50	0	50	14	86	65	14	64	40
57	100	96	64	0	7	11	89	81	22	57	68
52	90	72	24	0	59	21	62	77	7	29	33
37	92	85	46	8	19	36	67	70	13	46	45
30	100	97	35	0	45	19	81	55	10	43	39
32	100	61	40	0	32	19	56	91	8	29	46
D	100	93	14	0	86	0	62	44	14	69	48
M	100	100	15	0	65	5	65	—	—	45	40
L	100	100	25	0	10	0	0	—	—	60	10
A	100	94	24	0	76	24	88	78	27	40	65

Table 3. Physiological tolerance indices for temperature, pH, and salt for Gulf of Alaska and Cook Inlet bacterial communities

	Water			Sediment		
Station	P_T	P_H	P_S	P_T	P_H	P_S
156	0.74	0.40	0.10	—	—	—
159	0.65	0.23	0.00	—	—	—
145	0.76	0.42	0.24	—	—	—
134	—	—	—	0.57	0.66	0.18
137	—	—	—	0.67	0.44	0.09
121	—	—	—	0.54	0.45	0.23
101	0.80	0.73	0.45	0.71	0.60	0.21
204	0.63	0.57	0.21	0.67	0.46	0.14
215	0.85	0.61	0.40	0.70	0.42	0.12
225	—	—	—	0.68	0.56	0.14
245	0.83	0.61	0.30	—	—	—
265	0.85	0.47	0.41	—	—	—
266	0.76	0.54	0.33	—	—	—
229	0.83	0.55	0.58	0.72	0.41	0.21
105	0.72	0.45	0.25	0.77	0.60	0.27
1	0.71	0.55	0.35	0.71	0.53	0.40
4	0.72	0.53	0.33	0.77	0.65	0.58
53	0.72	0.50	0.53	0.70	0.55	0.28
57	0.80	0.77	0.74	0.69	0.62	0.23
52	0.99	0.76	0.90	0.72	0.59	0.47
37	—	—	—	0.70	0.60	0.50
30	0.83	0.60	0.73	0.65	0.54	0.26
32	—	—	—	0.80	0.61	0.52
D	0.82	0.52	0.60	0.86	0.50	0.58
M	0.90	0.62	0.77	0.79	0.44	0.63
L	0.83	0.65	0.43	0.82	0.60	0.74
K	0.91	0.60	0.59	—	—	—
E	0.76	0.61	0.63	—	—	—
A	0.80	0.52	0.42	0.69	0.46	0.25
F	0.70	0.51	0.62	—	—	—

communities sampled are more tolerant to changes in temperature, over the experimental range, than to changes in either pH or salinity over the ranges tested. Direct comparison of tolerance indices for different environmental factors must be made with caution, however, as the calculated numerical value of the indices are dependent on the selected ranges of experimental values for each factor.

Nutrient Utilization Indices

The nutrient utilization indices are shown in Tables 4 and 6. Carbohydrates and amino acids generally had the highest utilization indices. All of the carbohydrates tested could be used by the bacterial communities in 11 different samples. Usually less than half of

Table 4. Nutrient Utilization indices for Gulf of Alaska and Cook Inlet bacterial communities for various substrate classes[a]

	Water						Sediment					
Station	N_c	N_a	N_{ca}	N_{aa}	N_h	N_T	N_c	N_a	N_{ca}	N_{aa}	N_h	N_T
156	0.38	0.54	0.48	0.55	0.00	0.45	—	—	—	—	—	—
159	0.69	0.38	0.33	0.70	0.14	0.49	—	—	—	—	—	—
145	0.81	0.54	0.55	0.85	0.29	0.65	—	—	—	—	—	—
134	—	—	—	—	—	—	0.75	0.46	0.45	0.75	0.00	0.54
137	—	—	—	—	—	—	0.75	0.38	0.59	0.55	0.29	0.55
121	—	—	—	—	—	—	0.88	0.46	0.62	0.70	0.00	0.61
101	—	—	—	—	—	—	0.94	0.69	0.69	1.00	0.71	0.81
204	0.64	0.31	0.41	0.90	0.00	0.52	0.93	0.23	0.28	0.55	0.00	0.42
215	0.71	0.38	0.55	0.55	0.00	0.51	0.93	0.31	0.59	0.80	0.00	0.60
225	—	—	—	—	—	—	0.79	0.31	0.41	0.95	0.00	0.55
245	0.64	0.31	0.41	0.65	0.00	0.46	—	—	—	—	—	—
265	1.00	0.38	0.66	0.90	0.00	0.67	—	—	—	—	—	—
266	1.00	0.31	0.66	0.85	0.00	0.65	—	—	—	—	—	—
229	0.79	0.15	0.28	0.50	0.00	0.37	0.93	0.31	0.34	0.60	0.00	0.47
105	0.57	0.15	0.28	0.45	0.00	0.33	0.64	0.23	0.31	0.75	0.00	0.43
1	0.69	0.46	0.69	0.70	0.14	0.61	0.88	0.54	0.66	0.85	0.43	0.71
4	1.00	0.23	0.48	0.40	0.00	0.48	0.94	0.46	0.69	0.75	0.29	0.68
53	1.00	0.31	0.62	0.55	0.00	0.58	0.94	0.62	0.62	0.65	0.00	0.64
57	0.63	0.15	0.55	0.20	0.00	0.38	1.00	0.69	0.66	0.60	0.00	0.66
52	0.81	0.38	0.69	0.75	0.14	0.63	0.81	0.38	0.69	0.85	0.00	0.65
37	—	—	—	—	—	—	0.81	0.38	0.59	0.65	0.00	0.56
30	0.81	0.08	0.50	0.42	0.00	0.43	0.94	0.62	0.86	0.74	0.00	0.73
32	—	—	—	—	—	—	0.88	0.69	0.69	0.85	0.43	0.74
D	1.00	0.62	0.79	0.65	0.14	0.72	0.94	0.54	0.69	0.40	0.00	0.59
M	1.00	0.46	0.76	0.90	0.00	0.72	1.00	0.31	0.52	0.70	0.00	0.57
L	0.79	0.46	0.52	0.85	0.00	0.59	1.00	0.85	0.72	0.95	0.00	0.78
K	0.29	0.08	0.21	0.40	0.00	0.23	—	—	—	—	—	—
E	0.94	0.15	0.59	0.30	0.00	0.47	—	—	—	—	—	—
A	1.00	0.31	0.66	0.75	0.00	0.64	0.94	0.46	0.62	0.75	0.00	0.64
F	0.94	0.77	0.72	0.40	0.29	0.66	—	—	—	—	—	—

[a] N_c, carbohydrates; N_a, alcohols; N_{ca}, carboxylic acids; N_{aa}, amino acids; N_h, hydrocarbons; N_T, all substrates tested.

the alcohols and about half of the carboxylic acids tested could be used by the bacterial communities. Few individual hydrocarbon substrates could be utilized; in about 70% of the samples, the populations of the communities tested showed a complete lack of capability of utilizing any hydrocarbons. The total substrate utilization (N_T) values were somewhat lower in offshore waters (mean $N_T = 0.51$) than in offshore sediments (mean $N_T = 0.61$) or in intertidal samples (mean $N_T = 0.61$).

Taxonomic Diversity

The Shannon Weaver diversity indices (H') and equitability indices (J') are in Tables 5 and 6. There was no significant difference ($\alpha = 0.2$) between the taxonomic diveristy H'

Table 5. Taxonomic diversity of Gulf of Alaska and Cook Inlet bacterial communities

	Water		Sediment	
Station	H′	J′	H′	J′
156	3.3	0.87	—	—
159	2.2	0.56	—	—
145	3.3	0.83	—	—
134	—	—	3.6	0.81
137	—	—	3.4	0.80
121	—	—	3.7	0.90
101	0.0	0.00	4.1	0.93
204	3.0	0.70	3.7	0.86
215	3.3	0.76	3.7	0.87
225	—	—	3.8	0.89
245	2.9	0.68	—	—
265	4.0	0.98	—	—
266	4.1	0.95	—	—
229	1.4	0.32	3.4	0.79
105	4.1	0.99	2.9	0.67
1	4.4	0.90	3.9	0.86
4	4.0	0.94	3.9	0.80
53	4.3	0.94	4.2	0.94
57	1.1	0.29	3.5	0.72
52	2.3	0.48	4.6	0.95
37	—	—	4.2	0.89
30	2.9	0.71	4.1	0.83
32	—	—	3.8	0.79
D	4.1	0.83	4.5	0.91
M	2.6	0.60	3.9	0.90
L	2.8	0.65	1.4	0.32
K	0.3	0.70	—	—
E	2.6	0.56	—	—
A	4.2	0.94	3.6	0.81
F	4.1	0.85	—	—

values for offshore water and offshore sediment communities, although the mean H′ value of offshore water communities was 3.0, compared to an H′ value of 3.8 for offshore sediment communities. Likewise, there was no significant difference in taxonomic diversities between intertidal water and intertidal sediment bacterial communities, although the mean H' for intertidal water (3.0) was lower than for intertidal sediment (3.4). The equitability values also were higher for offshore sediment (mean $J' = 0.84$) than for offshore waters (mean $J' = 0.70$), but this difference was significant only at the $\alpha = 0.1$ level. Particularly high H' and J' values were found in surface waters at the upper end of Cook Inlet (stations 265 and 266) and in a contiguous region southeast of the entrance to Cook Inlet (stations 1, 4, 53, and 105). Extremely low taxonomic diversities were found in water samples from stations 101 and K. There was no significant relationship between population size and taxonomic diversity in these communities.

Table 6. Summary of Physiological tolerance indices, nutrient utilization indices, and taxonomic diversities showing mean (and SD) values

	Intertidal	Offshore	West of Kodiak Island	Cook Inlet	East of Kodiak Island
			Water		
P_T	0.82 (0.07)	0.78 (0.09)	0.72 (0.06)	0.78 (0.08)	0.80 (0.11)
P_H	0.58 (0.06)	0.55 (0.14)	0.35 (0.01)	0.57 (0.09)	0.62 (0.12)
P_S	0.58 (0.12)	0.41 (0.24)	0.11 (0.12)	0.57 (0.09)	0.61 (0.25)
N_c	0.85 (0.26)	0.76 (0.18)	0.63 (0.22)	0.76 (0.17)	0.82 (0.15)
N_a	0.41 (0.25)	0.32 (0.14)	0.49 (0.09)	0.28 (0.10)	0.27 (0.14)
N_{ca}	0.61 (0.20)	0.51 (0.14)	0.45 (0.11)	0.46 (0.16)	0.59 (0.09)
N_{aa}	0.61 (0.24)	0.62 (0.20)	0.70 (0.15)	0.69 (0.20)	0.50 (0.21)
N_h	0.06 (0.11)	0.04 (0.90)	0.14 (0.15)	0.00 (0.00)	0.05 (0.07)
N_T	0.58 (0.18)	0.51 (0.11)	0.53 (0.11)	0.50 (0.13)	0.52 (0.10)
H'	3.0 (1.4)	3.0 (1.2)	2.9 (0.6)	2.9 (1.5)	3.0 (1.4)
J'	0.64 (0.30)	0.70 (0.30)	0.75 (0.17)	0.67 (0.35)	0.71 (0.27)
			Sediment		
P_T	0.79 (0.07)	0.69 (0.06)	0.59 (0.07)	0.71 (0.04)	0.72 (0.05)
P_H	0.50 (0.07)	0.55 (0.08)	0.52 (0.12)	0.51 (0.09)	0.59 (0.04)
P_S	0.55 (0.21)	0.28 (0.15)	0.17 (0.07)	0.18 (0.06)	0.41 (0.13)
N_c	0.97 (0.03)	0.87 (0.09)	0.79 (0.08)	0.86 (0.12)	0.90 (0.07)
N_a	0.54 (0.23)	0.46 (0.16)	0.43 (0.05)	0.35 (0.17)	0.55 (0.13)
N_{ca}	0.64 (0.09)	0.57 (0.16)	0.55 (0.09)	0.44 (0.17)	0.68 (0.08)
N_{aa}	0.70 (0.23)	0.74 (0.13)	0.67 (0.10)	0.78 (0.18)	0.74 (0.10)
N_h	0.00 (0.00)	0.13 (0.22)	0.10 (0.17)	0.12 (0.29)	0.14 (0.20)
N_T	0.65 (0.12)	0.61 (0.11)	0.57 (0.04)	0.55 (0.15)	0.67 (0.06)
H'	3.4 (1.4)	3.8 (0.4)	3.6 (0.2)	3.6 (0.4)	4.0 (0.3)
J'	0.74 (0.28)	0.84 (0.08)	0.84 (0.06)	0.84 (0.09)	0.85 (0.08)

Discussion

A high state of diversity was found to be a characteristic of bacterial communities in the Gulf of Alaska; the maintenance of high diversity appears to be an adaptive feature of subarctic marine bacterial communities. The measured taxonomic diversity indices for Gulf of Alaska bacterial communities were similar to those previously found for Arctic marine bacterial communities during summer [7]. Although seasonal differences in taxonomic diversity were found in arctic waters, no significant seasonal differences in taxonomic diversity indices were found for bacterial communities in subarctic Gulf of Alaska waters sampled in March and October. The calculated Shannon diversity indices for Gulf of Alaska bacterial communities were comparable to those reported by Martin and Bianchi [12] ($H' \approx 4$) for oligotrophic marine waters of the French Mediterranean region [12].

We hypothesized that taxonomic diversity of the heterotrophic bacterial community would be lower in surface waters than in sediments due to "stress" from irregular fluctuations of temperature and salinity, high light intensities, and low concentrations of available nutrients. This study may support this hypothesis since taxonomic diversity in offshore waters was lower than in offshore sediments, but the difference was not statistically significant indicating further studies conducted with larger numbers of isolates will be needed to establish the validity of this hypothesis. In Arctic marine ecosystems taxonomic diversity was significantly lower in surface water than in sediment [7]. We also hypothesized that taxonomic diversity would be lower for bacterial communities within intertidal habitats than for offshore communities; this was not found to be the case. The regular tidal fluxes do not appear to severely stress intertidal bacterial communities. Additionally, we postulated that there would be an inverse relationship between population size and taxonomic diversity; high population sizes should reflect competitive success of a limited number of populations. No significant correlation, however, was found between population size and diversity for Gulf of Alaska bacterial communities.

The question of interpretation of diversity indices must be raised. What does a diversity index say about the community? Communities with low taxonomic diversities are relatively homogeneous; they are specialized and generally have low genetic heterogeneity. Communities with high diversities are heterogeneous and have high informational content within the gene pool of the community. Although there is no simple relationship between community stability and diversity, overly specialized communities are particularly susceptible to disruption by environmental perturbations, whereas diverse communities are better adapted for self-maintenance in fluctuating environments. Communities existing under severe environmental stress generally are quite specialized and thus have low diversities. From our experience, an H' value of <3.0 appears to represent relatively "low" diversity for bacterial communities, indicative of some form of environmental "stress," which exerts selective pressure on the bacterial community and results in the predominance of specialized populations.

Diversity indices have been used for assessing environmental stress caused by pollution [1, 16, 17, 22]. Communities in ecosystems characterized by a lack of environmental variability, e.g., in benthic deep ocean trenches, and those under natural stress, e.g., in polar ice caps, similarly, may have low diversities. High diversities are expected for communities under biological accommodation. The diversity index reflects the informational content within the community and the "status" of the community, but does not define the specific causal factors responsible for establishing a particular level of informational heterogeneity.

The Shannon Weaver index used in this study is a general diversity index, i.e., it measures both the species richness (number of different "species") and the evenness (distribution of individuals within "species") components of diversity. H′ is theoretically sensitive to the sampled population size, especially when fewer than 100 representatives of the community are sampled. Bianchi (paper presented 2nd International Microbial Ecology Symposium, Sept. 1980, Warwick, England) has found, though, only relatively small differences (<0.4) in H' values for marine bacterial communities when the actual sample size used in the calculation was varied between 20 and 150 strains. Because H' is sensitive to changes in rare species, its use has been criticized [18, 22]. In our study the selection of representatives of the community (isolates) follows screening (plating), which eliminates rare species; we thus are really

calculating diversity of the major (dominant) populations within the community. The random selection of isolates from dilution plates also means that each individual in this study represents between 10^2 and 10^5 organisms in the original sample, depending on the concentration of bacteria in the sample. This fact can be used for justifying the validity of the results of this study even though only a limited number of strains were examined for each sample.

The selection procedures, however, raise an additional problem for diversity measurements; all plating procedures are selective and thus exclude a portion of the community from the study. We found that marine agar 2216 gave higher counts than other media, including MSWYE and low nutrient media, for these samples (unpublished data). We thus considered marine agar 2216 to be the "least selective," since it permitted growth of the highest proportion of populations in the community of any of the media tested. Horowitz and Atlas (unpublished data) have found that taxonomic diversities for populations capable of growth on low nutrient media are significantly lower than for populations capable of growth on marine agar.

The equitability index (J') estimates the evenness with which importance is apportioned between species. Questions have been raised about the appropriateness of using J' unless the entire community is censused [18], an impossibility for bacterial communities. It has been pointed out that J' is sensitive to changes in the number of species, especially if one utilizes the actual number of species observed for calculating J'; in our study the number of possible taxa in the community exceeds the sizes of the sampled populations, and therefore the maximal diversity used in calculating the denominator for J' was based on the assumption that each bacterial isolate could represent a different taxon; this assumption decreases the sensitivity of J' to small changes in the number of taxa observed.

The physiological tolerance indices developed in this study assess the abilities of the members of the bacterial community to grow under extreme conditions and not just simply to survive. The high physiological tolerance indices for Gulf of Alaska bacterial communities are somewhat surprising considering the relatively low annual variations in temperature, salinity, and pH which occur in these subarctic marine ecosystems. Most populations were quite tolerant of fluctuations in temperature, salinity, and pH, beyond the limits to which they ever are exposed naturally. The lower salinity tolerance indices in the western Gulf of Alaska compared to those in Cook Inlet and the eastern Gulf correlate with expected areas of freshwater input; little runoff should occur from the Aleutian Islands, while east of Kodiak Island there are major river sources of freshwater. The salinity tolerance indices also indicate that intertidal communities are more tolerant than offshore communities to variations in salinity; this is adaptive since nearshore communities are subjected to greater fluctuations in salinity than offshore communities. The lack of statistically significant differences in physiological tolerance indices between water and sediment communities may reflect extensive turnover in the water column, which was suggested by the temperature and salinity (density) measurements.

The nutritional versatility index (N_T) developed in this study is virtually synonymous with the average carbonaceous compound index (UAI) developed by Martin and Bianchi [12], although different substrates were used in determination of the two indices. Average UAI values for oligotrophic Mediterranean waters were found to be approximately 40% (equivalent to an N_T value of approximately 0.40). Martin and Bianchi [12] reported increases of UAI values to 52–57% during peak phytoplankton bloom, i.e., higher UAI values occurred during a period of organic enrichment than

under oligotrophic conditions. The mean N_T value of 0.53 for offshore Gulf of Alaska waters is somewhat higher than the UAI of 0.40 reported for oligotrophic Mediterranean waters; direct comparison, however, is not possible in an absolute sense since different substrates were used in the calculations. The higher N_T values found for Gulf of Alaska offshore sediment and intertidal communities than for offshore waters support the hypothesis that low nutrient ("oligotrophic") conditions support low nutritional versatility, whereas environments with higher nutrient availabilities support bacterial communities with higher nutritional versatilities.

Employing a large number of biochemically diverse substrates permits factoring the nutritional versatility into utilization indices for individual classes of compounds. The similar utilization indices for amino acids and carbohydrates are noteworthy, as are the similar proportions of bacterial populations exhibiting extracellular proteolytic and saccharolytic activities. In other regions of the Pacific Ocean, proteolytic capacities have been found to far exceed saccharolytic activities for bacterial populations [29]. The nutritional utilization indices presumably reflect substrate utilization patterns within the natural habitats of these communities. This suggests that the bacterial communities may be deriving their energy from phytoplankton-produced nutrients, which are rich in carbohydrates.

In summary, this study indicates that the maintenance of a high degree of informational heterogeneity is characteristic of bacterial communities, even those occurring in relatively stable environments such as marine ecosystems. It appears to be of adaptive advantage to maintain the capabilities to tolerate physiological stress beyond the range to which the habitat is ever subjected and to maintain a nutritionally versatile community in marine ecosystems.

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