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Genetic signatures of ecological diversity along an urbanization gradient

Ryan P. Kelly [Corresp., 1] , James L O'Donnell [1] , Natalie C. Lowell [2] , Andrew O. Shelton [3] , Jameal F. Samhouri [3] , Shannon M. Hennessey [4] , Blake E. Feist [3] , Gregory D. Williams [3]

[1] School of Marine and Environmental Affairs, University of Washington, Seattle, Washington, United States of America

[2] School of Aquatic and Fishery Sciences, University of Washington, Seattle, Washington, United States of America

[3] Northwest Fisheries Science Center, NOAA Fisheries, Seattle, Washington, United States of America

[4] Department of Integrative Biology, Oregon State University, Corvallis, Oregon, United States of America

Corresponding Author: Ryan P. Kelly
Email address: rpkelly@uw.edu

Despite decades of work in environmental science and ecology, estimating human influences on ecosystems remains challenging. This is partly due to complex chains of causation among ecosystem elements, exacerbated by the difficulty of collecting biological data at sufficient spatial, temporal, and taxonomic scales. Here, we demonstrate the utility of environmental DNA (eDNA) for quantifying associations between human land use and changes in an adjacent ecosystem. We analyze metazoan eDNA sequences from water sampled in nearshore marine eelgrass communities and assess the relationship between these ecological communities and the degree of urbanization in the surrounding watershed. Counter to conventional wisdom, we find strongly increasing richness and decreasing beta diversity with greater urbanization, and similar trends in the diversity of life histories with urbanization. We also find evidence that urbanization influences nearshore communities at local (hundreds of meters) rather than regional (tens of km) scales. Given that different survey methods sample different components of an ecosystem, we then discuss the advantages of eDNA—which we use here to detect hundreds of taxa simultaneously—as a complement to traditional ecological sampling, particularly in the context of broad ecological assessments where exhaustive manual sampling is impractical. Genetic data are a powerful means of uncovering human-ecosystem interactions that might otherwise remain hidden; nevertheless, no sampling method reveals the whole of a biological community.

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Genetic Signatures of Ecological Diversity Along an Urbanization Gradient

Ryan P. Kelly*[1], James L. O'Donnell[1], Natalie C. Lowell[2], Andrew O. Shelton[3], Jameal F. Samhouri[3], Shannon M. Hennessey[4], Blake E. Feist[3], Gregory D. Williams[3]

[1]School of Marine and Environmental Affairs, University of Washington, 3707 Brooklyn Ave NE, Seattle, Washington 98105, USA
[2]School of Aquatic and Fishery Sciences, University of Washington, 1122 NE Boat St, Seattle, Washington 98105, USA
[3] NOAA Fisheries, Northwest Fisheries Science Center, 2725 Montlake Blvd E, Seattle, Washington 98112, USA
[4] Department of Integrative Biology, Oregon State University, 3029 Cordley Hall, Corvallis, Oregon 97331, USA

*Corresponding author: rpkelly@uw.edu

22   Introduction
23   An enduring question of environmental science and ecology is how to measure the effects of human
24   activities on nearby biological communities and ecosystems. While in some cases such impacts are so
25   obvious that in-depth sampling is unnecessary to reveal them—such as paving over a wetland or
26   clear-cutting a rainforest—many human activities are likely to have more subtle effects on the
27   surrounding system. More adequately measuring human impacts is a core challenge as human
28   demands on natural resources continue to grow; such measurement is a prerequisite for identifying
29   sustainable development pathways.
30
31   The difficulty of surveying ecological communities generally results in a depth-vs.-breadth (i.e.,
32   specificity, (Rice & Rochet, 2005)) tradeoff in sampling strategy. For example, one might
33   comprehensively survey indicator taxa with the idea that they reflect larger changes to the ecological
34   community (Niemi & McDonald, 2004), or instead build limited data from many taxa into
35   multimetric indices in an attempt to reflect some more holistic sense of ecosystem integrity (Karr,
36   1981; Weisberg et al., 1997). Environmental DNA (eDNA) could substantially improve upon
37   existing survey methods by mitigating this tradeoff (Karr, 1981; Weisberg et al., 1997) by providing
38   in-depth views of ecosystems at levels of effort comparable to traditional sampling. Indeed, microbial
39   ecology has used these same core techniques for a decade or more (Tyson et al., 2004; Venter et al.,
40   2004; Yutin et al., 2007). Sequencing the diagnostic traces of genetic material in environmental
41   samples makes it possible to detect hundreds or thousands of animals, plants, and other organisms
42   from target habitats on ecological time scales of hours to days (Thomsen et al., 2012; Turner et al.,
43   2014). Yet although the rapid rise of eDNA as a tool for ecological studies has featured
44   methodological leaps and assessments of performance (Thomsen et al., 2012; Ficetola et al., 2014;
45   Thomsen & Willerslev, 2015; Evans et al., 2016), the value that community-level eDNA methods
46   add to traditional ecological sampling is just beginning to be apparent.
47
48   Measuring the influence of urban development on surrounding ecosystems is one application for
49   which the broad scope of eDNA sampling may be particularly useful, in part because of the many
50   pathways through which correlates of urbanization are likely to influence nearby ecological
51   communities. Accordingly, it may be difficult to identify diffuse urban impacts using traditional
52   ecological sampling alone, a particularly pressing problem as coastal urbanization increases globally
53   (Neumann et al., 2015). For example, in Puget Sound, Washington, USA, as in many coastal areas,
54   homeowners modify or harden their shorelines with concrete or other materials to protect their
55   properties from erosion(Scyphers, Picou & Powers, 2015). Permitting for shoreline armoring can
56   create conflicts between individual property rights and the communal benefits that arise from
57   unarmored shoreline, which include storm- and flood mitigation, habitat, waterline access, and other
58   services. Laborious manual sampling has documented some shifts in ecology as a result of shoreline
59   armoring (Heerhartz et al., 2014), but the ability to detect the ecosystem effects of any stressor
60   depends strongly upon the choice of taxa sampled. Making such informed decisions about the scope
61   of sampling is a general problem in ecology and environmental sciences.
62
63   We assessed the effects of upland watershed urbanization on nearshore estuarine eelgrass (Zostera
64   marina) communities in Puget Sound, Washington, USA using eDNA sampling at four pairs of more-
65   and less-urban sites (Fig. 1). Puget Sound has experienced rapid urbanization over the past century,
66   its human population increasing nearly six-fold since 1920 (Minnesota Population Center, 2011), and
67   nearly 4 million people live within 20 km of its shore (Bright et al., 2012). Although preserving

68 biogenic eelgrass habitat is now a policy priority for state and federal agencies (Puget Sound
69 Partnership, 2011; US Army Corps of Engineers, 2012), the effect of such urbanization on eelgrass-
70 associated fauna has been difficult to characterize with traditional sampling techniques (e.g., (Blake,
71 Duffy & Richardson, 2014) in Chesapeake Bay). As such, the steep urbanization gradient of Puget
72 Sound makes a compelling setting for evaluating eDNA as a means of detecting ecological
73 differences associated with human development. Here, we report significant changes in community
74 composition, diversity, and life-history composition associated with upland urbanization, as
75 measured by the genetic signatures of animals detected in the water.
76
77 ## Methods
78
79 We selected 8 sites in nearshore eelgrass habitats adjacent to watersheds along a gradient of
80 urbanization in Puget Sound, Washington, USA (Fig. 1). We employed a paired study design, in
81 which each more-urbanized site had a companion less-urbanized site at approximately the same
82 latitude (Fig. 1), controlling for well-known geographic, oceanographic, and ecological gradients
83 within the Sound (Dethier, 2010). These were a subset of the sampling sites described in (Samhouri
84 et al.), and included Big Gulch Creek (BG), Clearwater Casino (CC), Clinton-Whidbey (CW),
85 Manchester (MA), Pipers Creek (PC), Redondo Beach Cold Creek (RB), Sinclair Inlet (SI), and
86 Shingle Mill Creek (SM). Further site details and coordinates are given in (Samhouri et al.).
87
88 **Environmental Setting**
89 We chose sites on the basis of watershed-scale patterns of urbanization as further described in
90 (Samhouri et al.). All watershed basins were less than 1,000 ha, and contained perennial streams
91 (Puget Sound Nearshore Ecosystem Restoration Project, 2010). We used three different geospatial
92 data layers that captured various aspects of terrestrial urbanization—imperviousness (Fry et al.,
93 2011), roadways (OpenStreetMap, 2013), and percent developed land cover (NOAA, 2013)—as well
94 as percent shoreline armoring (Puget Sound Nearshore Ecosystem Restoration Project, 2010), to
95 characterize urbanization at each site. Each of these individual metrics positively covaried and
96 ordination techniques did not result in an index that was significantly more useful than any one
97 urbanization variable alone. We therefore simply used imperviousness (the area-weighted mean
98 percent cover of impervious surface) here as a proxy for human population and other urbanization-
99 related parameters. This layer represents highly- to completely impermeable surfaces such as
100 building roofs, concrete or asphalt roads and parking lots, concrete, asphalt or brick sidewalks,
101 pedestrian walkways, and malls. We used Environmental Systems Research Institute's (Esri) ArcGIS
102 software suite (v. 10.1) for all spatial analyses. Within site pairs, more-urban sites had higher values
103 of imperviousness than their less-urban counterparts. Other environmental variables such as sea-
104 surface temperature (mean, max, SD) and salinity did not systematically vary with urbanization
105 across our sites.
106
107 **eDNA Collection, Extraction, and Sequencing**
108 In July 2014, we collected 1-liter water samples for eDNA analysis at each of three transects within
109 each site, and kept these on ice until they could be processed in the lab (within hours of collection).
110 We filtered samples onto cellulose acetate filters (47mm diameter; 0.45um pore size) under vacuum
111 pressure, and preserved the filter at room temperature in Longmire's buffer following Renshaw et al.
112 (Renshaw et al., 2015). Deionized water (1-liter) served as a negative control for filtering. We
113 extracted total DNA from the filters using the phenol:chloroform:isoamyl alcohol protocol in
114 (Renshaw et al., 2015), resuspended the eluate in 200uL water, and used 1uL of diluted DNA extract

115 (1:100, diluted to reduce amplification inhibition) as template for PCR. Total DNA recovered from
116 samples (quantified using a Qubit fluorometer) was uncorrelated with site urbanization, indicating
117 our results were not due to an accumulation of eDNA in environments near urban sites. See
118 Supplementary Methods for additional sampling details.
119
120 We designed a novel set of primers using ecoPrimers (Riaz et al., 2011) to amplify approximately
121 114-140bp of mitochondrial 16S DNA from metazoans exclusively. These primers effectively
122 amplify most major animal phyla—including representatives from Chordata, Arthropoda, Mollusca,
123 Echinodermata, Nemertea, and others—while excluding non-metazoans entirely. Their sequences are
124 as follows (5' to 3'): 16s_Metazoa_fwd AGTTACYYTAGGGATAACAGCG; 16s_Metazoa_rev
125 CCGGTCTGAACTCAGATCAYGT.
126
127 We generated amplicons using a two-step PCR procedure, described in (O'Donnell James L. et al.,
128 2016), to avoid the taxon-specific amplification bias that results from the use of differentially indexed
129 PCR primers (commonly used to include multiple samples onto the same high-throughput sequencing
130 run to minimize costs). The specific PCR protocol is included in Supplementary Methods.
131
132 Each of the 24 environmental samples (3 samples/site, 8 sites) was amplified in a total of four PCR
133 reactions, twice with each of two distinct indexed primer sets (see Supplementary Methods for
134 indexing details), for a total of 24×4 = 96 individual sets of amplicons for sequencing. All but one of
135 the environmental samples (from site CW) was sequenced successfully. We also sequenced four
136 positive (Tilapia; *Oreochromis niloticus* tissue) and three negative controls, treated the same way
137 (twice with each of two indexed primers, for a total of 16 replicates of positive controls and 12
138 replicates of negative controls). Using tissue-derived DNA as a positive control allowed us to assess
139 non-amplifications as deriving from sample-specific, rather than PCR-condition-specific causes, and
140 selecting a non-native species as the tissue source allowed us to identify putative cross-contamination
141 among samples (all Tilapia sequences should derive from the laboratory rather than the field). 150bp
142 paired-end sequencing was carried out on an Illumina Nextseq.
143
144 **Sequence Processing and Bioinformatics**
145 We processed the Nextseq reads with a custom Unix-based script (O'Donnell, 2015), which calls
146 existing third-party scripts to move from raw sequence data to a quality-controlled dataset of
147 operational taxonomic units (OTUs). See Supplementary Methods for further bioinformatics details.
148
149 **Contamination Removal and Sequencing-Depth Normalization**
150 We used a Bayesian site-occupancy modeling method to estimate the probability of the OTU
151 representing a true positive detection (Ficetola et al., 2014; Lahoz-Monfort, Guillera-Arroita &
152 Tingley, 2015), fitting a binomial distribution to OTU occurrences across replicates of each
153 environmental sample, and rarefied OTUs in each sample using the smallest number of reads we
154 observed in a single sample (124,041 reads; (Gotelli & Colwell, 2001)) to standardize estimates of
155 taxon richness across samples. We generated 1000 rarefied datasets, and unless otherwise specified
156 below, we report results from one representative rarefied dataset consisting of $11.8×10^6$ reads reads
157 representing 1664 unique OTUs. The results do not depend significantly on the choice of rarefaction
158 replicates; for example, replicates differed only trivially in OTU richness (mean = 1662, sd = 9.5)
159 and did not show different spatial trends among replicates. For beta and gamma diversity measures,
160 in particular, OTU identity is of importance, and accordingly we show data derived from the entire
161 set of rarefaction replicates. Finally, for each water sample, we then averaged across the four PCR

162 replicates to estimate the abundance of each OTU. The complete eDNA dataset and analytical scripts
163 are publicly available on Dryad (Accession: doi:10.5061/dryad.04tq4). See Supplementary
164 Methods for further sequence processing details.
165
166 Our results do not depend strongly on decontamination or normalization procedures. Analyses of raw
167 OTU data (with no decontamination or normalization), of only the most common 100 OTUs, and of
168 only the least-common 500 OTUs, all produce the same trends in the quality-controlled and
169 normalized data (Suppl. Fig. 1). Similarly, rarefaction replicates retain the same strong trends
170 observed in our representative single replicate (Suppl. Fig. 2).
171
172 **Taxonomic Annotation of eDNA Sequences**
173 We annotated the final set of OTU sequences using the command-line BLAST+ software (Camacho
174 et al., 2009), searching against the complete NCBI nucleotide database (as of 12 October, 2015), with
175 word size = 7 and up to 1000 hits per query sequence retained. Those with no hits at e = 10^{-13} (<
176 ca. 85% identity) or better were treated as unannotated. Conflicting sequence annotations were
177 resolved using the last common ancestor algorithm implemented in MEGAN (Huson et al., 2011).
178 Disagreement among hits for a given OTU (i.e., where a single OTU is an equally good match to >1
179 taxon) was generally resolved at the level of taxonomic Family (83.2% of reads; Supp. Table 1).
180
181 **Data Analysis, Community Composition, and Diversity**
182 Although amplicon sequencing produces read counts that may contain valuable information about
183 target species abundances (Evans et al., 2016; Port et al., 2016) it remains difficult to interpret the
184 results of amplicon studies in the context of quantitative ecology because the precise relationship
185 between amplicon abundance and taxon abundance remains unknown and likely varies among taxa
186 (Evans et al., 2016). Accordingly, our analyses used presence/absence information derived from
187 sequence count data.
188
189 To assess the appropriateness of the spatial scale scale of sampling, we apportioned the observed
190 variance in ecological distance (Jaccard) among sites, among transects (within sites), and among PCR
191 replicates using a PERMANOVA. We calculated alpha diversity (= richness, or "density", sensu
192 (Gotelli & Colwell, 2001)) at both the OTU level and at the level of taxonomic family, treating
193 individual transects as replicates within a geographic site. We calculated beta diversity (sensu
194 Whittaker 1960, a measure of faunal change) both among transects within sites and among sites
195 (using transect means within sites to calculate the latter), focusing on OTUs because of the loss of
196 resolution associated with incomplete taxonomic annotation. We used Raup-Crick dissimilarity
197 (Chase et al., 2011) to ensure the observed beta diversity trends were not strictly dependent upon
198 changes in alpha diversity. We then evaluated gamma diversity (richness across sites within a region)
199 by generating an accumulation curve for three sets of sites: more-urban (N = 4 sites), less-urban (N =
200 4 sites), and all sites (N = 8). We sampled each set of sites (with replacement) 25 times at each step in
201 the accumulation curve to capture the distribution of site-specific richness.
202
203 We evaluated the relationships between diversity metrics and urbanization using linear and
204 generalized linear regression, as well as mixed-effects models. Our data were nested, with three
205 transect samples per site, and with each site having a single imperviousness value. To avoid
206 pseudoreplication among transects, we used site means for linear and generalized linear regressions.
207 For the mixed-effects models, we considered imperviousness as a fixed covariate and both site pair
208 and site identity as a random intercept terms.

209
210   To approximate life-history diversity, we organized all OTUs for which a Family-level annotation
211   was possible and classified each according to the following natural history attributes: Category
212   (epifauna, infauna, demersal, pelagic, terrestrial); Habitat (terrestrial, freshwater, intertidal, subtidal);
213   and Mobility (motile, sessile) using available reference materials such as (Kozloff, 1983). In some
214   cases, Families included species with a range of classifications (e.g., Cardiidae are a bivalve family
215   which includes infaunal and epifaunal cockles found both intertidal and subtidal habitats, with a
216   range of motility); in such cases the Family was listed as having both attributes. In all, there were 19
217   unique life-history niches that combinations of these attributes described (e.g., "Sessile Intertidal
218   Epifauna", etc.; Suppl. Table 2). We used these classifications to assess trends in the richness of these
219   life-history groups with respect to imperviousness, and in a principal components analysis to assess
220   differences in faunas among sites.
221
222   Finally, we used logistic regression and binomial tests to identify particular taxa, OTUs, and life-
223   history characteristics significantly associated with imperviousness. We conducted all analyses in R
224   v3.2.2 (R Core Team, 2015).
225
226   Results
227   Our representative rarefied eDNA (16s mtDNA) dataset recovered 1664 operational taxonomic units
228   (OTUs; mean of 1000 rarefaction replicates = 1662 OTUs ± 9.5) from a wide array of taxa
229   characteristic of the Puget Sound estuarine environment, with 10 animal phyla represented across 27
230   Classes, 65 Orders, and 135 Families (Table 1). Detections included iconic groups such as
231   *Metacarcinus* (i.e., *Cancer*) crabs, birds of prey (Accipitridae), and marine mammals (Delphinidae),
232   with the bulk of unique OTUs reflecting molluscs (45.1%), chordates (20.2%), and arthropods
233   (15.9%). 92% of reads (70% of OTUs) could be annotated with high confidence (e $<10^{-32}$). These
234   annotations included many animal taxa common to Puget Sound or the surrounding environment
235   (Table 1; see Supplemental Table 1 for full Family-level annotations).
236
237   The total variance in community-level ecological distance was attributable to differences among sites
238   (38.6%), among transects within sites (45.4%), or among PCR replicates of the same water samples
239   (15.9%; PERMANOVA with Jaccard distance, p < 0.001, 999 permutations, using OTU presence-
240   absence data). These results are consistent with earlier work in nearshore habitats (Port et al., 2016),
241   reflecting differences in eDNA profiles at spatial scales on the order of tens to hundreds of meters
242   (here, between transects separated by ca. 50-100m) and limited variability due to PCR and
243   sequencing processes. Ordination of OTU data shows transect samples largely, but not exclusively,
244   clustering within geographic sites (Suppl. Fig. 3).
245
246   **OTU Diversity and Urbanization**
247
248   OTU richness increased significantly with upland imperviousness (Fig. 2). Family-level richness
249   reflected the overall richness trends (Fig. 2). The results were highly robust to different
250   decontamination or normalization procedures (Suppl. Figs. 1 and 2).
251
252   Our paired sampling design controlled for potentially confounding geographically associated
253   differences among sites. We observe the same strong positive OTU richness correlation with
254   imperviousness in all 4 site pairs (Fig. 2), evidence that some aspect of urbanization—rather than
255   confounding spatial differences among site pairs—explains the observed pattern. A mixed-effects

256 model showed that imperviousness had a positive effect on richness after accounting for pair and site
257 identity (p = 0.018).
258
259 We calculated beta diversity (faunal turnover) at two different hierarchical scales: between sites and
260 among transects within sites. Consistent with the high level of heterogeneity we observed among
261 transects within sites, between-site beta diversity was uniformly high and did not differ for more- or
262 less-urban sites (Whittaker's beta (1960); Wilcoxon test, p = 0.58). Focusing on the individual
263 transects, however, revealed a strong decrease in within-site beta diversity with urbanization across
264 all four site pairs: communities became more homogeneous (transects within sites became more
265 similar) as watershed imperviousness increased (Fig. 2). Whittaker's beta (Whittaker, 1960)
266 decreased from a mean of 0.816 when imperviousness was less than 10% to a mean of 0.546 when
267 imperviousness was greater than 25% (R^2 = 0.93, p 8.4×10^{-5}). Raup-Crick dissimilarity among
268 transects showed a similar trend, indicating that the urbanization-associated trend in transect-to-
269 transect variation in eDNA composition was greater than expected due to changes in alpha diversity
270 alone.
271
272 Consistent with the trend in richness, more-urban sites had consistently higher gamma diversity than
273 less-urban sites, as reflected in the completely non-overlapping OTU accumulation curves in those
274 sets of sites (Fig 2). In total, more-urban sites had 1295 unique OTUs in 116 Families, while less-
275 urban sites had 790 OTUs from 80 Families, respectively.
276
277 **Life-History Diversity and Urbanization**
278
279 Assessing individual characteristics of habitat and mobility, taxa with differing natural history
280 characteristics were differentially associated with urbanization. For example, OTU richness tripled
281 with greater urbanization among sessile taxa (p = 1.7×10^{-5}), but motile taxa increased only
282 nonsignificantly (p = 0.054). Similarly, OTU richness in intertidal (p = 7.5×10^{-6}) and subtidal (p =
283 3×10^{-5}) taxa increased with imperviousness, terrestrial taxa showed no such trend (p = 0.16).
284
285 Community shifts among natural history types reflected richness changes by taxonomic groups. At
286 both the OTU- and Family level, eDNA richness increased with urbanization, most notably among
287 bivalves and gastropods (Suppl. Fig. 4). Family-level bivalve richness rose, for example, from an
288 average of 5 Families (37 OTUs) at <10% imperviousness to 7.4 Families (111 OTUs) at >25%
289 imperviousness (Poisson GLM with log-link, p<0.01 at family level, 10^{-16} at OTU level). Other
290 taxa showed a more gradual increase in richness with imperviousness (Suppl. Fig. 5), resulting in an
291 overall increase in the number of taxonomic Families. No abundant Family declined with
292 imperviousness.
293
294 Combining ecological characteristics into tri-variate life-history categories (e.g., "intertidal sessile
295 epifauna") revealed 19 unique Family categories present. Life-history richness increased with
296 urbanization (Suppl. Fig. 6; R^2 = 0.74, p = 0.006), from a mean of 12.5 life histories per site in less-
297 urban sites to a mean of 14.7 in more-urban sites, due to the concomitant increase in taxon richness at
298 more-urban sites. Normalizing by the number of Families present at each site reveals a strong
299 decrease in occupied life-histories-per-taxon with urbanization, from a mean of 0.66 in less-urban
300 sites to 0.47 in more-urban sites (although the trend is nonsignficant; Suppl. Fig. 6; R^2 = 0.38, p =
301 0.1). Ordination of the life histories results in identifiable sites and urbanization categories (Suppl.
302 Fig. 7), similar to the ordination plot for OTUs.

303
304   Beyond community measures, we identified 46 individual OTUs—again dominated by bivalves (33
305   OTUs from 5 families)—that were positively correlated ($p < 0.01$; logistic regression) with upland
306   imperviousness. Gastropods (5; limpets), urchins or sand dollars (7; not classifiable to family level),
307   and one fish OTU comprised the remaining 13 OTUs. Conversely, a single OTU was negatively
308   correlated with imperviousness (a mytilid mussel OTU). Providing some direct indication of human
309   influence on the nearshore Puget Sound, human OTU richness increased significantly with
310   imperviousness ($p = 0.01$; Poisson GLM), as did richness in selected taxa cultivated commercially
311   (*Panopea*, $p = 5 \times 10^{-4}$; *Bos*, $p = 0.005$) or introduced taxa (*Mya*, $p = 5.9 \times 10^{-6}$).
312

313   ## Discussion
314   All organisms leave behind residual genetic signatures in their environments, which provide the
315   opportunity to explore patterns of diversity and community structure that may not be possible
316   otherwise. Here, we recovered these signatures from nearshore estuarine habitats along an urban
317   gradient, revealing strong trends in the diversity of animals and ecological roles present. While alpha
318   (site richness) and gamma (regional richness) diversity strongly increased with upland urbanization,
319   more-urban sites were significantly more homogeneous (within sites) than less-urban sites. Life-
320   history diversity largely paralleled these same trends, with a greater richness of ecological life
321   histories among taxa found in more urban areas, but greater redundancy in life-history niches among
322   these taxa. Taken together, our results suggest that more urbanized upland areas support larger suites
323   of species, with less compositional variation, in and around downstream eelgrass habitats. Further,
324   we find evidence that the mechanisms of land-sea interaction act at watershed scales, rather than at
325   the larger scale of Puget Sound. These results also substantiate the idea that eDNA can be a powerful
326   addition to traditional means of assessing human-ecosystem interactions.
327
328
329   **Trends in Diversity and Ecological Function with Urbanization**
330   Although dense urban areas do not necessarily decrease biodiversity in general (Ives et al., 2016) and
331   the effects of urbanization on species richness appear to be taxon- and spatial-scale-specific (Shochat
332   et al., 2006), the positive richness trend we see in Puget Sound 16s eDNA is nevertheless striking.
333   Several plausible mechanisms could explain the increase in 16s eDNA richness, although our study
334   design prevents us from assessing causation explicitly.
335
336   One likely explanation for the trend is the interaction between fauna sampled with eDNA and the
337   kinds of habitat that are more common near urban settlements. Our study design attempted to sample
338   identical habitats across all sites, however, there may be unobserved differences in habitats. For
339   example, our results may reflect an increase in availability of muddy habitats associated with
340   urbanization, and a concomitant increase in richness within those habitat patches.
341
342   A second plausible mechanism is that greater anthropogenic nutrient inputs into urban areas yields
343   greater productivity. Urbanization greatly increases total nitrogen fluxes into rivers and estuaries
344   (Rabalais et al., 2009; Mohamedali et al., 2011), and increased primary productivity, which may
345   result from such fertilization, is generally—but not strictly—associated with increased secondary
346   productivity (Leslie et al., 2005) and taxonomic diversity (Mittelbach et al., 2001; Whittaker &
347   Heegaard, 2003). However, Puget Sound, like many coastal systems, is dominated by marine derived
348   nutrients (Mackas & Harrison, 1997; Mohamedali et al., 2011), suggesting that any fertilization effect
349   from small watersheds such as those we focus on here is unimportant. Each of the urban sites we

350 sampled also has a wastewater treatment facility in the vicinity. However, all outflows from
351 treatment facilities occur in deep water offshore, far from our sampling areas, making any effect of
352 fertilization indirect at best. Wastewater treatment facilities could also increase richness by
353 concentrating genetic material originating elsewhere. However, although the increase in human
354 OTUs we observe is consistent with this hypothesis, the great majority of DNA recovered stems from
355 Puget Sound species rather than taxa likely to be dominant in human waste streams and none of our
356 results is driven by exogenous eDNA.
357
358 Intriguingly, as eDNA communities increased in richness with urbanization, they also became more
359 homogeneous. Others have found that increased subtidal sedimentation—associated with the kind of
360 low-energy environments we sampled here—tended to make rocky reef communities more similar to
361 one another (Balata, Piazzi & Benedetti-Cecchi, 2007), and nutrient enrichment can have the same
362 effect in lakes (Donohue et al., 2009). Our results are consistent with the idea that urbanization tends
363 to homogenize communities even though the total number of unique taxa may increase (Urban et al.,
364 2006; Piazzi & Balata, 2008). A similar effect is also associated with non-indigenous species
365 introductions (Rosenzweig, 2001), but non-indigenous species do not drive the trends we observe
366 here. Although a comprehensive list of native taxa is not available against which to compare our
367 results, the annotated Families are nearly all familiar native taxa from Puget Sound; moreover, the
368 trends we report are consistent across even small subsets of the data (Suppl. Figs 1 & 2), indicating
369 our results do not depend upon a small set of potentially non-indigenous taxa.
370
371 More generally, beta diversity can help disentangle the ecological forces behind community
372 assembly(Condit et al., 2002; Tuomisto, Ruokolainen & Yli-Halla, 2003; Dornelas, Connolly &
373 Hughes, 2006; Chase, 2007, 2010; Chase & Myers, 2011), by distinguishing niche-related
374 deterministic processes from stochastic ones. Our observations are consistent with the idea that that
375 deterministic, possibly niche-related, processes significantly influence Puget Sound nearshore
376 communities: transect-to-transect beta-diversity declined steadily with an environmental gradient of
377 urbanization independent of geographic space, and per-taxon life-history richness similarly declined
378 (albeit nonsignificantly) across this same environmental gradient.
379
380 We expect different ecological patterns to be apparent at different spatial scales, and conversely, the
381 scales of ecological patterns provide hints about the mechanisms driving those patterns (Levin,
382 1992). Given the site- and transect-level differences we observed, it seems likely that the mechanisms
383 mediating the human-ecosystem interactions in Puget Sound occur at the watershed scale (~100s of
384 meters), rather than at larger scales of urbanization (e.g., Puget Sound scale, 10s of km). Urbanization
385 does not appear to homogenize communities across sites; more-urban sites were just as different from
386 one another as less-urban sites were, and the gamma diversity accumulation curve indicated that
387 additional urbanized sites continued to feature new OTUs. The real differences associated with
388 urbanization occurred within sites, with more-urban sites being more homogeneous (i.e., smaller
389 differences among transects) than less-urban sites. In sum, we did not observe a generalized "urban"
390 fauna at urban sites. Instead, each urbanized site had a distinct ecological community, exhibiting
391 greater richness, lower spatial variability, and greater life-history redundancy than a similar less-
392 urban site, but without a shared, characteristic community.
393
394 Regardless of the precise mechanism, the eDNA data reveal a strong signal of land-sea interaction
395 (Samhouri & Levin, 2012). Especially in light of ever-increasing human population density in coastal

396 areas worldwide (Neumann et al., 2015), our results suggest that eDNA can be a powerful tool for
397 uncovering human-ecosystem interactions that might otherwise remain hidden.
398
399 **eDNA as an Emerging Tool for Ecological Analysis: Scale and Selectivity**
400
401 Ecology and related disciplines depend upon techniques to sample and describe communities,
402 ecosystems, and their properties. However, any one set of samples yields a necessarily biased view of
403 the world; ten different sampling methods can yield ten different results even with small numbers of
404 target taxa (Valentini et al., 2015). This selectivity is usually intentional—e.g., settlement plates are
405 designed to sample bryozoans rather than seals—but where unintentional, such selectivity can bias
406 results in ways that often remain unexplored (Baker et al., 2016).
407
408 The rise of eDNA sampling has led to studies comparing molecular techniques either to traditional
409 methods or to known communities. Single-taxon qPCR studies have compared favorably with
410 traditional surveys in terms of detection rates (Jerde et al., 2011; Takahara et al., 2012; Eichmiller,
411 Bajer & Sorensen, 2014a; Laramie, Pilliod & Goldberg, 2015), with sequence-based (i.e.,
412 metabarcoding) analyses proving more difficult to interpret relative to traditional sampling, in part
413 because of difficulty of comparing detection rates across methods (Cowart et al., 2015). eDNA is an
414 in-depth sampling technique that yields interesting and repeatable results; however, the absence of
415 eDNA detection does not imply absence of taxon of interest (Roussel et al., 2015). One eDNA locus,
416 or even several loci, will not reveal all of the taxa present in an area. Indeed, eDNA sampling with a
417 different genetic locus—or even a different set of primers at the same locus—would have yielded a
418 different suite of taxa (e.g., (Cowart et al., 2015)).
419
420 Consistent with earlier observations from a study of *Zostera* communities (Cowart et al., 2015), our
421 single eDNA locus failed to detect epifauna known from the sampled sites. Hippolytid and crangonid
422 shrimp, littorinid snails, idoteid isopods, and others were common in the field but absent from the
423 eDNA (Samhouri et al.), likely due to amplification bias and primer mismatches. Such performance
424 does not make eDNA inappropriate for biodiversity monitoring, but rather put sequenced-based
425 sampling in the company of every other sampling technique (Shelton et al., 2016). Because the "true"
426 community remains unknown (Shelton et al., 2016), it is impossible to evaluate error rate in an
427 absolute sense for any field-based method. Given that nearly all (>99%) of the taxa we detect here
428 are known from local waters or the surrounding area, our false positive rate for eDNA appears to be
429 very low. We suggest that community-level eDNA surveys be viewed in a light appropriate to any
430 new sampling technique: biased relative to some unknown true value, but significantly
431 complementing existing imperfect sampling techniques such as tow nets and other manual
432 collections.
433
434 Finally, our results suggest that eDNA recovers fine-scale differences in ecological communities,
435 such that transects tens of meters apart can be as different as transects kilometres apart. Nearly half
436 (45%) of the variance in ecological distance was due to differences between transects at the same
437 sampling site, consistent with the fine-grained spatial resolution reported by (Port et al., 2016) in
438 another nearshore eDNA amplicon study. This observation supports a growing sense that eDNA may
439 travel only limited distances away from its sources, depending upon the environmental context
440 (Eichmiller, Bajer & Sorensen, 2014a; Deiner & Altermatt, 2014; Laramie, Pilliod & Goldberg,
441 2015), and provide further evidence that eDNA variation at small spatial scales is more likely signal
442 than noise. Nevertheless, it is not obvious why eDNA might exhibit such variability on the order of

443 tens of meters (in this study and in others; (Eichmiller, Bajer & Sorensen, 2014b; Port et al., 2016)),
444 but simultaneously feature the genetic signatures of species that are not in the immediate vicinity.
445 Examples here include terrestrial and aquatic taxa, whose DNA must have travelled at least some
446 distance into the intertidal habitats sampled. One explanation is that—if genetic material is detectable
447 as a steady-state balance of generation, degradation, advection, and diffusion away from a point
448 source—such transportation is to be expected at low levels, even when the bulk of genetic material
449 remains close to its source. Consistent with this model, the great majority of taxa in our data are
450 marine, with non-marine taxa only at low levels (6% of reads including human DNA; 3% not
451 including human DNA; see Suppl. Table 1).
452
453 **Conclusion**
454
455 Sampling using eDNA sequencing offers a breadth of taxonomic coverage valuable for both basic
456 and applied ecology. Our results demonstrate the power of this technique for assessing human-
457 ecosystem interactions in a nearshore environment, revealing significant trends in animal diversity
458 and life history likely linked to human alteration of upland habitats. Like all sampling methods,
459 eDNA offers a view of the world that is both biased and incomplete, in the sense that surveys using a
460 given gene will detect some taxa and not others. Traditional sampling has analogous drawbacks.
461 Here, data from a single genetic locus provided a reasonably holistic view of the Puget Sound
462 nearshore ecosystem—encompassing taxa as diverse as high-intertidal barnacles, birds of prey, and
463 subtidal bivalves, from a wide variety of ecologically-linked habitats—that strongly suggests
464 urbanization has generated unexpected consequences for a large number of nearshore taxa,
465 particularly those with sessile lifestyles. Consistent with (Samhouri et al.; Blake, Duffy &
466 Richardson, 2014; Ives et al., 2016), we see these results as a counterexample to the idea that humans
467 uniformly decrease biodiversity. Rather, the observation that more urbanized areas support larger, but
468 more homogeneous, suites of species indicates a more nuanced effect of human alteration on
469 nearshore communities.
470

Additional Information

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Figure captions

489
490 FIGURE1

491   Study site sampling locations and associated stream basins. Matched site pairs share a stream basin color.
492   More urban sites are open boxes, less urban are black boxes. Two-letter codes correspond to site names in
493   the Methods. Brown shading indicates areas with greater than 50% imperviousness.
494
495   FIGURE 2
496   **Top Row**: Rarefied OTU richness and imperviousness—a proxy for urbanization—in Puget Sound. Analysis of
497   a single focal rarefaction draw. *Left* : Rarefied 16s eDNA richness (solid trendline reflects OTUs; dashed
498   trendline reflects taxonomic Families). Site means (larger circles) among transect-level data points (smaller
499   circles). Family data shifted slightly for clarity. *Right*: The same data by site pair (N = 4 pairs of more- and
500   less-urban sites), means plotted. Red lines indicate significant trends, grey lines indicate non-significant
501   trends. Legends correspond to 2-letter site codes in Fig. 1. **Middle Row**: *Left*: Mean among-transect (within-
502   site) Whittaker's beta diversity for each of 1000 rarefaction draws from the overall OTU dataset, rarefied to
503   create comparable sample sizes (N = 1.3×10^5 OTUs per transect). Linear regression on site means,
504   R^2=0.95, p = 3.38×10^{-5}. *Right*: Site means highlight the site-pair trends for single focal rarefaction draw.
505   **Bottom Row**: Regional (gamma) diversity, in OTUs-per-site, as an accumulation curve. Boxplots show
506   variance due to sampling each each set of sites (with replacement) 1000 times from a pool of 1000 rarefaction
507   draws from the overall OTU dataset, rarefied to create comparable sample sizes (N = 1.3×10^5 OTUs per
508   transect). Best-fit logarithmic curves shown for more-urban sites (N = 4), less-urban sites (N = 4), and all sites
509   (N = 8).
510
511
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676

Figure 1

Study site sampling locations and associated stream basins.

Study site sampling locations and associated stream basins. Matched site pairs share a stream basin color. More urban sites are open boxes, less urban are black boxes. Two-letter codes correspond to site names in the Methods. Brown shading indicates areas with greater than 50% imperviousness.
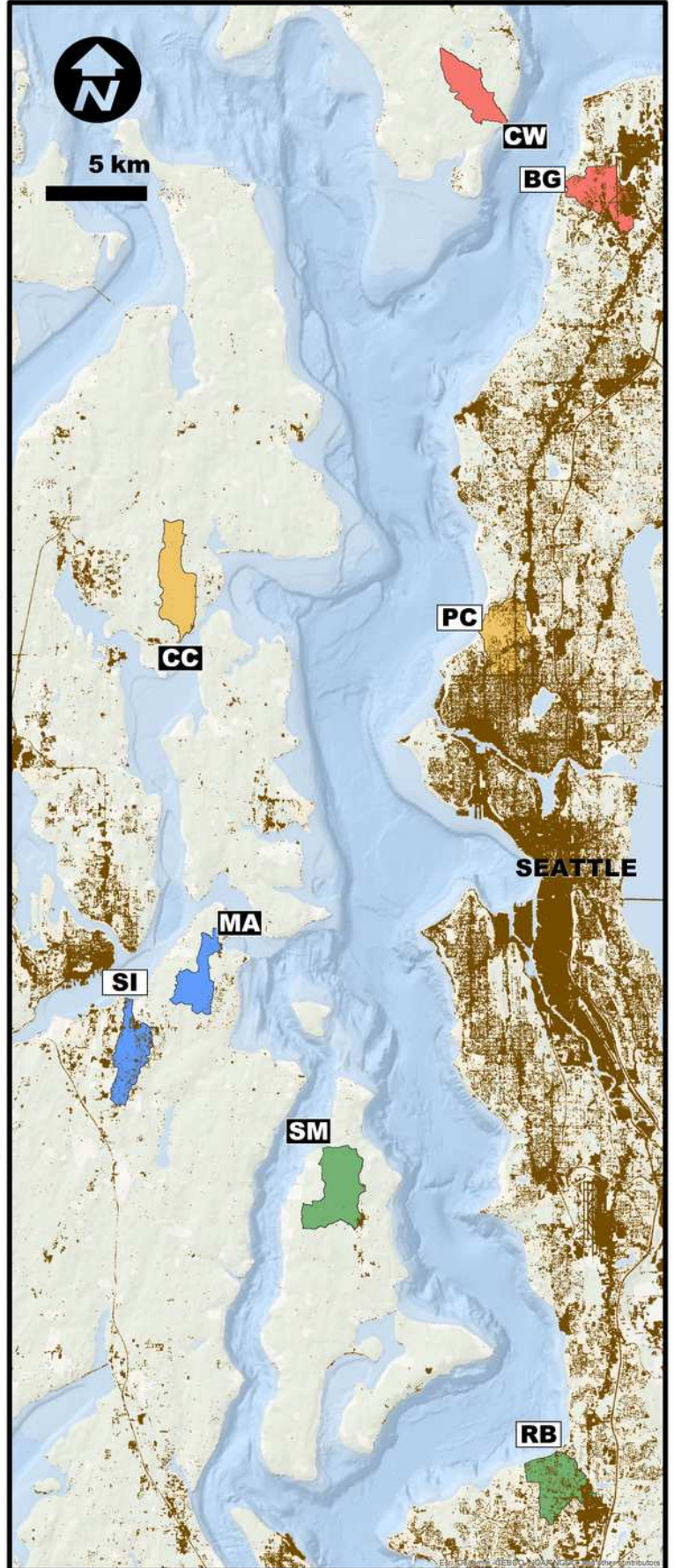
Figure 2 (on next page)

Alpha, beta, and gamma diversity recovered from water samples in Puget Sound along an urbanization gradient.

Top Row: Rarefied OTU richness and imperviousness—a proxy for urbanization—in Puget Sound. Analysis of a single focal rarefaction draw. **Left** : Rarefied 16s eDNA richness (solid trendline reflects OTUs; dashed trendline reflects taxonomic Families). Site means (larger circles) among transect-level data points (smaller circles). Family data shifted slightly for clarity. **Right**: The same data by site pair (N = 4 pairs of more- and less-urban sites), means plotted. Red lines indicate significant trends, grey lines indicate non-significant trends. Legends correspond to 2-letter site codes in Fig. 1. **Middle Row**: **Left**: Mean among-transect (within-site) Whittaker's beta diversity for each of 1000 rarefaction draws from the overall OTU dataset, rarefied to create comparable sample sizes (N = 1.3×10^5 OTUs per transect). Linear regression on site means, $R^2 = 0.95$, p = 3.38×10^{-5}. **Right**: Site means highlight the site-pair trends for single focal rarefaction draw. **Bottom Row**: Regional (gamma) diversity, in OTUs-per-site, as an accumulation curve. Boxplots show variance due to sampling each each set of sites (with replacement) 1000 times from a pool of 1000 rarefaction draws from the overall OTU dataset, rarefied to create comparable sample sizes (N = 1.3×10^5 OTUs per transect). Best-fit logarithmic curves shown for more-urban sites (N = 4), less-urban sites (N = 4), and all sites (N = 8).
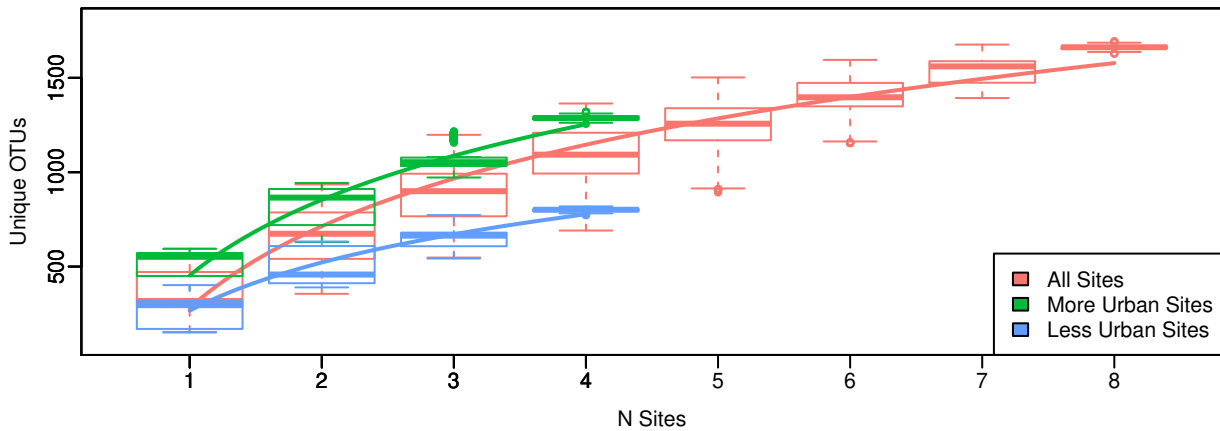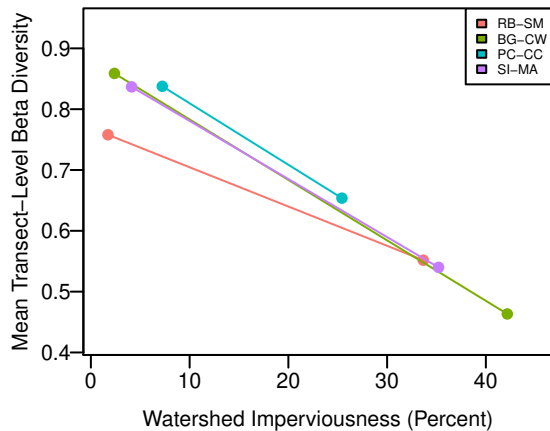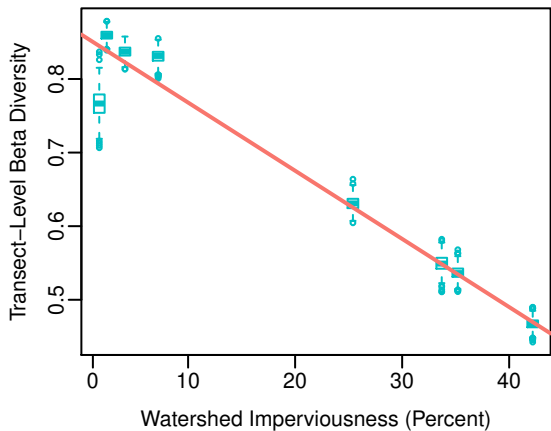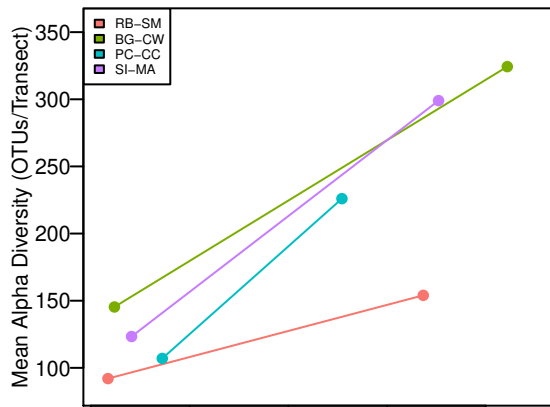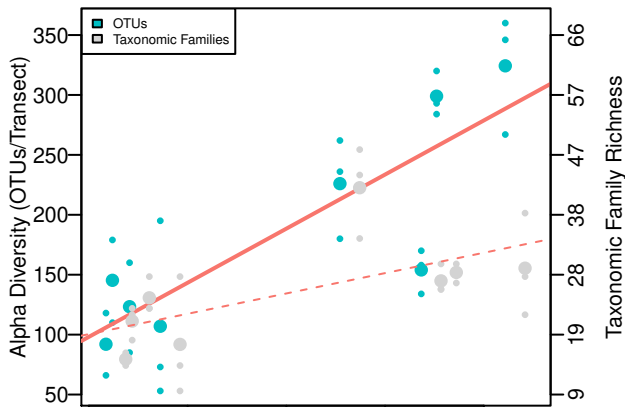
Table 1_(on next page)

Summary of 16s Read Annotations

Summary of taxonomic annotations for 16S reads; for full annotations, see Supplementary Material.

Tables

PHYLUM	CLASSES	ORDERS	FAMILIES	OTHER RANK
MOLLUSCA	3	6	34	9
ARTHROPODA	6	13	29	10
CHORDATA	5	28	37	28
BRYOZOA	1	2	10	2
ECHINODERMATA	5	8	13	4
NEMERTEA	3	2	8	0
HEMICHORDATA	1	1	1	0
ENTOPROCTA	1	1	1	0
PORIFERA	1	2	2	0

TABLE1

Summary of taxonomic annotations for 16S reads; for full annotations, see Supplementary Material.