Research article

Exposure to synthetic hydraulic fracturing waste influences the mucosal bacterial community structure of the brook trout (*Salvelinus fontinalis*) epidermis

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Supplementary

Figure S1. Box and whisker plots illustrating taxonomic richness for 4 experimental hydraulic fracturing waste treatments (control, low, medium and high). Note differences in y-axis scales.

Table S1. Statistical output comparing brook trout epidermal bacterial community structure (Analysis of Similarity, ANOSIM; Community Richness ANOVA; and Shannon-Weaver Diversity ANOVA) across 4 experimental hydraulic fracturing waste treatments (control, low, medium, and high) and at three taxonomic levels (OTUs, genus, and family). Bold and italicized text represents significant differences among treatments.

| Community metric          | Day | OTU Test statistic | P-value | Genus Test statistic | P-value | Family Test statistic | P-value |
|---------------------------|-----|--------------------|---------|----------------------|---------|-----------------------|---------|
| Analysis of Similarity    | 15  | $R = 0.28$         | 0.001   | $R = 0.17$           | 0.001   | $R = 0.12$            | 0.002   |
| (ANOSIM)                  |     |                    |         |                      |         |                       |         |
| Richness (ANOVA)          | 15  | $F_{3,32} = 1.06$   | 0.38    | $F_{3,32} = 0.27$    | 0.84    | $F_{3,32} = 0.99$     | 0.41    |
| Shannon-Weaver Diversity  | 15  | $F_{3,32} = 1.21$   | 0.32    | $F_{3,32} = 4.17$    | 0.01    | $F_{3,32} = 6.29$     | 0.001   |
| (ANOVA)                   |     |                    |         |                      |         |                       |         |

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Table S2. Ranges of ammonium and nitrite concentrations in 4 hydraulic fracturing waste treatments and the flow-through holding tank on day 13 of experimental exposure.

| Treatment               | Ammonium (mg NH$_4^+$) | Nitrite (mg NO$_2^-$) |
|-------------------------|-------------------------|-----------------------|
| Holding tank (flow through) | <0.2                  | <0.05                 |
| Control                 | 0.4–1.5                 | 0.5–0.8               |
| Low                     | 0.8–1.5                 | 0.2–0.8               |
| Medium                  | 0.8–1.0                 | >0.8                  |
| High                    | 1.5–2.0                 | 0.3–0.8               |

Supplemental Material A (microbiol-04-03-413-s2): Relative sequence abundance of bacterial taxa (OTUs) from 4 HF waste exposure treatments (control, low, medium, and high) implemented by Krona software [1] (HTML files,). See Table 1 for specific treatment concentrations.

References

1. Ondov BD, Bergman NH, Phillippy AM (2011) Interactive metagenomic visualization in a Web browser. *BMC Bioinformatics* 12: 385.

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