Table S1. PCR protocols and results for amplifying DNA of Rocky Mountain tailed frogs (*Ascaphus montanus*) and Idaho giant salamanders (*Dicamptodon aterrimus*) from stream water.

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| Extraction protocol | PCR protocol | Results |
| *Phase 1 (early fall), Nasty Creek only, one 10-L and two 5-L samples* |
| MoBio Extraction: UltraClean® Soil DNA isolation kit (MoBio Laboratories, Inc.)  | PCR protocol 1: 1 µl DNA sample, 1X AmpliTaq Gold PCR Buffer (Applied Biosystems), 3.0 mM MgCl2, 0.4 mM of each dNTP, 0.2 µM of ASMO primers or 0.3µM of Dicamp primers (Table 2), 0.4 units of AmpliTaq Gold DNA polymerase in a 10 µl reaction. Initial incubation at 94ºC for 15 minutes followed by 50 cycles of 94ºC for 1 minute, a touchdown step from 58-50ºC that decreased by 0.5ºC per cycle until the lower temperature was reached for 30 seconds, and 72ºC for 30 seconds, finishing at 72ºC for 5 minutes. | No product, not inhibited |
| DNeasy extraction: DNeasy Tissue and Blood Kit (Qiagen, Inc.) | PCR protocol 1 | Inhibited |
| DNeasy extraction | PCR Protocol 2: PCR Protocol 1 with addition of 0.75 mg/ml bovine serum albumin (BSA) | Both species detected in 10L sample only. |
| DNeasy extraction | PCR Protocol 3: 1X Qiagen Multiplex PCR Master Mix (Qiagen, Inc.), 0.5X Q solution, 0.2 µM of ASMO primers and/or 0.3 µM of Dicamp primers, and 1 µl extracted DNA in a 7 µl reaction. Initial incubation at 95ºC for 15 minutes followed by 50 cycles of 94ºC for 30 seconds, a touchdown step from 58-50ºC that decreased by 0.5ºC per cycle until the lower temperature was reached for 90 seconds, and 72ºC for 60 seconds, finishing at 60ºC for 30 minutes. | Both species detected in all samples.  |
| *Phase 2 (early spring), 5 streams sampled, 5L each.* |
| DNeasy extraction | PCR Protocol 3 (multiplex) | Weak amplification for Idaho giant salamanders, no detection of Rocky Mountain tailed frogs. |
| DNeasy extraction, QIAshredder (Qiagen, Inc.)  | PCR Protocol 4: PCR Protocol 3 (multiplex) without Q solution and with 55 total cycles | Rocky Mountain tailed frogs detected in 4/5 streams, Idaho giant salamanders in 5/5 streams. |
| DNeasy extraction, QIAshredder (Qiagen, Inc.)  | PCR Protocol 5: PCR Protocol 4 with Qiagen Multiplex Plus PCR kit. | Rocky Mountain tailed frogs detected in 2/5 streams, Idaho giant salamanders in 4/5 streams. |