

1 **Supplementary Information**

2 **Environmental RNA reflects seasonal changes in the metabolic activity of stream salamanders**

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26 **Supplementary Table S1.** Results of the linear mixed-effects models (LMMs) assessing the influence
 27 of season, species, their interaction, sampling day, and lateral sampling position on log-transformed eNA
 28 concentrations. Separate models were fitted for eDNA and eRNA. Stream was included as a random
 29 intercept. Significant P-values ($P < 0.05$) are shown in bold.

Term	eDNA		eRNA	
	Estimate ± SE	P	Estimate ± SE	P
Intercept	2 ± 0.1	< 0.001	0.2 ± 0.06	0.05
Season (winter)	-0.3 ± 0.07	< 0.001	-0.07 ± 0.04	0.09
Species (<i>G. porphyriticus</i>)	-0.01 ± 0.06	0.9	0.3 ± 0.04	< 0.001
Season × species	0.8 ± 0.1	< 0.001	-0.3 ± 0.06	< 0.001
Day (2)	-0.05 ± 0.06	0.4	-0.009 ± 0.04	0.8
Day (3)	0.007 ± 0.06	0.9	0.06 ± 0.04	0.08
Position (left)	0.06 ± 0.06	0.3	-0.03 ± 0.04	0.3
Position (right)	0.03 ± 0.06	0.6	-0.03 ± 0.04	0.4

30 SE: Standard error

31 **Supplementary Table S2.** Seasonal contrasts (fall – winter) in log-transformed eDNA and eRNA
32 concentrations derived from the LMMs presented in Supplementary Table S1. For each species,
33 estimated marginal means were computed and contrasted between fall and winter. Positive contrast
34 values indicate higher concentrations in fall relative to winter. Results are averaged over sampling day
35 and lateral sampling position. Significant P-values ($P < 0.05$) are shown in bold.

Species	eDNA (fall – winter)		eRNA (fall – winter)	
	Estimate ± SE	P	Estimate ± SE	P
<i>E. bislineata</i>	0.3 ± 0.07	< 0.001	0.07 ± 0.04	0.09
<i>G. porphyriticus</i>	1.1 ± 0.07	< 0.001	0.4 ± 0.04	< 0.001

36 SE: Standard error