

Supplementary material

Intraspecific competition reduces the quantity of excreted nutrients in tadpoles

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Quantifying the amount of mineralised nutrients from egestion in tadpoles

This experiment tested the quantity of mineralised nutrients from egesta. The method consists of incubating a known amount of egesta and by quantifying the concentration of nutrients released in the water column (Lies and Haglund 2007). We collected tadpoles from the same paddy fields as for the excretion experiment. Upon capture, the tadpoles were first transferred in plastic tubs with aged distilled water to remove attached materials, and subsequently transferred into 30 × 35-cm plastic trays containing distilled water. Fecal pellets were collected as they deposited at the bottom of the trays. We did not separate tadpoles by species because it was difficult to gather enough pellets for some species due to their low abundance. The solutions containing pellets were transported to the laboratory where they were shaken and divided into two equal parts, and filtered through Whatman GF/A glass microfibre filters. One filter with egested materials was incubated in 4-L rectangular container with 2.5 L of distilled water, and the second filter was dried to estimate weight of fecal pellets. We replicated the procedure three times. The containers were covered with transparent plastic film to limit air exchange, and were placed in a room maintained at 24°C. We sampled 20 mL of water from each container on days 0, 1, 4, 8, 12, 16, and 20. We sampled water column at half depth, and the syringe was used to gently mix the water carefully before water sampling. Water samples were filtered through Gelman AE filters and were analysed for ammonia and TDP as for excretion (cf. manuscript). Concentrations of nutrients N and P released in the water column are expressed by μmol nutrient per amount of dry fecal pellet (μmol nutrient g^{-1} pellet). To determine initial elemental composition of fecal pellets, samples of dry fecal pellets ($n = 2$) were analysed for C and N on a CHN analyser (Flash EA 1112 elemental analyser, ThermoFisher Scientific, Yokohama, Japan), and P with HCl digestion followed by soluble reactive P (SRP) analysis using HACH Phosphorus Reactive and Total TNTplus 843.

Tadpole egesta were nutritionally poor (0.61% N, 0.02% P, 7.5% C, C/N =12.29, dry mass). N- and P-bound nutrients were mineralised at different rates: concentration in N steadily increased in the incubating water column and reached values that were almost 20 times (on average 105 $\mu\text{mol N g}^{-1}$ dry egesta) higher than its value at Day 1 ($\chi^2 = 92.84$, d.f. = 6, $P < 0.001$), indicating that egesta were latent sources of nutrients. Concentration in P was relatively low and tended to show a hump shaped pattern with a maximum concentration at day 4 reaching 2.93 $\mu\text{mol P g}^{-1}$ dry egesta; the amount of P in the water column significantly varied ($\chi^2 = 20.47$, d.f. = 6, $P = 0.002$) during the 20 days of incubation (Fig. S1).

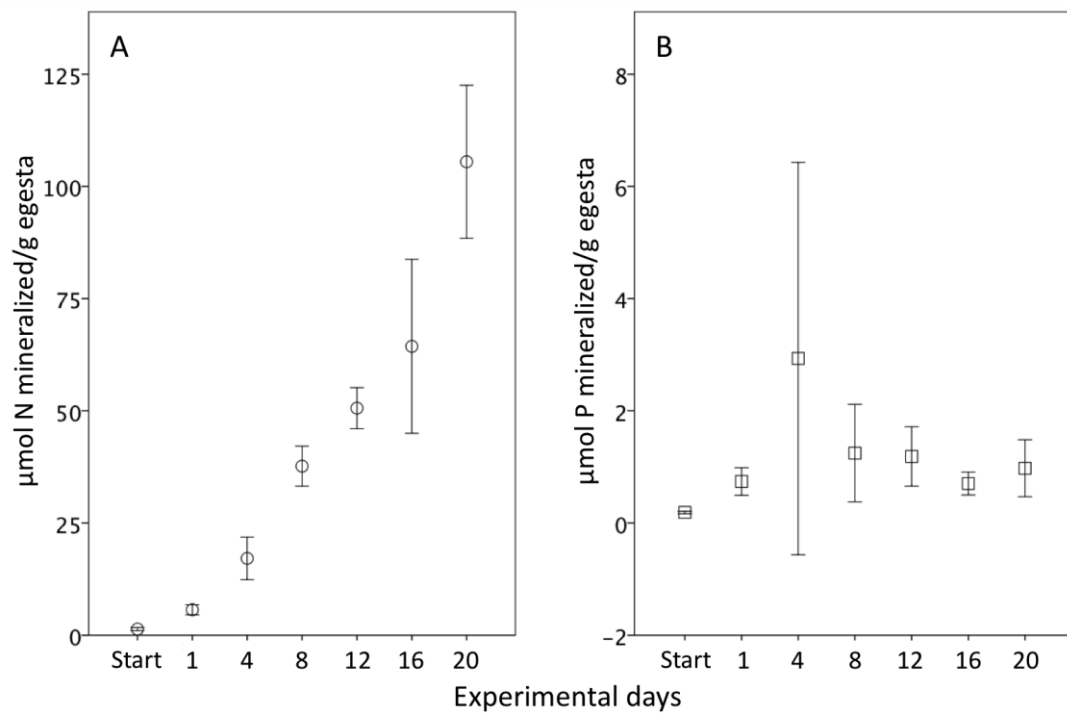


Fig. S1. Amount of N (a) and P (b) mineralised from tadpole egesta in a 20-day laboratory incubation experiment. N and P were calculated from ammonia-N and total dissolved P respectively. Values represent mean \pm s.d., $n = 3$.

References

Liess and Haglund 2007

Liess, A., and Haglund, A.-L. (2007). Periphyton responds differentially to nutrients recycled in dissolved or faecal pellet form by the snail grazer *Theodoxus fluviatilis*. *Freshwater Biology* **52**(10), 1997–2008. [doi:10.1111/j.1365-2427.2007.01825.x](https://doi.org/10.1111/j.1365-2427.2007.01825.x)