## Towards understanding stable isotope signatures in stressed systems

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## Abstract

Stable isotope analysis (SIA) is a valuable tool in ecotoxicology because  $\delta^{13}$ C and  $\delta^{15}$ N may provide insights into the trophic transfer of contaminants in a food web. The relationship between a species' trophic position (TP, determined from  $\delta^{15}$ N) and internal concentration of biomagnifying contaminants can be established and used for regulatory purposes. However, the exposure of organisms to xenobiotics incurs physiological costs, and the stable isotope signature of a consumer reflects not only diet but also a physiological state. The latter raises questions regarding the interpretation of stable isotope signatures in contaminated areas. Therefore, the aim of this Thesis was to evaluate the behaviour of consumers' stable isotope signatures in stressed systems, with a primary focus on the effects of environmental contaminants.

In **paper I**, the physiological costs of chemical exposure were found to alter incorporation rates of dietary nitrogen and carbon in a consumer by influencing both growth and metabolic turnover, with resulting changes in isotope signatures relative to a control system. In **paper II**, the diet-consumer discrimination factors for <sup>15</sup>N and <sup>13</sup>C were confirmed to increase under chemical exposure mediated via increased metabolic costs. However, the physiological response was low and translated into only minor shifts in the  $\delta^{13}$ C and  $\delta^{15}$ N. The predictability of exposure effects on the stable isotope signatures was demonstrated in **paper III**, in which animals exposed to a chemical with a known mode of action presented expected effects on elemental composition, body size, biomarkers of oxidative stress and the stable isotope signatures. Moreover, consumers' oxidative balance was found to be related to their  $\delta^{15}$ N values, thus providing evidence of the kinetic isotope effect on the oxidative status. However, despite the alterations in stable isotope signatures observed in laboratory settings (**papers I-III**), the effect of xenobiotics on the TP estimates was nil or minor in the field-collected animals. Moreover, the TP values were not significantly different between the animals in the contaminated and the reference habitats because of the high overall uncertainties in the TP estimates (**paper IV**). Also, the TP estimates based on  $\delta^{15}$ N values in amino acids. Therefore, the latter method appears more sensitive towards xenobiotics (and, possibly, other environmental stressors) and thus less suitable for TP assessment in contaminated areas.

This Thesis improved the overall understanding of the applicability of SIA in stressed systems by establishing relationships between various exposure regimes, physiological responses and the stable isotope signatures in consumers. In model species at low trophic levels, the exposure to xenobiotics was found to significantly affect  $\delta^{13}$ C and  $\delta^{15}$ N values, which can be expected whenever physiological responses are detected. However, because of the overall high uncertainty in TP estimates, no significant differences between contaminated and control systems were detected, although the estimated TP were consistently higher in the contaminated systems. Future research should focus on higher trophic levels, in which effects of a greater magnitude can be expected. Moreover, the effects in entire food webs should be addressed rather than single prey–consumer relationships as well as other environmental variables that may contribute to the stable isotope variability in and among systems under various environmental pressures.

**Keywords:** Stable isotope analysis, trophic position, chemical exposure, oxidative stress, Daphnia magna, Gammarus spp., Limecola balthica.

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