



SPME Fiber and Twister in a Closed System for Volatile Organic Compound Analysis in *Lissotriton vulgaris*.

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Volatile organic compounds (VOCs) are hypothesized to play a critical role in amphibian ecology, potentially contributing to communication, defense, and attraction. However, their study is constrained by technical challenges, such as animal manipulation and low concentrations. We developed a novel *in vivo* methodological approach to analyze VOCs in three populations of the common newt (*Lissotriton vulgaris*) in Germany. The system consisted of a closed glass cylinder allowing the simultaneous sampling with SPME fibers and Twisters. To our knowledge, this is the first reported method for field-based volatile collection in amphibians, particularly caudates (newts and salamanders), where knowledge of VOC production is almost completely lacking. Two adult specimens were sampled in the glass cylinder (n=6 per population, 3 females, 3 males, 2 individuals per cylinder), and extracts were analyzed by GC-MS. Data were processed in LabSolutions, analyzed in MZmine, and manually annotated using NIST 23 databases for fragment comparison, with retention indices calculated for compound confirmation. SPME provided the higher number of annotated compounds with monoterpenes and sesquiterpenes as the main chemical classes. In turn, Twister, applied for the first time in live animals, identified alcohols, ketones, and alkanes, offering a complementary profile. SPME exhibited greater sensitivity for trace compounds but had fragile fibers and higher noise, whereas Twister offered robust adsorption and field versatility. This study demonstrates the feasibility of the closed system sampling approach and the complementarity of SPME and Twister, providing an innovative tool for ecological research.

Keywords: SPME Fiber, Twister, Volatile Organic Compounds, Closed System

