Fish-mediated inducible defenses in *Daphnia* spp. – identification of the kairomone and effects on physiology, behavior and morphology

by Meike Hahn

Abstract

In standing water bodies, herbivorous zooplankton of the genus *Daphnia* plays a crucial role by linking trophic levels: They graze on phytoplankton, and are subject to predation by planktivorous vertebrates and invertebrates. In the context of predation non-consumptive effects on prey have gotten into focus. In *Daphnia*, these non-consumptive effects include defenses induced by the presence of predator-borne chemical cues, so-called kairomones. *Daphnia* express a variety of these defenses like changes in morphology, life history, and behavior, which protect them from predation. In case of the species *D. lumholtzi*, which invaded large parts of North America over the last decades, invasion success has even been attributed to the morphological defenses *D. lumholtzi* expresses in response to fish kairomones. Elongated helmet and tail spines protect the invasive species from fish predation. Although kairomones are the chemical key to this and other ecologically relevant inducible defenses in *Daphnia* spp., only a few of them have been identified so far but none of them are exuded by fish.

The major goal of this dissertation was to identify fish-derived kairomones, responsible for the induction of defenses in *Daphnia* spp. In response to fish, particularly large *Daphnia* perform diel vertical migration (DVM). This predator avoidance strategy is characterized by daily repeating migrations through the water column that cause the animals to remain in the darker and colder hypolimnion during the day, where they are protected from visually oriented predators. At night they return to the epilimnion, profiting of higher temperatures and food availability. Despite the DVM associated advantage of reduced predation, migrating *Daphnia* experience metabolic costs due to lower experienced ambient temperatures. Under decreased temperatures, *Daphnia* possess comparatively higher concentrations of polyunsaturated fatty acids (PUFAs), which stabilize the membrane fluidity of these poikilothermic animals. Such membrane adjustments are called homeoviscous adaptation. The availability of essential PUFAs such as eicosapentaenoic acid (EPA) limits *Daphnia* growth and reproduction, especially at lower temperatures. Likewise, EPA limits the amplitude of

DVM and reproduction at alternating and on average reduced temperatures, as experienced under DVM.

In the studies, presented within this dissertation, I investigated how DVM-associated factors affect the fatty acid profile of *Daphnia magna* and their offspring. In a full-factorial experimental design, I elucidated the combined influence of the abiotic factors light and temperature (simulated DVM), and for the first time also the effect of fish cues. Relative tissue concentrations of fatty acids were estimated by gas chromatography coupled to flame ionization detection and revealed that that simulated DVM leads to an increased transgenerational EPA allocation. This allocation manifested as increased EPA concentrations in the offspring at the expense of EPA in mothers. The interaction of simulated DVM and the fish cue as well affected fatty acid patterns of the offspring generation, resulting in increased relative tissue concentrations of EPA in offspring, which does not experience simulated DVM. These results imply that *D. magna* offspring adapts to impending DVM through transgenerational resource allocation that may later on enable them to express the full DVM amplitude. Fatty acid limitation may therefore be less severe offspring than in mothers. The involvement of the fish cue in this physiological adaptation reconfirms its major impact on *Daphnia*.

Although a number of chemical properties of the fish-borne kairomone, inducing DVM and life history changes, have already been discovered by studying extract of fish incubation water (EFI), so far its identity remains unknown. This dissertation targets the identification of this kairomone. I identified for the first time the chemical structure of the kairomone as the bile salt 5α-cyprinol sulfate (CPS) by bioassay-guided fractionation of EFI from roach (*Rutilus rutilus*). In a small-scale experiment, I confined the DVM-inducing activity of the extract to a single fraction, gained from high performance liquid chromatography (HPLC). Using CPS purified from carp bile (*Cyprinus carpio*), I demonstrated that this compound is present at a sufficiently high concentration in EFI to account for the kairomone activity. For DVM induction a threshold concentration of 100 pM was estimated and the relevance of other bile salts as kairomones was excluded by bioassays with purchasable bile compounds.

To date, there have been no studies on kairomones of freshwater metazooplankton that confirm their relevance under natural conditions. In order to gain field evidence for the kairomone role of CPS, I investigated the influence of CPS on the vertical distribution of a natural *Daphnia* population in a mesocosm approach in a mesotrophic lake and concomitantly monitored the CPS concentrations in the mesocosms over the course of the experiment by

HPLC-MS. The results showed that CPS induces DVM in a natural *Daphnia* population as well, albeit at higher concentrations than observed in laboratory bioassays. However, *in situ* concentrations within the lake were high enough to achieve this threshold concentration. CPS concentrations within the mesocosms decreased over time. This gives a first indication of rapid microbial degradation of CPS, which is considered a prerequisite for a reliable chemical cue.

Based on the generated knowledge that the group of bile salts may be promising in the search for fish kairomones, which has since been corroborated by further experiments, I aimed on identifying the fish kairomone that induces morphological defenses in the invasive species *D. lumholtzi*. In order to identify the kairomone I used an analogous experimental approach as used to identify the DVM-inducing kairomone. I analyzed the same HPLC fractions of EFI by testing their effect on morphological traits of *D. lumholtzi*. In that way, I could show that CPS is the only compound in EFI, which is present at sufficiently high concentrations to induce morphological defenses in *D. lumholtzi*. More specifically, CPS induced an increase of the relative helmet length at a threshold concentration of 10 pM, whereas its effect on the relative tail spine length remains inconclusive.

To sum it up, within this dissertation transgenerational physiological effects in *D. magna* are revealed for fish cues and abiotic factors – both accompanying DVM. A fish kairomone is identified and its relevance in multiple *Daphnia* species as well as in multiple inducible defenses is demonstrated, which suggests a high evolutional conservancy of the underlying molecular mechanism of predator recognition. The identification of the kairomone allows for the performance of standardized experiments and investigations of kairomone dynamics in waterbodies.