Abstract P1.03: Consumer diversity effects in multispecies predator-prey systems: Relevance of inter- and intraspecific consumer trait variation

During the first funding period our project investigated the relevance of inter- and intraspecific consumer trait variation for understanding the consequences of the loss of functional diversity on trophic dynamics and ecosystem functioning. Using a freshwater ciliate consumer and microalgal prey system, we applied a combination of experimental approaches (batch, semi-continuous and chemostat-experiments), live observations, molecular techniques and statistical modelling. Despite delays in molecular and experimental work due to technical difficulties regarding the molecular assay and a PhD student quitting after 2 years, we were able to fulfil the majority of the aims proposed in the project: We developed a molecular assay to distinguish the different ciliate strains used in our study (1), and we conducted various experiments to investigate the effects of inter- and intraspecific consumer trait variation and prey diversity on consumer competition and trophic dynamics (2), and the interplay of inducible offences and defences (phenotypic plasticity) in an intraguild predation system (3). The latter aspect was not part of the initial project proposal, but arose from insights derived from first experiments.

1) Development of a molecular assay to distinguish different strains of the ciliate species Coleps hirtus. Trait variation among heterospecific and conspecific organisms may substantially affect community and food web dynamics. While the relevance of competition and feeding traits have been widely studied for different consumer species, studies on intraspecific differences are more scarce, partly owing to difficulties in distinguishing different clones of the same species. To address this problem, we collaborated with Prof. Dr. Thorsten Brinkhoff (AG Aquatic Microbial Ecology, ICBM, Oldenburg) to design a specific primer pair (product: 535 bps) that binds upstream and downstream from the rRNA ITS-1 and ITS-2 regions of the strains. Individual strains were clearly distinguishable from one another after sequenced ITS amplicons were compared via sequence alignment. In addition, PCR products generated from the same primer pair with added GC clamps were analysed with denaturing gradient gel electrophoresis (DGGE). Differences in band migration distance allowed the identification of individual strains (Joanne Yong, Master Thesis, published as part of Flöder et al., 2021).

2) Effects of inter- and intraspecific consumer trait variation and prey diversity on consumer competition and trophic dynamics.

Consumer diversity effects on ecosystem functioning are highly context dependent and are determined by consumer specialization and other consumer and prey specific traits such as growth and grazing rates. In microbial microcosms, we investigated the effects of algal prey diversity on the production, evenness and grazing rates of 4 ciliate consumers, differing in grazing preferences and rates. Prey diversity increased prey biovolume in the absence of consumers and had opposing effects on different consumers, depending on their specialization and their preferred prey. Consumers profited from prey mixtures compared to monocultures of non-preferred prey, but responded negatively if preferred prey species were offered together with other species. Prey diversity increased consumer evenness by preventing dominance of specific consumers, demonstrating that the loss of prey species may have cascading effects resulting in reduced consumer diversity. Our study emphasizes that not only the degree of specialization but also the selectivity for certain prey species within the dietary niche may alter the consequences of changing prey diversity in a food web context (Wohlgemuth *et al.*, 2017).

Focusing on the effects of intraspecific consumer trait variation, we investigated competitive interactions between the ciliates *Euplotes octocarinatus* and *Coleps hirtus* in a nitrogen-limited

chemostat system. The ciliates competed for two microalgae (Cryptomonas sp. (Cry) and Navicula pelliculosa (Nav)), and the bacteria present in the cultures over a period of 33 days. We used monoclonal Euplotes and three different Coleps strains (Col 1, Col 2, Col 3) that could be distinguished by the newly developed rRNA-based molecular assay. Experimental treatments comprised two-species mixtures of Euplotes and one or all of the three different Coleps strains, respectively. The experiment was complemented by a carbon budget model estimating energetic and biochemical constraints for the growth of the different ciliates. Intraspecific variation in selectivity and maximum ingestion rates for the different algae significantly altered the competitive outcome between the two ciliate species. As Nav quickly escaped top-down control and likely reached a state of low food quality, ciliate competition was strongly determined by the preference of different Coleps clones for Cry as opposed to feeding on Nav. In addition, the ability of Euplotes to use bacteria as an alternative food source strengthened its persistence once Cry was depleted. Overall, trait variation on both trophic levels co-determined the population dynamics and the outcome of species competition. The change in algal food quality turned a previously advantageous consumer trait into a disadvantageous one, showing that trait values may be beneficial in one setting and disadvantageous in another, which suggests that the resulting effects are context dependent. Via context dependency, intraspecific variation might ensure the overall fitness of a species in variable and changing environments, thus contributing to community stability (Flöder et al., 2021).

3) Interplay of inducible offences and defences (phenotypic plasticity) in an intraguild predation system

A short-term microcosm experiment was conducted to investigate the effects of inter- and intraspecific consumer trait variation on consumer diversity effects. We created three levels of ciliate diversity, all feeding on a 3-species microalgal prey mixture. Ciliates differed in consumer specialisation, feeding on one (specialist S: Euplotes octocarinatus), two (intermediate I: Coleps hirtus) or all three (generalist G: Stylonychia sp.) micro-algal species. Intraspecific trait variation was incorporated by including three different clones of I and setting up ciliate combinations with either monoclonal or polyclonal populations of I. Both inter- and intraspecific consumer diversity decreased prey evenness and increased total ciliate biovolume. On the species level, total ciliate biovolume was high wherever G was included, indicating a positive selection effect for a competitively superior species. Polyclonal I monocultures exceeded the biovolume of all monoclonal ones (transgressive overyielding) based on complementary differences of clone-specific feeding niches. This effect was also observed in multi-species combinations. In addition to feeding on all prey species, G exhibited an inducible offense, forming giant cells that fed on other ciliates. The specialist S responded with an inducible defence, escaping predation by the intraguild predator. Overall, our study demonstrated highly complex trophic interactions driven by consumer selectivity, grazing rates, selective feeding and phenotypic plasticity, and indicated that both interand intraspecific consumer trait variation determine the consequences of consumer diversity loss on ecosystem functioning (Flöder et al., 2018).

Following up the intraguild predation of *Stylonychia*, we first evaluated potential intraspecific differences in edibility of different *Coleps* strains when fed to the intraguild predator (IGP) using DGGE (see above). *Stylonychia* demonstrated highly selective feeding on different *Coleps* strains, which could be confirmed in a subsequent short-term (5 days) experiment (Julia Schmidt, Research Practical). Feeding interactions of the IGP with its ciliate competitors *Coleps* and *Euplotes* in the absence and presence of an alternative food source, the microalgal prey *Cryptomonas* sp., were further investigated in a short-term (8 days) experiment, incubating monocultures of all three

ciliates, as well as two- and three- ciliate species combinations with and without the microalgae. *Stylonychia* quickly formed giant cells feeding on the other ciliates, thus successfully accessing an additional food source. While *Coleps* showed no phenotypic plasticity and was quickly lost, *Euplotes* survived in all species combinations, providing evidence that its inducible defence mechanism reduced the grazing pressure of *Stylonychia* and facilitated its survival. The presence of the microalgae as alternative food source further reduced the grazing pressure on *Euplotes* (Rebecca Schröter, Bachelor Thesis; Flöder et al., in prep.).

Overall, our experiments demonstrated that intraspecific consumer trait variation might have equally strong effects within and across trophic levels than interspecific trait variation. Trophic interactions in our consumer-prey systems were strongly determined by consumer specific traits such as selectivity, grazing rates (including selective feeding within dietary niches), switching of food sources, and phenotypic plasticity (induced offense and defense). Especially the latter have not extensively been studied so far regarding feedback effects on food web dynamics, which we further addressed in the second phase of DynaTrait. Experiments of the first phase revealed that the intraguild predator Stylonychia dominated quickly and outcompeted other ciliates through its inducible offense. However, when subjected to prey depletion, the population decreased quickly and collapsed, indicating a low starvation resistance. Euplotes, on the other hand, exhibited a high starvation resistance, but could not compete with Stylonychia under the homogeneous experimental conditions chosen in the first phase of the project. In the second project phase, we focused on the trade-off between consumer starvation resistance and maximum grazing rate and investigated the relevance of associated consumer traits in response to altered resource regimes. This trade-off has rarely been studied within the framework of biomass-trait feedbacks, but is highly relevant in natural systems characterized by continuously altered environmental conditions.

References

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