Abstract P2.03: The relevance of consumer competition and feeding traits, as well as their trade-offs, in determining multispecies trophic interactions

In the second funding period of DynaTrait, we studied the effect of inter- and intraspecific consumer trait variation on the consumer trade-off between starvation resistance and grazing rate in response to altered regimes of resource supply, again using a combination of laboratory experiments (continuous and semi-continuous culture techniques), molecular and modelling approaches. We focussed on two types of starvation resistance: 1) the ability to reduce basal metabolism at the expense of reproduction, and 2) the ability of photosynthetic carbon fixation in addition to phagotrophy (mixotrophy). Furthermore, we investigated how inducible offense (formation of giant morphotypes capable of intraguild predation) and inducible defence (decreasing grazing susceptibility) interact with this trade-off by altering species interactions. Unfortunately, we were not able to develop a molecular assay to distinguish between different strains of *Euplotes octocarinatus*, which was planned as a continuation of our successful cooperation with Prof. Dr Thorsten Brinkhoff (AG Aquatic Microbial Ecology, ICBM, Oldenburg). This was in part due to restrictions regarding laboratory access and consumables related to the Covid-19 pandemic. However, we have successfully conducted a series of experiments investigating both mechanisms of starvation resistance under resource fluctuations as well as the interplay of inducible defences and offences.

The relevance of adaptive trait variation in nutritional strategies (heterotrophy, mixotrophy) for determining ciliate – microalgae trophic interactions

In the first part of the second phase, we studied performance and competition of purely heterotrophic and algal symbiont-bearing mixotrophic ciliates of the genera *Euplotes* (E) and *Coleps* (C) under different light and prey regimes to evaluate the effect of starvation resistance via mixotrophy on food web dynamics under homogenous and heterogeneous resource supply. We used a heterotrophic and a mixotrophic form of *Euplotes* and *Coleps*, respectively (E_{mix}, C_{mix}, E_{het}, C_{het}), all feeding on the microalgae *Cryptomonas* sp.. Starting with a short-term (9 days) experiment to estimate the parameter range and the extent of trait variation, we set up ciliate monocultures and two-genus mixtures (E_{mix} + C_{mix}, E_{het} + C_{mix}, E_{mix} + C_{het}, E_{het} + C_{het}) and exposed them to low and high prey concentrations and light intensities in a fully factorial design. Ciliate competition and biovolume production were significantly and interactively determined by species combination, light intensity and prey concentration, the latter being the most important factor. Results indicated that genus-specific effects were as strong as trait variation in nutritional mode (mixotrophy versus heterotrophy). This aspect was further investigated in a 48 day chemostat experiment focusing on the relevance of the consumer's starvation resistance via mixotrophic nutrition for species coexistence under constant and fluctuating regimes of resource supply. Using the same ciliate species as before, we set up all possible combinations of heterotrophs and mixotrophs (E_{mix} + E_{het}, E_{mix} + C_{het}, C_{mix} + C_{het}, C_{mix} + E_{het}) providing prey either continuously or in pulses under constant or fluctuating light conditions. Treatments entailed periods of resource depletion in fluctuating environments, but overall provided the same amount of prey and light. To facilitate handling and sample processing, this experiment was split into two runs of 48 days each comprising two species combinations, respectively. During the first weeks of the experiment, prey and light regimes interactively affected the proportions of mixotrophs and heterotrophs, although these effects were highly species-specific. Overall, resource fluctuations promoted consumer coexistence. Mixotrophs benefited from phases with high light intensity in treatments with fluctuating light, while prey pulses promoted both, the mixotrophic and the heterotrophic *Coleps*. This was, however, enhanced for the mixotroph under high light conditions. In the second half of the experiment, the outcome of competition seemed to be strongly determined by the ciliates' ability to utilize bacteria in addition to algal prey, resulting in a dominance of *Euplotes* over *Coleps* irrespective of their nutritional mode. This finding emphasizes that the relevance of different traits and trade-offs for food web dynamics may change over time, even though environmental forcing remains the same. Overall, this experiment demonstrated highly complex trophic interactions driven by consumer-specific nutritional mode, growth- and grazing rates, and enhances our understanding of the adaptive potential of consumers that are subjected to resource fluctuations, and its importance for ecosystem processes and functioning (Flöder et al. in prep.). This experiment is complemented by a mathematical model comprising photoautotrophic algae and heterotrophic bacteria that are bottom-up limited by light, inorganic nutrients and/or organic carbon and further top-down regulated by mixotrophic and heterotrophic ciliates. With this model we would like to elucidate how the population growth of mixotrophs and heterotrophs depends on light and prey availability, what role bacteria play for the outcome of ciliate competition, and the relevance of the degree of mixotrophy (fraction of phagotrophy versus photosynthesis for ciliate nutrition) for trophic dynamics (Master Thesis, in progress).

We are also currently investigating an eco-evolutionary consumer-resource model that considers mixotrophy as a dynamic trait. Depending on the density of the photoautotrophic prey, the mixotrophic species either invests more energy into the production of the photosynthetic equipment (at low prey densities) or into the active searching for the prey and subsequent feeding (high densities of the prey). Our preliminary results show that rapid evolution of the mixotrophic trait may give rise to antiphase cycles among the autotrophic prey and the mixotrophic consumer species (Schulze et al., in prep). This extends previous theory of eco-evolutionary dynamics showing that not only trait variation in the prey but also in the predator level can give rise to altered predator-prey dynamics.

The results of our competition experiment exemplify the potentially important role of externally generated resource fluctuations for the coexistence of different consumer species. However, species coexistence may also be enabled through internally generated resource fluctuations when associated with a gleaner-opportunist at the consumer level (Klauschies & Gaedke, 2020). While high abundances of the resources are giving fast-growing opportunist species a growth advantage, low abundances of the resources are promoting the survival of starvation resistant gleaner species instead. Nevertheless, this mechanism may only weakly stabilize species coexistence in systems exhibiting top-heavy biomass distributions across trophic levels as nutrient retention by consumers is likely to stabilize any internally generated consumer-resource fluctuations and thereby preventing temporal niche differentiation among the consumers (Klauschies & Gaedke, 2020).

Effects of resource supply mode and food quality on the interplay of inducible defence and offence and resulting predator-prey interactions – relevance of inter- and intraspecific consumer trait variation

In order to elucidate the role of food quality for interactive effects of the giant-forming intraguild predator *Stylonychia* (inducible offense) and its potentially defended intraguild prey *Euplotes* (inducible defense), we set up ciliates in monoculture and in combination and fed them either with high quality prey (*Cryptomonas* sp.), low quality prey (bacteria) or a mixture of both prey types (*Cryptomonas + bacteria*) in a factorial design, all prey treatments containing the same amount of carbon. The experiment was performed using semi-continuous cultures for 32 days. Ciliate samples have been analysed using a FlowCam to estimate size changes in both ciliates and follow their phenotypic plasticity directly in live samples. The experimental work is completed and the samples are being processed (Madeleine Hamm, Bachelor Thesis).

In a subsequent study, we investigated the intraspecific extent of the inducible defense in *Euplotes octocarinatus* and the relevance of this trait variation for trophic dynamics with the IGP *Stylonychia* under different regimes of resource supply (homogenous versus heterogenous). The extent of phenotypic plasticity of 10 different *Euplotes* strains was determined in short-term experiments, testing for cell size changes in response to exposure to the chemical cue released by *Stylonychia* (using freeze-dried *Stylonychia* to prevent feeding). Four strains that differed in initial cell size and in their plastic response were chosen to create five *Euplotes* – *Stylonychia* combinations (4 monoclonal, 1 polyclonal), providing prey either continuously or in pulses (*Cryptomonas*, fed in equal amounts every 2nd day or every 10th day). Both, intraspecific trait variation and *Cryptomonas* supply mode affected the predator-prey interaction of *Euplotes* and *Stylonychia*. The intraguild predator *Stylonychia* grew faster after a *Cryptomonas* pulse, which was followed by a decline in population size due to resource depletion and the formation of a large number of resting stages, before the next pulse was given. The *Euplotes* strain that had shown the highest plasticity was superior to the other strains and the polyclonal culture under both modes of *Cryptomonas* supply. The pulsed supply, however, was connected to a higher individual biovolume of this strain. In combination with the fluctuations in the *Stylonychia* population, this led to a larger *Euplotes* population during the initial phase of the experiment and to a longer survival time. Overall, this study demonstrated that intraspecific consumer trait variation strongly determined predator-prey dynamics and enhanced coexistence among consumers (Fenja-Marie Möller, Bachelor Thesis).

We currently further investigate the relevance of intraspecific consumer trait variation for food web dynamics in a tri-trophic system by including mono- and polyclonal cultures of *Euplotes* and *Coleps* under continuous and pulsed prey supply with or without the IGP *Stylonychia*. Based on previously exhibited differences in edibility to the IGP and in starvation resistance in response to resource depletion, we expect high trait variation and resource heterogeneity to increase consumer coexistence. The experiment is currently running (Miriam Christa Schedl, Master Thesis, Schedl et al., in prep.).

Inspired by our experimental findings, we also developed a model to investigate the impact of trait adaptation on species coexistence in an IGP module, in which each of the species, i.e. basal prey, intraguild prey and intraguild predator can adapt in response to the prevailing selection pressure. Our model results show that sufficiently fast and large trait adaptation may strongly promote species coexistence and stabilize the corresponding population dynamics of the different species (Li, Klauschies and Gaedke, in prep.).

References

Klauschies, T. and Gaedke, U. (2020) Nutrient retention by predators undermines predator coexistence on one prey. *Theoretical Ecology,* **13**.