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 four 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 dependence, 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 inter- and 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).

Overall, our experiments demonstrated that intraspecific consumer trait variation might have equally strong effects within and across trophic levels as 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.

~~**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) 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 defense (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 defenses and offenses.~~

~~*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~~

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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 growth rates and total 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 therefore 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}), respectively, 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 as under constant conditions. To facilitate handling and sample processing, this experiment was split into two runs of 48 days, each comprising two species combinations, respectively. Competition between mixotrophic and heterotrophic ciliates in our experiment was significantly affected by prey supply, while the effect of light supply depended on species combination. Whether mixotrophs or heterotrophs dominated in competition thus strongly depended on the genera of the competing species, driven by species-specific differences in the minimum resource requirements that are associated with feeding on shared prey, nutrient uptake, light harvesting and access to additional resources such as bacteria. All treatment effect depended on time, reflected in varied population dynamics of heterotrophs and mixotrophs in different treatments in the first 16 days of the experiment, while irrespective of light and prey supply, *Euplotes* dominated both mixed genus combinations by the end of the experiment, the heterotroph the same-genus *Coleps* mixture, whereas it was the mixotroph in the *Euplotes* mixture. Overall, genus specific differences in response to resource supply mode led to faster competitive exclusion in mixed-genus combinations than in more similar species of the same genus. Fluctuations in resource supply did not alter the qualitative outcome of species competition, but influenced the shape of the population dynamics throughout the experiment and promoted species coexistence in some species combinations. Overall, our study demonstrated that genus- or species-specific traits other than related to nutritional mode may override the relevance of acquired phototrophy by heterotrophs in competitive interactions. Furthermore, it revealed that the potential advantage of photosynthetic carbon fixation of symbiont-bearing mixotrophs in competition with pure heterotrophs may differ greatly among different mixotrophs, playing out under different environmental conditions and depending on the specific requirements of the species. Complex trophic interactions determine the outcome of competition, which can only be understood by taking on a multidimensional trait perspective (Flöder *et al.*, revised manuscript under review).

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

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~~stabilize any internally generated consumer-resource fluctuations and thereby preventing temporal niche differentiation among the consumers (Klauschies & Gaedke, 2020).~~

~~In line with our experimental work, we also developed and analyzed a trait-based model comprising a mixotrophic consumer (e.g. a ciliate or mixotrophic algae) and an autotrophic (algae) or heterotrophic resource (bacteria) competing for inorganic nutrients. The model involves trade-offs between autotrophic and heterotrophic growth for the mixotroph, and between defense capacity against predation and maximum growth rate for the resource. We investigated the population and trait dynamics for different scenarios, in which none, one or both species were able to adapt their traits in response to selection. Under specific combinations of fixed traits, either species could dominate. However, the mixotroph often gained dominance when it could adapt its trait to exert both strong predation and competition pressure on the resource. Trait adaptation in the resource promoted its dominance only when the mixotrophy trait was fixed, whereas it played a minor role under coadaptation. Moreover, antiphase cycles often emerged when both species adapted their traits independently or interactively, with the species dominating that was able to adapt to the current selection pressure. Overall, our findings demonstrate that trait adaptation in mixotrophs substantially affects species composition and the shape and stability of population dynamics in food webs (Li *et al.*, submitted manuscript under review).~~

~~*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*~~

~~Focusing on the dynamics of reciprocal phenotypic plasticity, we investigated the intraspecific extent of the inducible defense in *Euplotes octocarinatus* and the relevance of this trait variation for trophic dynamics with the intraguild predator (IGP) *Stylonychia mytilus*, which is capable of an inducible offense, under different regimes of resource supply (homogenous versus heterogenous). At first, we determined the extent of phenotypic plasticity in ten different *Euplotes* strains in short-term experiments, testing for defense-related cell size changes in response to exposure to the chemical cue released by *Stylonychia* (using freeze-dried *Stylonychia* to prevent feeding). The strains revealed significant differences in their width and length development. In a subsequent semi-continuous 30-day experiment, four of these strains were incubated in monoculture and mixture with *Stylonychia*, providing prey either continuously or in pulses (*Cryptomonas*, fed in equal amounts every 2nd day or every 10th day). The polyclonal *Euplotes* population outperformed monoclonal populations, except for the strongest one, which developed the most pronounced inducible defense, especially under pulsed prey supply, and retained the highest biovolume. Although *Stylonychia* did not exhibit size changes, it dominated all communities irrespective of clonal composition. Pulsed resource supply promoted biovolume production of both species. However, periods of resource depletion resulted in more *Stylonychia* resting cysts, allowing *Euplotes* to resume its growth. Overall, this study demonstrated that intraspecific consumer trait variation strongly determines predator-prey dynamics and enhances coexistence among consumers, in particular under variable environmental conditions (Fenja-Marie Möller, Bachelor Thesis, Möller *et al.*, under review).~~

~~We further investigated the relevance of intraspecific consumer trait variation for food web dynamics in a similar set-up with *Euplotes* and *Stylonychia*, but this time including an additional intraguild prey, i. e. the non-defended heterotrophic ciliate *Coleps hirtus*. Here, we increased food web complexity and intraspecific diversity even more by including mono- and polyclonal cultures of both *Euplotes* and *Coleps* that were set up alone and in two-genus mixtures under continuous (fed three~~

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times a week) and pulsed (fed every fortnight) prey supply with or without the IGP *Stylonychia*. Community development in this experiment was strongly affected by prey supply mode, with prey pulses supporting biovolume production of *Coleps* and *Stylonychia* and coexistence in some species combinations, while Euplotes didn't grow well in any of the treatments. Intraspecific consumer trait variation affected *Euplotes* more than *Coleps;* however, in contrast to prior experiments, none of the polyclonal populations were clearly promoted in comparison to monoclonal ones in different treatments (Miriam Christa Schedl, Master Thesis, Schedl *et al.*, in prep.).

In order to further elucidate the role of resource supply for the extent of inducible defense in *Euplotes* and inducible offense in *Stylonychia*, we conducted a series of experiments testing the effects of *Cryptomonas* concentration (microalgal prey) and the presence of another ciliate, i. e. *Coleps hirtus* (competitor for *Cryptomonas*, intraguild prey for *Stylonychia*) on the reciprocal phenotypic plasticity of the target species and their population dynamics. First results show that microalgal prey concentration strongly determines the extent of expressed induced defense in *Euplotes* and that both, increasing *Cryptomonas* and increasing *Euplotes* concentrations have a positive effect on *Stylonychia* cell size (Lena Loh, Master Thesis, Loh *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 (Li et al. 2023). The adaptive IGP model accounts for both width and speed of trait adaptation within each species, allowing prey and predator species to mutually adjust their species specific defensive and offensive strategies to each other interactively. We compared species persistence, community dynamics, and the occurrence of bistability between different versions of our IGP model where species were either able or unable to adapt their traits in response to selection along a gradient of enrichment represented by carrying capacity of the basal prey. We found that trait adaptation within multiple species greatly enhanced the coexistence of all three species in the module. A larger width of trait adaptation facilitated species coexistence independent of the speed of trait adaptation at lower enrichment levels, while a sufficiently large and fast trait adaptation promoted species coexistence at higher enrichment levels. Increasing the speed of trait adaptation buffered the detrimental effects of enrichment on the temporal variability of biomasses of all species. Finally, the bistability encountered in the non-adaptive IGP model was strongly reduced when allowing trait adaptation (Li et al. 2023). These findings resolve the contradiction between the empirical evidence of the widespread occurrence of IGP and the theoretical predictions that IGP should only occur under restricted conditions and lead to unstable community dynamics, which broaden the mechanisms underlying the maintenance of IGP modules in nature. Generally, by revealing the eco-evolutionary feedbacks among complex trophic interactions, this study demonstrates that intraspecific diversity may enhance interspecific diversity and stabilize food web dynamics.

Synthesis

In the synthesis phase of DynaTrait, we contributed to a review on flexibility in aquatic food web interactions by linking different scales and approaches in aquatic ecology. Trophic interactions are highly flexible, changing on temporal scales from diurnal to evolutionary times due to phenotypic plasticity, rapid evolution and species sorting. Small-scale experimental and theoretical approaches have demonstrated a high relevance of this flexibility for community dynamics and ecosystem processes; but the extent to which they drive dynamics, functioning and responses to global change in

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~~more complex communities is still poorly understood. Differences in methodology, focus and communication between research disciplines limit our ability to project effects of flexible trophic interactions onto larger spatial and temporal scales. To bridge this gap, we proposed a general framework for upscaling our knowledge on flexible interactions from small-scale research to large-scale model projections. Building on examples from aquatic communities, we used this framework to show how mechanisms demonstrated on the small scale may be linked to ecosystem functions relevant in large-scale (e.g. global ocean) models. We argue for incorporating flexibility in large-scale process-based models in order to improve their realism and predictive power, and discuss challenges and promising ways forward for achieving this (Van Velzen et al., in prep.).~~

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