Trait variability and defence costs in coupled bitrophic phytoplankton – biofilm systems: effects on predator-prey dynamics and coexistence

The project investigates the effect of trait variation on predator-prey dynamics. The systems consist of a highly plastic prey bacterium and specialized protozoa grazers. The general state of the art and background for the project was provided in the initial application of the first and second funding period. Here we provide a brief summary, updated by novel findings gained during the two funding periods of DynaTrait.

1. Phase 1. Effect of trait variability on the dynamics of coupled, bi-trophic plankton-biofilm systems.

Within DynaTrait Phase 1, we aimed to develop a coupled experimental plankton–biofilm systems to investigate dynamic effects on trait variation. Due to its contrasting phenotypes (i.e. biofilm and plankton) *Pseudomonas putida* was used as prey for two specialised predators, one feeding on suspended prey, *Paramecium tetraurelia*, and the other feeding on biofilm-associated bacteria, *Acanthamoeba castellanii*.

Four specific hypotheses were addressed within the first funding period of the project (ca. 2014-2017): **(H1)** Lack of trait variation within the predator guild results in accumulation of "predator resistant" prey biomass, which gets either locked in the biofilm or the plankton depending on the food spectrum of the specialized predator. **(H2)** An increased loss rate affecting the defended morphotypes implies a decreased net population growth rate and enhanced costs of defense. This will alter the dynamics of the system and may lead to cyclic dynamics in biomasses of predator and prey phenotypes. (**H3)** Trait variation of the predator leads to a higher biomass allocation towards the total predator guild with dynamic allocation to plankton vs. biofilm (prey guild) and plankton vs. biofilm feeders (predator guild). **(H4**) If the predator guild shows trait variation (i.e. grazing on different prey phenotypes), then the system shows an efficient carbon flux from the first to the second trophic level, which will be reflected in the overall carbon metabolism of the total community.

The development of the hypotheses H1, H3 and H4 was achieved using batch experiments and mathematical modeling. Details of experimental settings can be found in the publication of Seiler et al. 2017.

Results from batch experiments, starting with either only planktonic bacteria or with additional pre-grown biofilms, (Fig.1 A-B) confirmed that grazing resistance in bacterial phenotypes is dynamic and depends on the feeding trait variation of the grazer either on plankton prey (*P. tetraurelia*) or on biofilm prey (*A. castellanii*) (**H1**). When a single grazing type is present, the non-preferred prey phenotype (i.e. either biofilm or plankton) is

stimulated, while the edible prey phenotype decreased (**H3**). Although an slight decrease of planktonic prey biovolume in presence of *A. castellanii* was observed (Fig 1. A-B), this effect was not significant (P > 0.05) and it can be explained (based on the mathematical model) by the migration of plankton cells into the biofilm (Fig 1. C-D). Interestingly, in the experiments, the plankton-feeder strongly stimulated the biofilm biomass. This stimulation of the resistant prey phenotype was not predicted by the model and it was not observed for the biofilm-feeders, suggesting the existence of additional mechanisms that stimulate biofilm formation besides selective feeding.

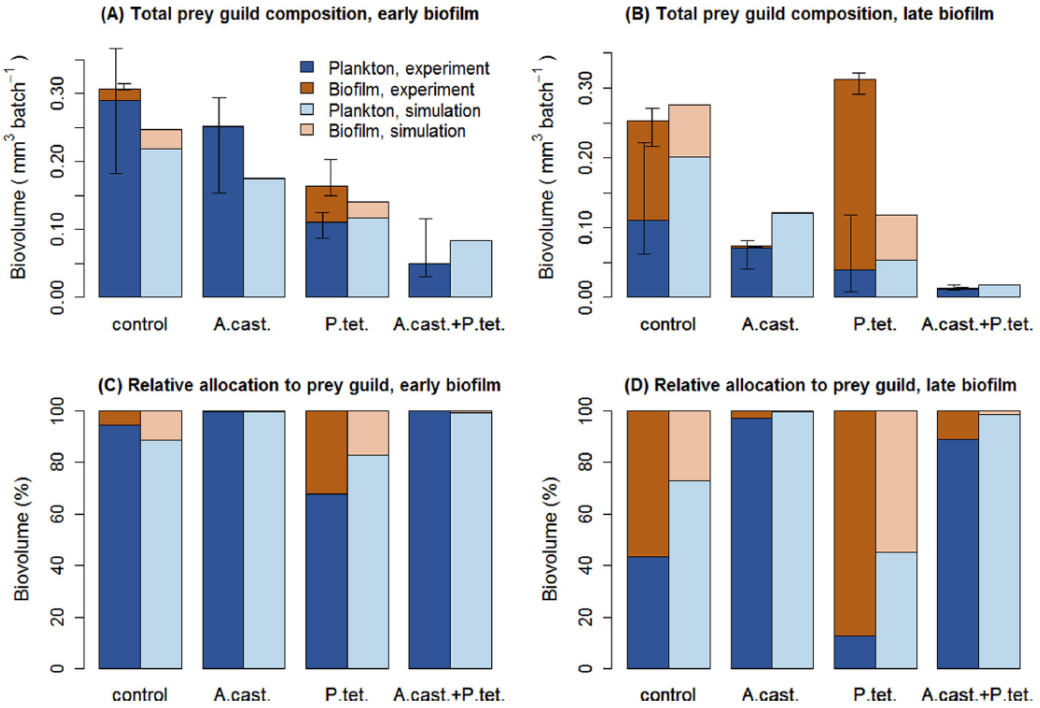

Figure 1 (source: Seiler et al.2017)**.** Treatment effects on the total prey guild composition (upper panels, **A** and **B**) and on the relative biovolume allocation to the prey guild (lower panels, **C** and **D**) in the early biofilm experiment (left panels, A and C) and the late biofilm experiment (right panels, B and D) after 3 or 4 days, respectively. Presented are experimental and simulation results for all treatments (predator-free control—'control', *Acanthamoeba castellanii* only—'A.cast', *Paramecium tetraurelia* only—'P.tet.', and *Acanthamoeba castellanii* and *Paramecium tetraurelia*—'A.cast. + P.tet.'). Depicted are medians and 0.25 and 0.75 quantiles (error bars).

Finally, in the presence of the two grazing types (i.e. biofilm and plankton feeders) efficient control of the total bacterial biomass is possible. Comparative analysis of the prey and predator biomass revealed that a lower total biomass in the prey guild feeds a higher total predator biomass in the full system treatment (two predators) compared to the one-predator treatments. This in turn results in a higher ratio between predator and prey (predator:prey) compared to the one-predator treatments. These results further indicate an enhanced carbon flow within the food web with variable predator traits (two-predator treatment) as biomass is not accumulated in the prey guild. This finding supports **H4**.

In parallel with experimental investigations, we used mathematical modelling to investigate **H3** in long-term dynamics. This demonstrated the critical importance of dynamic allocation within the prey and predator guilds: total prey biomass decreased and total predator biomass increased (confirming **H3**) but only when the prey phenotypes and the two predators showed compensatory or asynchronous dynamics. However, a very fast plastic response in the prey resulted in synchronization in prey dynamics, removing the potential for dynamic allocation in the predator guild, which reversed the above patterns. These findings were published in Oikos (van Velzen et al. 2018), and this paper received the Editor's Choice distinction for the issue in which it was published.

H2 could not be addressed experimentally in chemostat systems, but we performed simulations to test this hypothesis *in silico*. Dilution rates of planktonic and biofilm-associated bacteria were varied to test the impact of increased loss rates (implying increased costs of defense) on average biomasses and on the occurrence of predator-prey cycles. Simulation results showed that the system dynamics is dominated by planktonic processes, due to the fact that the plankton growth rate is much higher than the biofilm growth rate. This contradicts at least partially hypothesis 2 (trait variation in predators leads to dynamic allocation between plankton and biofilm predators).

2. Phase 2. Trait variability and defence costs in coupled bitrophic phytoplankton – biofilm systems: effects on predator-prey dynamics and coexistence

Based on the results obtained in the first funding period, we addressed advanced questions on how trait variation on the prey and on the predator level interact with each other, and how they together determine the temporal dynamics of the system and species coexistence. Specifically, three major aims were proposed: **(H1)** Trait variation in the predator guild of coupled plankton – biofilm systems strongly increase the carbon flow of the total system. **(H2)** Phenotypic plasticity in prey can drive indirect facilitation and coexistence between predators. Finally, **(H3)** the trait variation in predators increases the range of coexistence of two competing prey species with phenotypic plasticity each.

So far, we could show in the static batch system that the trait variation in the grazer guild leads to a higher predator/prey biomass ratio, strongly suggesting a higher carbon transfer to higher trophic levels (first phase results). The experimental proof in the dynamic system (chemostat systems) over many prey generations is still lacking. Nevertheless, we could address and confirm the hypothesis **(H1)** using a mathematical model for a chemostat system (Fig. 2). Carbon transfer to higher trophic levels was measured as the predator-prey biomass ratio (Fig. 2a) and the trophic transfer efficiency, i.e. predator production relative to prey production (Fig. 2b). Both measures showed consistent patterns: energy transfer to the

predator level was highest when trait variation was present in the predators, and lowest when only the biofilm-grazing predator was present (Fig. 2).

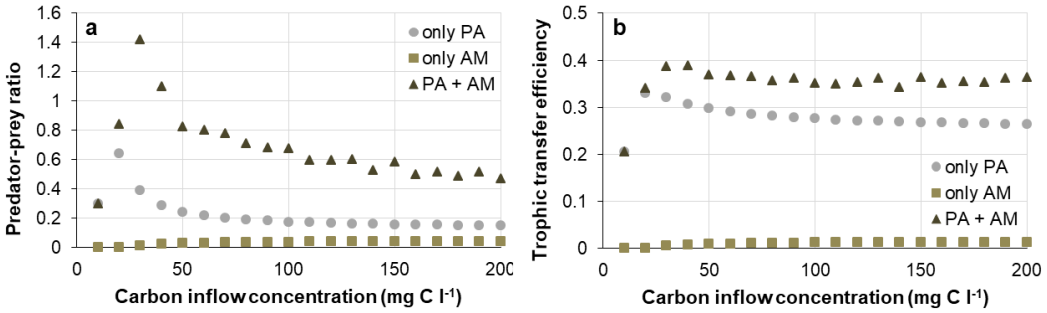

Figure 2: Simulation runs for hypothesis 1. All calculations are based on the average biomasses of the last 60 days of simulated chemostat runs of 200 days. In the presence of a single predator (only plankton predator Paramecium (PA) or only biofilm predator Amoeba (AM), energy transfer to the predators is low because the defended bacterial prey phenotype accumulates (BF in the case of plankton predator PA; PL in case of the biofilm predator AM). Trait variation in the predators (PA + AM) breaks down defense and yields a higher predator/prey ratio (**a**) and a higher trophic transfer efficiency (**b**).

The experimental investigation of the hypotheses about predator coexistence (**H2**, **H3**) was largely compromised by a number of factors. (a) Unexpectedly, long-term cultivation of the original planktonic predator (*P. tetraurelia*) in the newly designed chemostat system failed in spite of serious and time-consuming attempts to optimize medium and turbulence conditions. An alternative ciliate (*Tetrahymena pyriformis*) was successfully cultivated, however, it exhibited a mixed feeding behaviour consuming both planktonic cells and biofilm. We finally turned to a *Spumella*-like flagellate which proved to be very robust with a strictly planktonic feeding mode. As expected, biofilm formation was clearly promoted in the presence of *Spumella.* We were able to provide strong experimental evidence for a mechanism of induction (i.e. chemical interference) in addition to interactions solely rooted in selective grazing and competition among prey phenotypes. (b) Contrary to expectation, however, the steady state observed in the experimental systems consistently involved more than two bacterial phenotypes: biofilm, suspended cells, and filaments (of up to 250 µm length). Filaments always became the predominant phenotype after about 3 weeks and they were apparently inaccessible to both the planktonic and the biofilm predator. Obviously, plasticity of the prey was great enough to provide an ultimate defense under simultaneous grazing pressure on suspended single-cells and biofilm. (c) In our experimental systems, the biofilm feeder *Acanthamoeba castellanii* tended to form cysts after depletion of biofilm prey and the dormant stage was only reverted after prey recovery. This life cycle of *A. castellanii* contradicts at least partially **hypothesis 2** according to which phenotypic plasticity in prey can drive indirect facilitation and coexistence between predators.

In view of these unanticipated challenges and after spending a huge amount of time to fix the problems (ultimately unsuccessfully), the group decided to shift the focus target from prey defense by biofilm formation to alternative prey defense(s). Doing so, we were able to maintain the thematic focus of the project (i.e., effect of trait variation on predator-prey dynamics). For that purpose, mesocosms were set up to study the coexistence between *P. putida* (prey) and *Spumella* (predator) over periods of several weeks to months. To actively suppress biofilm formation, chemostats were replaced by a semi-continuous approach where part of the culture (10 to 50%) is transferred into a clean vessel supplemented with fresh medium once a day. This approach finally allowed for fascinating insights into the full spectrum of predation defenses by *P. putida*.

In all replicate experiments, a standard predator-prey dynamics was observed over the first 7 days. Then, however, flagellate densities dropped to very low levels around day 14 indicating growth inhibition (Fig. 3, A). The latter was confirmed in dedicated exposure tests where axenically grown flagellates were confronted with filtrate from bacteria-flagellate co-cultures. Apparently, when biofilm formation as a first-line defense is suppressed, *P. putida* excretes metabolites which provide a successful second-line defense against grazing losses. Nevertheless, in all replicate experiments, flagellates were able to recover (Fig. 3, B) and cell densities converged to a consistent steady state (Fig. 3, C) after filaments became the predominant bacterial phenotype.

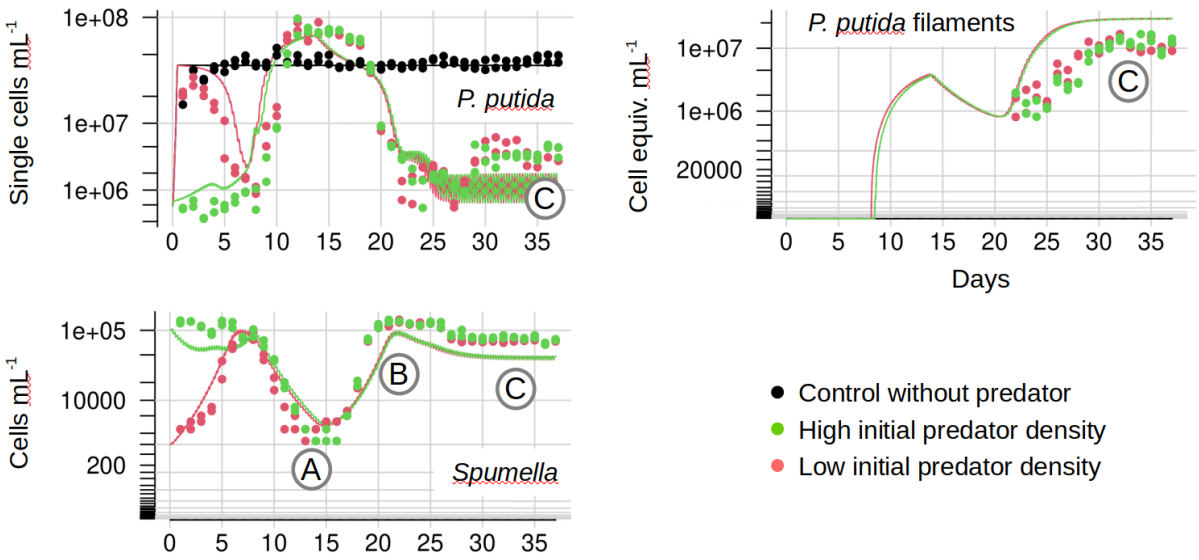

Figure 3. Observed predator-prey dynamics in semi-continuous cultures with 50% of volume transferred every day. Lines represent predictions by a preliminary ODE-based model.

To understand why filamentation (and not toxin production) consistently appeared as the ultimate bacterial response, selected filamentous isolates were whole-genome sequenced. It was found that filamentation was caused by point mutations in genes involved in septum formation such as, e.g., *ftsQ*. Hence, the observed “succession of bacterial defenses”

involves both an active response reflecting phenotypic plasticity (excretion of inhibitors) and an evolutionary mechanism (accumulation of favourable mutations).

To validate our current mechanistic understanding of the system, we implemented an ODE-based model accounting for the primary processes of bacterial growth, flagellate grazing, continuous dilution, and replenishment of utilizable carbon. It furthermore accounts for the production of siderophore-like secondary metabolites by bacteria that come with a dual effect of (a) better resource exploitation and (b) inhibition of flagellate growth. Up- and downregulation of metabolite production is assumed to be controlled by both flagellate density (predator sensing) and the abundance of non-defended single-celled bacteria (quorum sensing). Finally, the model considers the formation of bacterial filaments via mutation which continue to grow as either filaments (elongation) or single-cells (segregation) with defined probabilities. According to our current understanding, the considerable abundance of *Spumella* in steady state is primarily supported by single cells originating from filament segregation. A publication that combines the empirical results and model predictions shown in Fig. 3 is currently under preparation.

List of publications:

- Van Velzen E, Thieser T, Berendonk T, Weitere M, Gaedke U (2018). Inducible defense destabilizes predator-prey dynamics: the importance of multiple predators. Oikos. 00:1-12
- Seiler C, van Velzen E, Neu T, Gaedke U, Berendonk T, Weitere M (2017). Grazing resistance of bacterial biofilms: A matter of predators' feeding trait. Federation of European Microbiological Societies (FEMS) Microbiology Ecology 93(9), fix112.
- De la Cruz Barron M, Berendonk T, Van Velzen E, Weitere M, Kneis D (202X). The long way to steady-state: Successive adaptations in co-cultures of *Pseudomonas putida* and a *Spumella*-like flagellate. In preparation.