Ecoevolutionary feedbacks of phenotypic plasticity and mono- vs. polyclonal communities in bi-and tritrophic systems

Trophic interactions in aquatic communities are significantly affected by the performance of the interacting partners. Difference performance levels can depend on different traits such as edibility, reproductive rate or growth rate can significantly affect population dynamics and community structures. Trait variability in populations may result from either phenotypic plasticity or genetic diversity. A form of phenotypic plasticity are inducible defences which have been shown to protect the prey but additionally to dampen the oscillations of predator-prey population cycles. However, there is a coevolutionary adaptation in the form of inducible offenses in some predators which partly overcome the induced defenses. We here examined if trait variability and phenotypic plasticity on the consumer level stabilize trophic interactions more than trait variation on two (consumer and predator) trophic levels within a tritrophic system. For this, we used freshwater ciliates of the genus *Euplotes* feeding on the non-plastic algae *Chlorogonium*. *Euplotes* expresses defensive features by increasing their cell length and width in the presence of the predatory ciliate *Lembadion*. *Lembadion* can counter this kind of prey plasticity, by a plastic reaction to the enlarged prey and gradually adjust peristome size facilitating ingestion of defended prey.

To identify the Euplotes species, we developed a new method for species identification in ciliates. The classical silver staining (protein-silver-technique & pyridinatedsilver-carbonate method) is rather time consuming. Our new method is based on immunofluorescent staining and confocal imaging.

In a further step of our experiment, we measured the reaction norms in isolated strains of the species: *E. octocarinatus*, *E. daidaleos* and *E. aediculatus.* This was done by co-culturing them with different densities of the plastic predator Lembadion and the non-plastic predator *Stenostomum sphagnetorum* (Turbellaria) under abundant food conditions. In response to both predators, the smallest strain *E. octocarinatus* EO1 showed the relatively strongest defence. The medium sized *E. daidaleos* AS3 showed an intermediate increase in cell size and the largest *E. aediculatus* LV7, showed the smallest size increase. The increase of cell size of all three prey species is predator dependent and the presence of the small strain *Lembadion* clone L1 induces smaller traits than the large strain L2. Reaction norms of size adjustments in *Lembadion* were also measured by culturing it with above mentioned-prey

that were selected due to size differences (*E. octocarinatus* (EO1) as small prey, *E. daidaleos* (AS3) as medium prey and *E. aediculatus* (LV7) as large prey).

With this information, we performed monoclonal tritrophic long term experiments with the top predators *Lembadion spp.* (plastic) and *Stenostomum* (non-plastic), and the three different *Euplotes* species as differently sized prey. *Euplotes* was fed with the algae *Chlorogonium elongatum.* Over 50 days we measured the abundance of predator, prey and algae to study population dynamics. At the same time, we measured morphological traits for predator and prey. Population dynamics of *E. octocarinatus* (EO1 small) & E. *daidaleos* (AS3 medium) and the plastic predator *Lembadion* L1 showed predator prey oscillations with small amplitudes. When *Lembadion* L1 was co-cultured with *E. aediculatus* (LV7-large) it died out, as the induced size of *E. aediculatus* outgrows *Lembadion's* peristome thereby hampering food ingestion. When co-cultured with the larger and more plastic predator *Lembadion* L2, predator prey oscillations increased in amplitude. The small prey is heavily predated and defenses to not protect *Lembadion* L2 predation as population size is significantly reduced but, in our experiments, do not reach zero.

In comparison to the plastic predator *Lembadion* (clone L1 & L2) *Euplotes* species also defend against the non-plastic predator *Stenostomum*. Population dynamics of the small *Euplotes* species EO1 and AS3 show only weak oscillations. When provided with large size prey *E. aediculatus* LV7, *Stenostomum* apparently foraged stronger and LV7 almost died out.

We are now investigating the effect of prey's trait variability on population dynamics in polyclonal experiments. As species distinction and therefore population density determination is the limiting factor in these experiments, we implemented a faster molecular analytical method. By targeting a species-specific DNA sequence, we can quantity this unicellular organism's cell numbers using quantitative PCR in comparison to a given standard curve. These experiments are ongoing.

Publications

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- Bamberger V, Marks, A., Weiss, LC, Tollrian, R. (In prep.). Trait variability and phenotypic plasticity on one or two trophic levels affect population dynamics in a tritrophic system.
- Bamberger V, Tollrian, R., Weiss, LC, (In prep.). Monitoring ciliate population dynamics using genetic markers and cell number standard curves for quantitative PCR – a fast and efficient cell quantification system