The influence of environmental changes and individual trait variability (phenotypic plasticity) on biodiversity and ecosystem stability

With the continuing burning of fossil fuels, the degree, rate and intensity of anthropogenic environmental changes are further increasing. Pollution, but also massive changes in land use and the emission of greenhouse gases lead to a severe loss of habitat structure, changes in habitat chemistry and even climate change. Anthropogenically released CO_2 accumulates in the global carbon cycle and is anticipated to imbalance global carbon fluxes. For example, increased atmospheric CO_2 induces a net air-to-sea flux where the oceans take up large amounts of atmospheric CO_2 (i.e., leading to ocean acidification). Not only the oceans take up large amounts of atmospheric CO_2 but also freshwater carbon hydro-geochemistry is affected, giving rise to even higher amounts of pCO_2 in freshwater habitats. This development will, in the long run, harm ecosystems and can reduce biodiversity. Biodiversity is a major factor stabilizing ecosystems serving as a buffer against environmental perturbations. Biodiversity can be observed on all levels of biological organizations i.e. genetic, species and ecological. These general levels can be further subdivided, so that within one species not just different genotypes but also different phenotypes can be observed. This trait variability paired with phenotypic plasticity further complicates our understanding of how biodiversity contributes to ecosystem stability. Two scenarios are possible: 1) Phenotypic plasticity (trait variation) might increase the biodiversity because organisms are adapted to exist under a range of changing conditions. 2) Phenotypic plasticity (trait variation) might decrease biodiversity because it enables the plastic organism to exploit a wider niche space rather than allowing a specialization of different genotypes. We tested the influence of phenotypic variability on the genetic diversity in artificially composed populations subjected to natural (predation) and anthropogenic (elevated pCO_2) stressors. After isolating ten *Daphnia pulex* clones from a wild population, we performed a full-factorial life-table experiment and tested these *Daphnia* clones and their reaction norm to four treatments (**control**: animals were cultured in control medium M4 at norm pCO_2; **predator:** animals were cultured in control medium M4 and norm pCO_2 but in the presence of *Chaoborus* kairomone enriched medium; **pCO₂**: animals were cultured in M4 medium with elevated pCO_2 (12,000 µatm) and **pCO₂ + Chaoborus**: where animals were cultured in M4 medium with elevated pCO_2 (12,000 µatm) and *Chaoborus* kairomone enriched medium).

We observed a significant reduction in neckteeth expression through elevated pCO_2 in clones AF, AW and three and delays in reaching sexual maturity in clones AE, AH, AQ. Clutch size was significantly affected through CO_2 in the clones AF, AH, AW and AY.
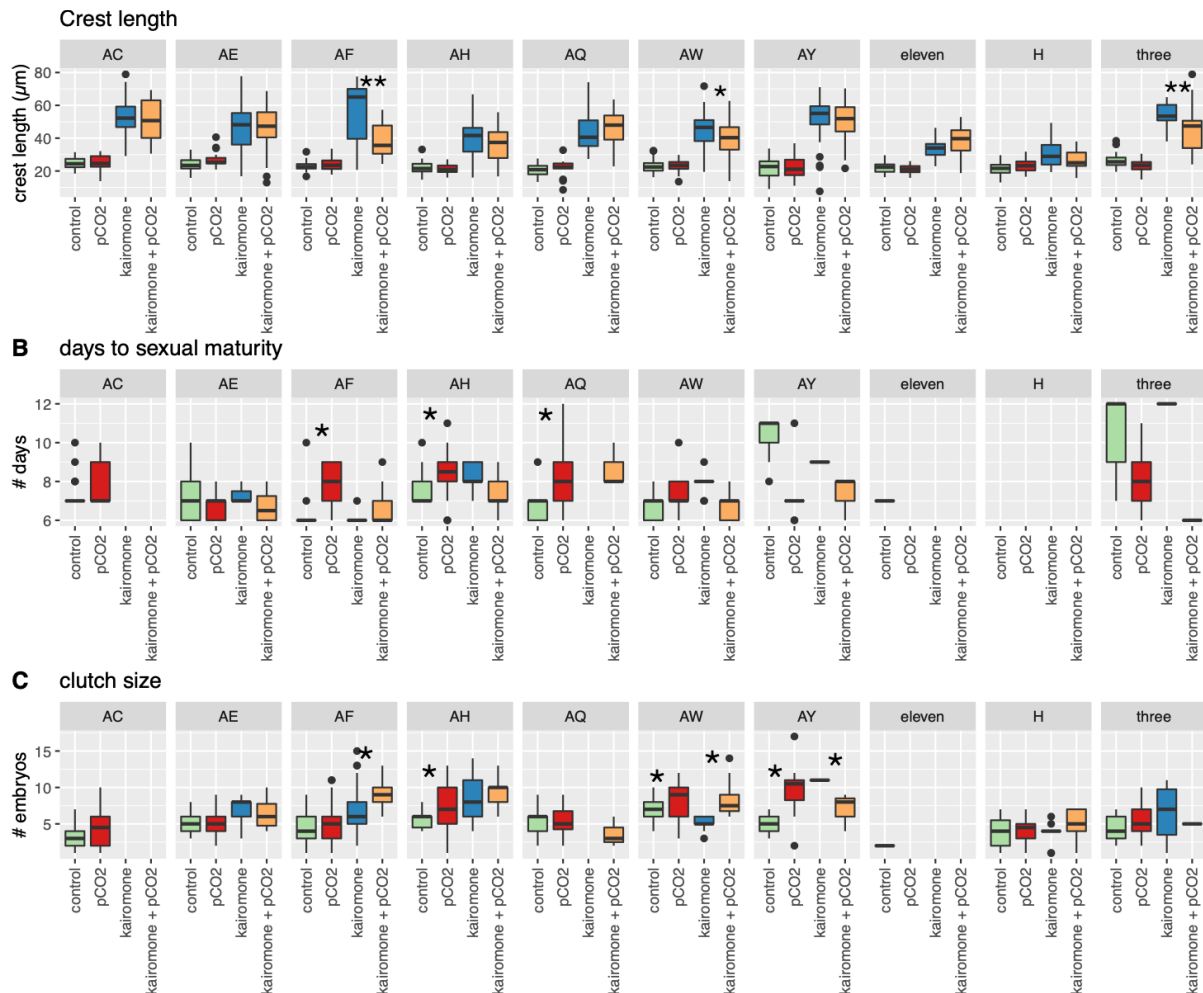

Figure 1: Life-table experiment results from ten clones. We analyzed A) crest length in the second juvenile instar. B) days to sexual maturity, and C) clutch size at sexual maturity.

Based on neckteeth expression levels, we divided these genotypes into strongly, intermediately and weakly defended clones (tab 1). This categorization we also analyzed when the clones were exposed to kairomones and in response to elevated levels of pCO_2. In this case, the strongly defended clones AF, and AE become intermediately defended, and the intermediately defended clone AH becomes weakly defended.
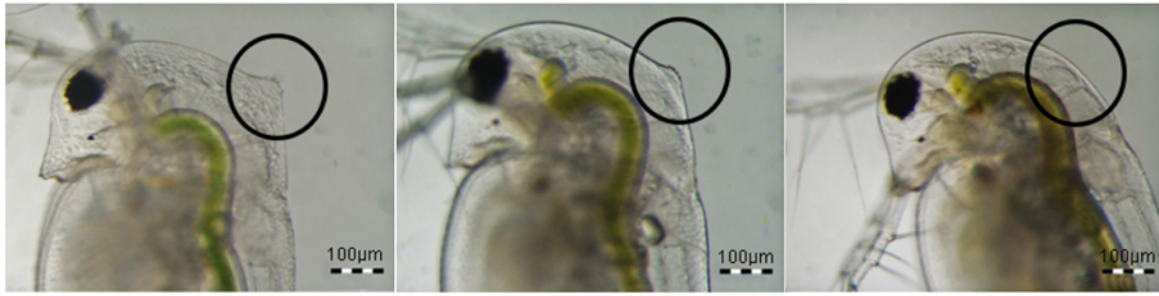
Figure. 2: Select categories of defense expression, ranging from strongly defended (left), to intermediately (middle) and weakly (undefended, right) genotypes.

Clones distinguished in defensive categories			Clones distinguished in defensive categories when exposed to elevated pCO_2		
Strong (>50µm)	Intermediate (<50µm>40µm)	Weak <40µm	Strong (>50µm)	Intermediate (<50µm>40µm)	Weak <40µm
AF	AW	H	AC	AW	H
Three	AQ	eleven	AY	AF	AH
AC	AH		Three	AQ	eleven
AY				AE	
AE					

Table. 1 : Different degrees of defense expression in ten *Daphnia* clones. We artificially divided these genotypes into subgroups of strongly (>50 µm crest expression), intermediate (<50 µm >40 µm crest expression), and weakly (>40µm) defended clones. The investigated clonal population consisted of 5 clones, that were strongly, 3 clones that were intermediately and 2 clones that were weakly defended. When exposed to elevated levels of pCO_2 the strongly defended clones AF, and AE become intermediately defended, and the intermediately defended clone AH becomes weakly defended.

Taken together, the clones AF, AE, AH, AW and three were affected by elevated levels of pCO2 with respect to defense expression.

To determine if this reduction in defense expression also reduces the animals' fitness, we measured survival rates in predation trials per clone (fig. 3). Here we exposed 20 individuals of each clone to one *Chaoborus* larva and determined the number of surviving *Daphnia* in four hours. We observed that especially the clones AC, three, AQ, and AW that had increased survival rate when exposed to *Chaoborus* kairomones, were significantly more predated when exposed to *Chaoborus* + pCO_2.

Between all clones, the clones AE and AH survive *Chaoborus* attacks better when not defended (control) and the clones AE an AQ show highest survival when exposed to CO_2 only. When defended the clones AQ and AC have the highest survival rates in comparison to the other clones (fig. 3b). Between the *Chaoborus* and CO_2 exposed clones, interestingly the clones AH and eleven have the highest survival rate between all clones. The lowest survival

rate between all clones was observed in AC, and AY, when they are not defended. AF and three in the CO₂ condition and not being defended, and AY, and H when defended. In the kairomone and CO₂ exposed condition, survival rate was lowest in clones three and AW.
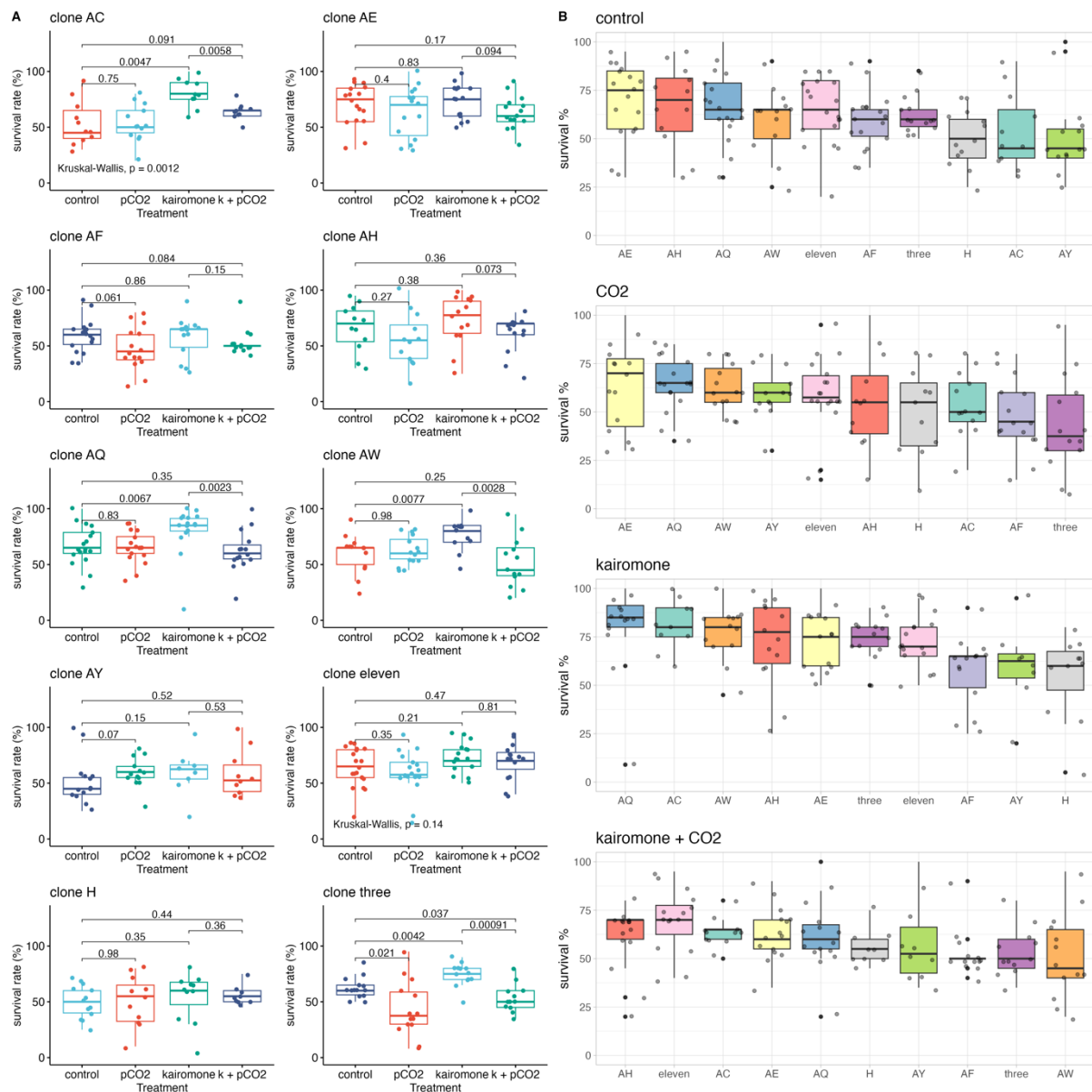

Figure. 3: Survival rates in clonal specific predation trials, sorted by treatment A) and sorted by clone per treatment B.)

We then wanted to test how phenotypic variability under these stressors affects the composition of an artificial *D. pulex* population. This population was composed of the same ten clones with the known traits. In these mesocosms we again exposed this artificial population to the four conditions described above but with three *Chaoborus* larvae as direct

predators. Within one experimental trial we replicated each treatment six times and performed in total three experimental trials.

We then analyzed the population composition after six weeks using microsatellites. We observed unexpectedly stable genotype distribution at the end of our experiments between treatments and replicates (fig. 4a). Clonal diversity declined in all treatments. However, no treatment resulted in particularly lower or higher genotypic diversity in the *Daphnia* populations (fig 4b). In the control and CO_2 mesocosms the clone AH clearly dominated to population repeatedly. Only in the mesocosm with predation where larvae were omnipresent and feeding on the clones, the dominant clone was AE. In combination with CO_2 however, the clone AH again dominates the population event though clone AH is only weakly defended when exposed to CO_2. The most unsuccessful clones in most of the mesocosms were repeatedly the clones H, AQ, three. Interestingly, even though clone eleven is weakly defended but it ranks second in the *Chaoborus* predated populations and not in the non-predated populations. This result is quite unexpected because also the life-history traits do not suggest that it propagates easily. In fact, it was one of the slowest growing clonal lines. The results of clone AH and eleven also mirror in our individual predation trials, where these clones reach intermediate ranks and well survive in *Chaoborus* predation trials. It is therefore, possible that these clones have other e.g. behavioral traits that render them less susceptible.

Clones AF, AC and AY which are strongly defended especially in the predation mesocosms neither dominate the population nor do they rank lastly. It therefore appears that being highly plastic does not always promise population dominance. In fact, the intermediately defended clone AH often dominated the population. In addition, this clone was affected by elevated pCO_2 (under pCO_2 AH is only weakly defended) but still it outcompeted the clone AE (which is continuously strongly defended) in the *Chaoborus* and pCO_2 mesocosm.
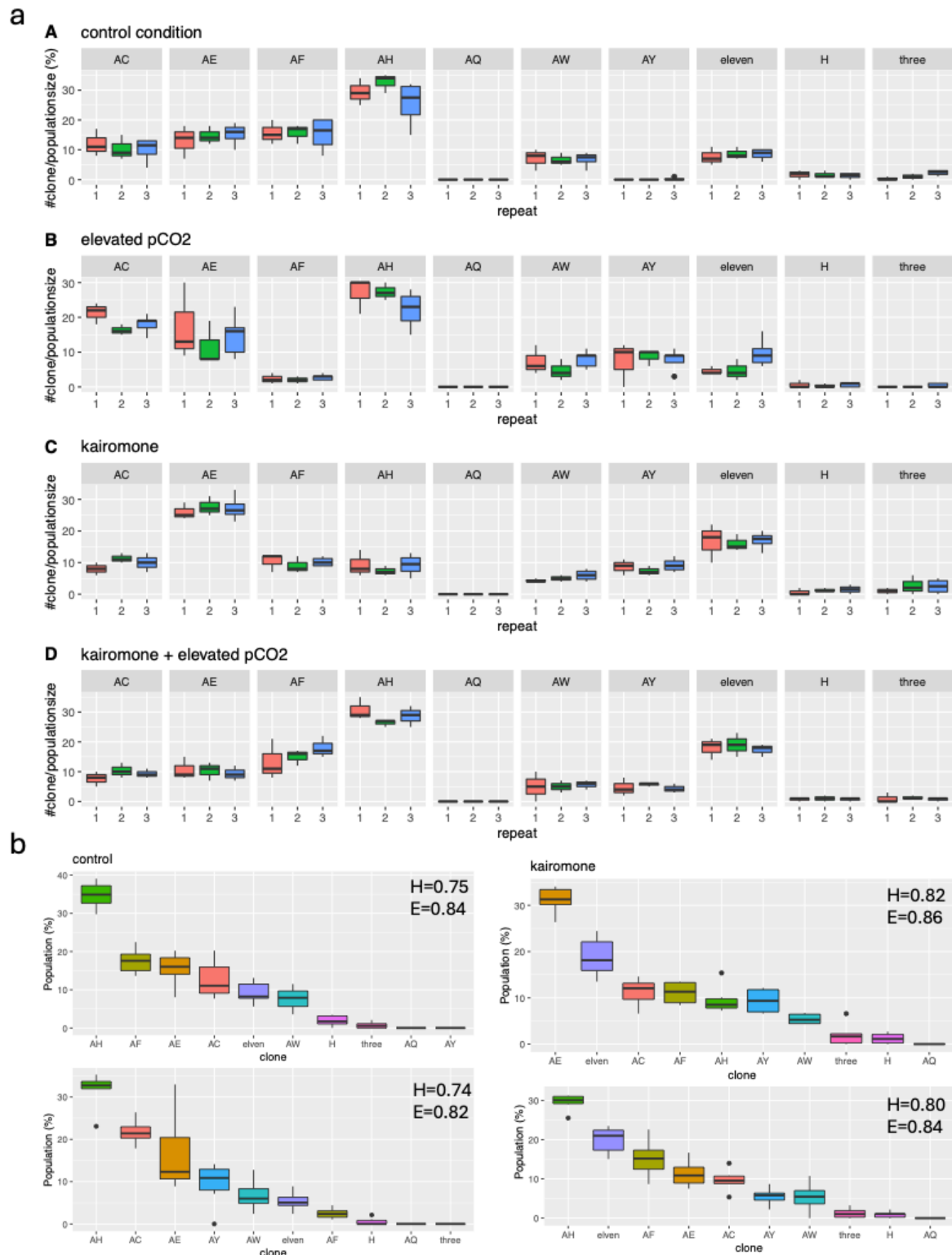
Figure 4: Experimental stability of the mesocosms population composition. a.) Over the three experimental trials, the populations were surprisingly stable between the individual replicates. b.) Population composition after six weeks. Clone AH was dominant in all treatments but the kairomone treatment, where clone AE was dominant.

In conclusion, we could show that within a species phenotypic trait variability can greatly vary between genotypes. We provide evidence, that anthropogenic disturbance (CO_2) can interfere with a species reaction to natural threats (predation). However, under our experimental conditions the treatments did not lead to clonal extinction but to a dominance of different clones. Being highly flexible did not guarantee higher fitness. In fact, we conclude that in general (with some exceptions) that clones that show intermediate reaction norms survive and reproduce at a higher rate than clones at the end of the reaction norm spectrum. Apparently, being average seems to be the cost-benefit optimized solution to the here tested moderate scenarios.

Publications

Laumen, C., Lampert, KP, Tollrian, R., Weiss, LC, (In prep.). Genetic diversity and trait diversity in experimental *Daphnia pulex* communities affected by predators and elevated CO_2

Laumen, C., Lampert, KP, Tollrian, R., Weiss, LC, (In prep). Sensory impairment by elevated levels of CO_2 leading to modified behavioral defenses in *Daphnia pulex*