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The effect of prey resources on evolutionary and ecological dynamics of prey (*Serratia marcescens*) and predator (*Tetrahymena thermophila*)

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TIIVISTELMÄ

Saaliin resurssien määrän ennustetaan vaikuttavan pedon ja saaliin väliseen ekologiseen suhteeseen ja evoluutioon saaliin puolustuskustannuksen kautta. Esim. puolustautumisen allokaatiokustannus laskee tyypillisesti saaliin kilpailukykyä olosuhteissa joissa resurssia on saatavilla vähän. Näitä ennusteita testattiin 14 viikkoa kestäneessä mikrokosmoskokeessa detritusta syövällä akvaattisella *Serratia marcescens* saalisbakteerilla ja *Tetrahymena thermophila* alkueläinpedolla. Laboratoriokokeessa manipuloitiin saaliin ravintoresurssien konsentraatiota (alhainen ja korkea ravintotaso) ja koeyhteisön rakennetta (käsittelyjä, joissa saaliit ja pedot elivät yksin ja yhdessä). Saaliiden ja petojen populaatiokoot mitattiin viikoittain. Lajien kilpailukykyä mitattiin 1-2 viikon välein erillisissä kokeissa, joissa yksin kasvaneita evolutiivisesti naiiveja saaliita ja petoja verrattiin yhdessä kasvaneisiin eli evolutiivisesti kokeneisiin saaliisiin ja petoihin. Pedot pienensivät saaliiden populaatiokokoja kummassakin ravintotasossa. Saaliin resurssien nelinkertaistaminen vaikutti lähinnä saaliiden biomassaan mutta ei kasvattanut petojen populaatiokokoja. Erillisissä mittauksissa havaittiin, että evolutiivisesti kokeneilla saaliilla oli keskimäärin alhaisempi kantokyky ja maksimaalinen kasvunopeus kuin evolutiivisesti naiiveilla saaliilla. Ero populaatioiden kantokyvyssä oli nähtävissä selkeämmin alhaisessa ravintotasossa. Nämä tulokset viittaavat allokaatiokustannukseen saaliin kilpailukyvyn ja pedolta puolustautumisen välillä, kun resurssit eivät riitä kumpaankin yhtä aikaa. Se, ettei saaliin ravinnon lisääminen kasvattanut pedon populaatiokokoja viittaa siihen, että saalis puolustautui myös korkeassa ravintotasossa, jossa sillä oli varaa allokoida yhtä aikaa kilpailukykyyn ja puolustautumiseen petoa vastaan. Tämän kokeen tuloksien mukaan ympäristön tuottavuus ja nopea evoluutio voivat yhdessä vaikuttaa siihen, kuinka tehokkaasti energia siirtyy trofiatasolta toiselle. Tulokset antavat lisätukea uudelle ajatukselle, jonka mukaan evolutiiviset ja ekologiset ilmiöt pystyvät vaikuttamaan toisiinsa samanaikaisesti.

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ABSTRACT

The availability of prey resources can affect ecology and evolution of predator and prey through defence of prey. For example, prey defence can lower the competitive ability of prey when resources are limiting. These predictions were tested in a long-term microcosm experiment with aquatic prey bacteria *Serratia marcescens* (~2400 generations) and predatory protozoa *Tetrahymena thermophila* (~800 generations). The structure of the experimental community and the resource concentration were manipulated in factorial experiment where prey and predator were let evolve alone and together in both low and high resource level. Population sizes of preys and predators were measured weekly. Competitive abilities of alone (evolutionary naïve) and together (evolutionary experienced) evolved prey and predator were evaluated in separate experiments in several time points during the experiment. Predators decreased prey population sizes in both resource levels. The four-fold increase in prey resources increased prey biomass but had no effect on predator population sizes. According to separate life history trait measurements the preys that evolved with predators had smaller carrying capacity and maximum growth rate than preys that evolved alone. In the case of carrying capacity this difference was clearer in low resource level suggesting a trade-off between allocation to defence against predators and competitive ability when resources are limiting. Since the strong increase in the resources of prey did not turn into biomass of predators implies that evolutionary experienced prey in high resource level could allocate simultaneously to defensive and growth abilities. Therefore the productivity of environment and rapid evolution of prey can affect how effectively energy is transferred to upper trophic levels. This study supports the new idea that evolutionary and ecological processes should be considered taking place at the same time-scale.

1. INTRODUCTION

Predator-prey interactions are prevalent in almost all ecosystems creating strong selection pressures for most of species. Usually ecologists have treated predator and prey populations consisting of homogenous sets of individuals, rather than genetically diverse populations capable of evolution (Johnson & Agrawal 2003). Recent findings however suggest that interactions between predator and prey populations can result from both ecological and rapid evolutionary responses of prey or both prey and predator (Nakajima & Kurihara 1994; Thompson 1998; Buckling & Rainey 2002; Yoshida et al. 2003). Despite the growing number of examples, ecological and evolutionary processes are still often thought to occur in different timescales. Thus, rapid evolution is seldom considered to explain the results of ecological phenomena.

Predation is expected to select prey individuals capable of defending themselves over those who are not (Abrams 2000). Further, prey's defensive ability is thought to select predators that can response prey defence by becoming better at consuming prey giving rise to the coevolutionary arm's race (Dawkins & Krebs 1979). The existence of predator-prey coevolution has long been debated. For example, Vermeij (1987 & 1994) has argued that only predators can affect evolution of predator-prey interaction through process called escalation. Escalation is a "top-down" interpretation of the way how organisms affect their evolution. The selection by enemies is seen stronger evolutionary force than the reciprocal selection by a species pair (i.e. predators are more efficient selective agents than preys). According to Vermeij's idea, number of enemies has increased over evolutionary time and that long evolutionary trends in morphology, ecology and behaviour are product of enemy-related adaptations (Vermeij 1994). However, hypothesis of escalation is based on paleobiological view and is more relevant on macroevolutionary scale while rapid antagonistic coevolution can occur within 100 bacterial generations (Buckling & Rainey 2002) and affect significantly ecological processes in nature (Thompson 1999).

Current theory predicts that the rapid evolution of prey is more likely than the evolution of predator (Abrams 2000). This is because predators are thought to exert stronger selection pressure on preys than preys on predators. This asymmetry is often described as life-dinner dichotomy where unsuccessful predation event means further reproduction opportunity for the prey but only missed lunch for the predator (Dawkins & Krebs 1979). In addition, typically preys have shorter generation time and larger population size than predators which allows their faster evolutionary response (Abrams 2000). Even though prey's evolution is thought to be more probable in theory, the presently available experimental data is not sufficient to exclude the possibility of predator's evolution or the coevolution of them both. Even if the evolution of predators has been difficult to demonstrate in practice some experiments have showed that e.g. bacteria and viruses can rapidly coevolve (Buckling et al. 2002; Brockhurst et al. 2003) as predicted by the arms-race theory (Dawkins & Krebs 1979).

Defence traits of prey are often costly reducing defending individual's competitive ability relative to non-defending individuals (Lenski 1988; Nakajima & Kurihara 1994; Yoshida et al. 2004). Therefore the evolutionary and co-evolutionary processes are likely to be constrained by the amount to which prey's competitive ability is decreased by its defensive ability. It is commonly assumed that the trade-offs play important part in predator-prey interaction because without limitation traits could evolve to infinite values (Abrams 2000). It is generally thought that in the absence of predators, competition for

resources (e.g. nutrients) acts as main force leading prey individuals to maximize their growth rate and thus fitness. However, the prey's success in the environment is also defined by their ability to withstand or defend against natural consumers and in the presence of predators preys should allocate energy also to costly defensive ability. How much the other traits are compromised because of prey defence, can depend on the amount of available energy in the environment. Therefore, the productivity of the environment (e.g. concentration of nutrients) can set ecological boundaries for the evolution of predator-prey interaction and affect how evolutionary responses feed back to the ecological level. Previously, rapid evolution has been observed to affect population dynamics (Yoshida et al. 2003, 2004), lead changes in community structure (Hairston et al. 1999), and to alter ecosystem processes (Elser et al. 2000; Gorokhova et al. 2002).

Most of the theoretical predictions of predator-prey relationship are dependent on model structure and specific parameter values. As a result, current theory does not present one but a variety of predictions. Even though the theory have addressed for years how evolutionary processes can affect the ecology of predator-prey interactions there is still a shortage of proper empirical data. One way to overcome the difficulties in experimental evolutionary research is to use microbes as study organisms in model laboratory systems (Jessup et al. 2004). In the laboratory, theoretical predictions can be examined rigorously in a biological system that is easily monitored in ways that would be difficult or impossible in the field (Lawton 1995). The laboratory experiments are easy to replicate and environmental variables can be manipulated one at a time (Elena & Lenski 2003). Microbes have also very short generation times, their genetics are well known and they are easy to propagate, enumerate and store for further analysis. Bacteria are also credible study organisms for predator-prey experiments because in natural communities their growth and survival are strongly constrained by the bacteria-consuming protozoa (Matz & Kjelleberg 2005). Bacteria are known to defend themselves against predation in many ways: escaping by swimming (Matz & Jürgens 2005), forming filamentous cell forms (Hahn & Höfle 1998; Hahn et al. 1999), being capable to withstand predator's digestive enzymes (Boenigk et al. 2001; Bukharin & Nemtseva 2001), growing as biofilm on biotic and abiotic surfaces (Fux et al. 2005; Matz et al. 2005) or forming aggregates too big to be grazed by protozoa (Jürgens & Matz 2002). Besides being useful study organisms microbes are of a great importance as pathogens, are responsible for many essential ecosystem services and industrial applications and represent most of the earth's biodiversity (Whitman et al. 1998). Therefore, it is very important to increase the understanding concerning the dynamics and mechanisms of microbial evolution (Elena & Lenski 2003).

In this experiment I investigated if the prey resource concentration affects the evolution of predator and prey and if these evolutionary changes feed back to the ecological level. Following questions were posed:

1.) Can the prey and the predator evolve in response to predation?
2.) Can the evolutionary changes differ depending on the resource level?
3.) Can the evolutionary dynamics affect to the ecological dynamics of the predator-prey interaction?

Most of the studies concerning evolution have relied on comparative studies (Elena & Lenski 2003). By using microbes, evolutionary process can be observed directly in long-term experiments lasting for thousands of generations (Lenski et al. 1991; Lenski & Travisano 1994). In this microcosm experiment, aquatic and heterotrophic prey bacteria

Serratia marcescens were grown in the absence and in the presence of predatory protozoa *Tetrahymena thermophila* in two different resource concentrations. Evolutionary changes of both prey and predator were measured weekly in separate experiments. According this study, predator-prey interaction can lead to rapid evolutionary changes in life history traits of prey. Tentative evidence for predator's adaptation was also found suggesting coevolution of prey and predator. The results also propose that rapid evolution can affect ecological dynamics in natural communities but the degree can depend on the quality of the environment.

2. MATERIALS AND METHODS

2.1 Study species

Predator, the aquatic ciliated protozoa *Tetrahymena thermophila* (Elliot 1974), was obtained from the American Type Culture Collection (ATCC strain number 30008). The strain is originally isolated from fresh water in Falmouth, MA, in 1952. Prior starting this experiment, the strain was cultured axenically for six months in proteose peptone-yeast extract medium (10 g of Bacto™ peptone and 2.5 g of Bacto™ yeast extract in 1 litre of distilled water; Becton, Dickinson and Co., Franklin Lakes, NJ). *Tetrahymena* has short generation time (at fastest 3 hours in above mentioned medium) and well-known biology (Hill 1972; Elliot 1974; Fenchel 1987). It has been used as model predator in many microcosm experiments (Nakajima & Kurihara 1994; Ammendola et al. 1998; Bukharin & Nemtseva 2001; Klobutcher et al 2006). *Tetrahymena* is typically 30 μm long but large changes in size can occur under stress, e.g., food depletion and thermal stress (Laakso et al. 2003). *Tetrahymena thermophila* feeds on particles (e.g. bacteria and nonliving particles) and macromolecules (pinocytosis) and it reproduces asexually through binary fission (Elliot 1974). The strain is likely to be genetically homogenous due to a long history of serial transfer culturing.

The prey, *Serratia marcescens*, is gram-negative, rod-shaped, typically 0.3–1.0 × 1.0–6.0 μm size bacterium and belongs to the family Enterobacteriaceae (Krieg & Holt 1984). *Serratia marcescens* is the type species of the genus. For our experiment *Serratia marcescens* strain was obtained from the American Type Culture Collection (type strain, ATCC number 13880). The strain is originally isolated from pond water. In our experimental conditions one *Serratia marcescens* generation lasts 1 hour at minimum. *Serratia marcescens* is facultative anaerobe, produces red pigment prodigiosin and is motile by peritrichous flagella. In liquid medium, the bacteria are short rods with few flagella and show classical swimming behaviour but it can also differentiate into long and highly flagellated swarming cells (Sharma & Anand 2002). The swarming cell forms of *Serratia liquefaciens* longer than 30 μm have shown to be resistant to predation by *Tetrahymena sp*. (Ammendola et al. 1998). *Serratia marcescens* is commonly found in freshwater environments, plants, insects, and mammals including humans (Balows et al. 1992). Healthy humans do not often become infected by *Serratia marcescens* while hospitalized patients get frequently infected (Balows et al. 1992).

2.2 The design of the evolution experiment

An aquatic microcosm experiment with three trophic level food chain was set up. The prey bacteria were fed on nonliving resource (plant detritus) and the predatory protozoa used the prey bacteria as their sole source of energy. Control treatments were set up where prey bacteria and predatory protozoa evolved alone (hereafter called as naïve preys and predators). Thus, alone evolved preys and predators (populations exposed to drift) could be compared later with the preys and predators that had accompanied evolutionary history (hereafter called as experienced preys and predators). Besides controlling the predator-prey interaction the resource environment was manipulated by setting it to two different resource concentrations (low and high, Figure 1.). Total amount of energy for the populations was same in low and high resource level treatments but in each low resource level treatment, the volume was fourfold compared to the high resource level treatment. Altogether 6 treatments were found in which prey (*Serratia marcescens*) and predator (*Tetrahymena thermophila*) evolved alone (control treatments; naïve prey and predator) and together (experienced prey and predator) in both low and high resource level (Figure 1). All treatments were replicated 6 times (a total of 36 microcosms). The evolution experiment lasted approximately 14 weeks (24.6 - 3.10.2004) giving a maximum of 800 *Tetrahymena* and 2400 *Serratia* generations.

2.3 Comparison between evolutionary naïve and experienced prey and predator

The life history traits of naïve and experienced preys and predators were compared from weekly samplings alongside the evolution experiment (Figure 1). In these additional experiments preys and predators were grown alone in the same nonliving resource where they had been evolving during the evolution experiment and their life history traits (indicated by the carrying capacity and maximum growth rate of populations) were determined. The carrying capacity and the maximum growth rate measured in the population level depict the ability of individual cells to compete in low and high resource environments respectively. These traits were selected for both prey and predator because they are easy to measure and are descriptive of their competitive ability.

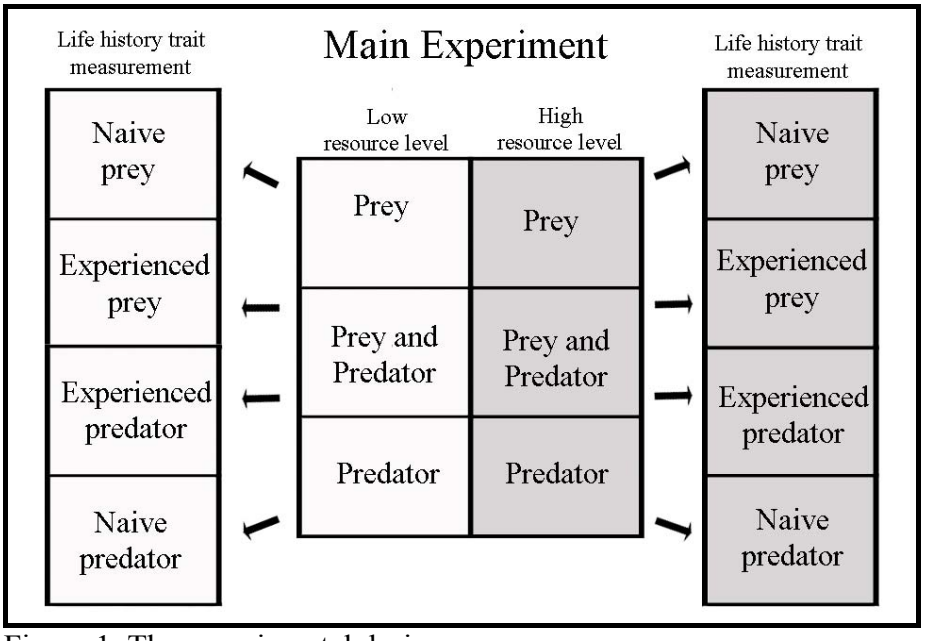

Figure 1. The experimental design

2.4 Culture media

Four different culture media were used during the experiment. In the evolution experiment bacteria populaions were grown in cereal leaf extract (abbreviated hereafter as "SPL"-medium containing 0.25g (low resource SPL) or 1g (high resource SPL) of cereal leaf powder in 1 litre of distilled water (abbreviated further as dH_2O); Ward's natural science, Rochester, NY.). SPL-medium was prepared as follows: one litre of dH_2O was heated to 100°C and 2g of cereal leaf powder was added to the water. After ten minutes of boiling, cereal leaf liquid was cooled down and filtered through glass microfibre filter (GF/C, Whatman). SPL-medium was diluted to 0.25 and 1g/L concentration, autoclaved (121°C for 25 minutes) and sterile phosphate buffer (47.172g of $K_2HPO_4 \cdot 3H_2O$, 12g of KH_2PO_4, 15 g of $(NH_4)_2SO_4$, 3g of $MgSO_4 \cdot 7H_2O$, 0.3g of NaCl and 0.684g of $CaCl_2 \cdot 2H_2O$ per 490 ml of dH_2O) added so that final pH of 7.5 was reached.

Agar plates were used to separate preys from the predators before performing life history trait measurements. The agar plates contained 10g of Difco™ nutrient broth, 2.5g of Bacto™ yeast extract and 15g of Bacto™ agar (Becton, Dickinson and Co., Franklin Lakes, NJ) in one litre of dH_2O. Because SPL-medium alone can not support growth of *Tetrahymena*, predators in control treatments (*Tetrahymena* alone) were fed with proteose peptone-yeast extract medium (abbreviated further as "PPY"-medium containing 10g of Bacto™ peptone and 2.5g of Bacto™ yeast extract (Becton, Dickinson and Co., Franklin Lakes, NJ) in 1 litre of dH_2O). This high resource PPY-medium was diluted fourfold to obtain low resource PPY-medium. When the study species' life history traits were measured in separate experiments *Tetrahymena* were grown in enhanced proteose peptone medium (abbreviated further as "ePP"-medium containing 10g of Bacto™ Peptone; Becton, Dickinson and Co., Franklin Lakes, NJ and 1g of liver concentrate; Sigma-Aldrich Corp., St. Louis, MO) in 1 litre of dH_2O. The ePP-medium supports up to 20-fold larger population sizes and faster growth for *Tetrahymena thermophila* than the PPY-medium (Elliott 1974). All culture media were sterilized in autoclave (121°C for 25 minutes) before the use.

2.5 Microcosms and sampling

Microcosms were modified from 250 ml polycarbonate cell culture bottles (Corning, Figure 2.) To allow gas exchange, the bottles were sealed with vent caps (non-wettable membrane with 0.2 μm pore size; Corning). The microcosms were connected via silicon tube (1 mm inner diameter) to separate resource stock bottles, 50-ml storage syringe, outflow tube (2 mm inner diameter) and to ten 5-ml syringes for sterile sampling which were used when changes in the prey's and predator's life history traits were assessed in additional batch-culture experiments. During the weeks when we did not assess species life history traits samples were taken through microcosms' outflow tube. To prevent microbial contamination between samplings the outflow tubes were submerged in 75 % ethanol which was replaced every week. The inflow and outflow tubes were heated to 80ºC with 10 cm long heating elements to further reduce the risk of contamination. Prior to sampling the microcosm was gently agitated, 50% of microcosm's volume was drawn aside to storage syringe, the sample taken and fresh resource medium added to the microcosm from the stock bottle. After sampling and renewal, the content of the storage syringe with remaining population was returned to the culture bottle. The sampling and subsequent renewal of resources was performed at seven days intervals.

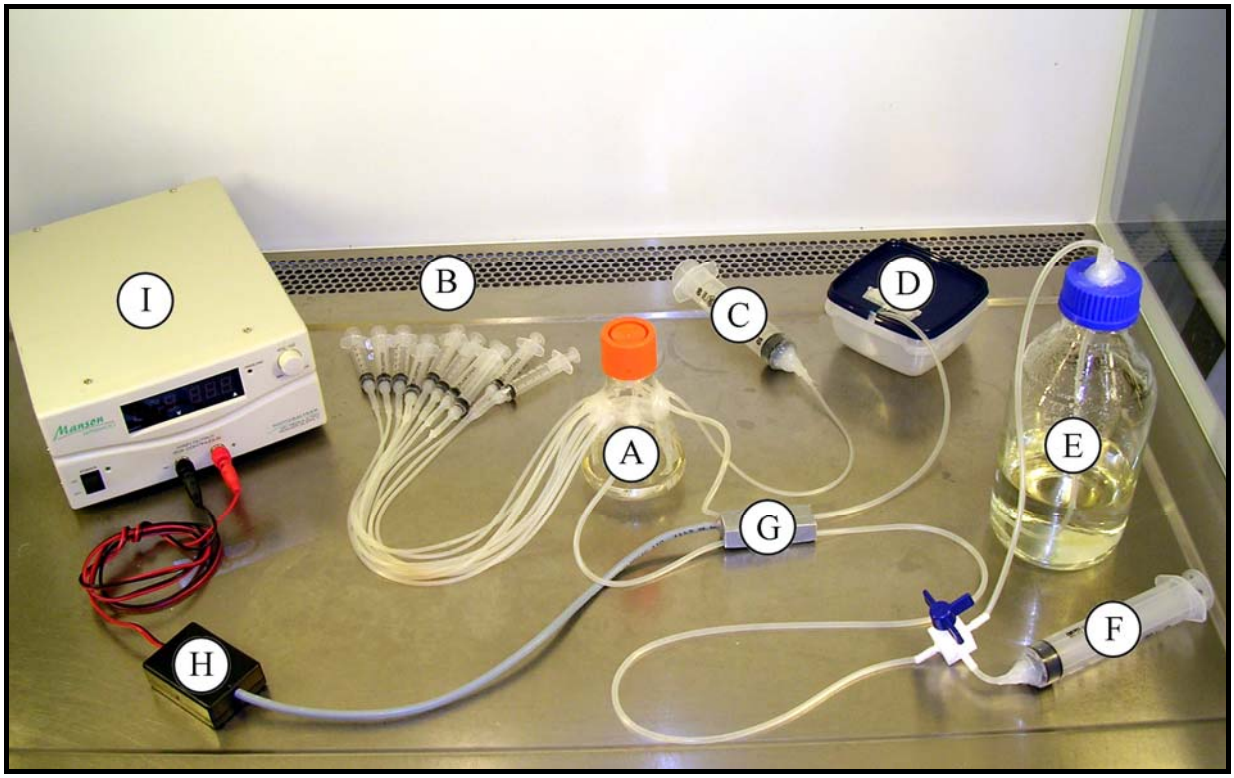

Figure 2. A: the microcosm, B: 10 sterile 5 ml syringes for sampling, C: a 50 ml storage syringe, D: Outflow tube submerged in 75 % alcohol, E: the resource stock bottle, F: a 50 ml syringe and 3-way valve for renewal of the resources, G: a heating element, H: a thermostat and I: a power supply for the heating element.

2.6 Establishment of the evolution experiment

Prior to starting the evolution experiment all microcosms, stock bottles and other attached parts were autoclaved at 121°C for 25 minutes. All microcosms that included prey were initiated from the same prey clone: one *Serratia marcescens* colony grown on agar plate was mixed into 50 ml of high resource SPL-medium and incubated in 25°C for 24 hours. 2 ml of this bacterized SPL-medium was inserted into 2 litre of low and 2 litre of high resource SPL-medium and incubated in 25°C for 24 hours after the bacterized SPL-media were measured into the microcosms in 30 ml (high resource treatments) and 120 ml (low resource treatments) volumes. Microcosms including predator were initiated from *Tetrahymena thermophila* stock medium (high resource concentration PPY-medium): one week prior the start of the experiment 10 ml sample of well-shaken *Tetrahymena* culture was added into 90 ml of high resource PPY-medium. When the experiment was started 1.5 ml of this *Tetrahymena* sample was added to the predator with prey and to the predator-alone treatments. Same amount of dead, autoclaved *Tetrahymena* was added to the prey-alone treatments to compensate for differences in nutrient and energy input between treatments. Some of the microcosm's stock bottles that included bacteria were contaminated within the first four days but were subsequently replaced with new ones. One replicate from the low resource level treatment with naïve *Tetrahymena* was contaminated after one week. However, replicate was re-initiated from one of the randomly chosen remaining microcosm within the same treatment.

2.7 Measurements from the weekly samplings

2.7.1 Determination of prey and predator population sizes

Population sizes of *Tetrahymena thermophila* were determined from one ml samples which were fixed with Lugol's solution (0.7% final concentration) and stored in 4ºC. 200 µl of fixed sample was inserted into a glass cuvette rack (depth 2.34 mm), individuals were let descend to the bottom and eight greyscale images were taken with an Olympus SZX microscope (40x magnification) connected to Panasonic WV-CL702 video camera. The images were captured with Matrox meteor II video capture board and Image Pro Plus™ (v.4.5, Medium Cybernetics Inc.) image analysis software. The individuals were identified and counted automatically based on the object's size and shape using an image recognition script written for the Image Pro Plus (J. Laakso, unpubl.). Population sizes had to be counted manually from 8 greyscale images in the treatments where *Tetrahymena* preyed on bacteria (in low and high resource level) because bacteria formed large aggregations which prevented the use of the automatic image recognition.

Population sizes of *Serratia marcescens* were measured as optical density at 600 nm wavelength on sterile microtitre plates (Nunclon™ Delta Surface 96-well) with spectrophotometer (Thermo labsystems Multiskan ascent; ascent software version 2.6). Population sizes of the 200 µl sub-samples were calculated as an average of 30 successive absorbance measurements performed at 2 minutes interval. Before determining the population size estimates the background absorbance of the SPL culture media was removed from the measurements. The microcosms' pH was monitored from weekly samplings with Denver instrument basic digital pH meter. The prey samples were also cryopreserved for further analyses as follows: 500 µl of prey sample was weekly mixed into cryopreservation medium containing 500 µl of glycerol (Riedel-de Haën, 86-88%, Sigma-Aldrich) and 100 µl of nutrient broth-yeast extract (same medium that was used for agar plates except for agar). After 30 minutes the cryogenic vials were agitated and transferred to -20ºC and stored later at -70ºC.

2.7.2 Determination of life history traits of prey

Before measuring the evolutionary changes in the life history traits of prey the bacteria were grown alone for 3 day period. This was done in order to separate genetic effects from the effects caused by physiological state of the bacteria at the moment of sampling. Life history traits of naïve and experienced preys, maximum growth rate and carrying capacity, were measured weekly for the first five weeks and thereafter every second week. Measurements were performed as follows: 100 µl of diluted sample of bacteria were plated on agar plates and after two day incubation, 20 colonies were randomly selected from every plate and mixed into SPL-medium. After 24 hours of incubation 10 µl of diluted naïve and experienced prey (that had been growing in the absence of predator for 3 days) were inoculated to 190 µl of fresh SPL-medium and their growth were measured with spectrophotometer (at 600 nm wavelength and 10 minutes intervals) giving a total of 432 measurements during next 3 days. The bacteria's growth was measured only in the same resource environment where they had been living in the evolution experiment. The carrying capacity (K) was determined as the final density of the prey's population size and the maximum growth rate ($r_{max)}$ was calculated as aslope of a linear regression of log transformed population size plotted against time where data

showed exponential growth. Some of the first data points were excluded because of the too strong background noise of the measurement device.

2.7.3 Determination of life history traits of predator

Life history traits (K and r_{max}) of naïve and experienced predators were measured after 5 weeks from the beginning of the experiment. 100 µl of sample of naïve and experienced predator were inoculated into 900 µl of 1% ePP-medium with 3.1 µl of antibiotic (10 000 units of penicillin and 10 mg of streptomycin per ml 0.9 % NaCl, Sigma-Aldrich) per 1 ml of ePP. We used the antibiotics to separate the predator from the prey in the treatments where they lived together. The concentration of antibiotics was tested prior to be optimal, i.e. being lethal to the prey but leaving predator unharmed. After 24h antibiotic treatment 571 µl sub-samples were inoculated into 8 ml of new, fresh 1% ePP-medium. After two days of incubation 1.5 ml sub-samples were inoculated into 30 ml of fresh 0.25% or 1% PPY-medium) and predators' growth measurements were started. The growth of protozoa was measured in the same resource environment where they had been living in the evolution experiment. The density in the predator inoculums were not standardized prior starting the growth measurement. However the inoculum's population size were measured and as it showed not to be statistically significant covariate in the ANOVA's model ($F_{1, 19} = 1.7$, $p = 0.2$) we left it out from further analysis. The batch cultures were sampled after 2, 4, 24, 76 and 100 hours and the predator's population sizes were determined as described above. Predator's K was determined as the final density of their population size at 76 h after the start of the measurement and the r_{max} was calculated from the linear regressions of log transformed population size against time (between 4 – 24 hours) where data showed exponential growth.

2.8 Data analysis

All data was analysed with repeated measures ANOVA or two-way ANOVA. The experimental design had two factors which both had two levels: factor for evolutionary experience (naïve or experienced preys and predators) and factor for resource concentration (low or high). All statistical analyses were performed using SPSS (v. 12.0.1, SPSS Inc., Chicago, IL). SPSS's missing value analysis (EM) were used to input three missing values of total 504 observations in pH-data and for four values of total 432 observations in prey's evolutionary data before proceeding to repeated measures ANOVA. Missing values in pH-data were due to a week when we had not enough sample for the pH measurement and in the prey's evolutionary data due to condensation of water vapour into the 96-well microtitre plate's lid.

3. RESULTS

3.1 Prey population size dynamics during the experiment

During the fourteen weeks the prey population sizes increased in all treatments except for prey with predator treatment in low resource level where the population size decreased (Greenhouse-Geisser corrected $F_{3.6, 72.1} = 5.13$, $p = 0.002$, Figure 3). Overall, the prey population sizes were larger in high resource level than in low resource level ($F_{1, 20} = 249$, $p < 0.001$). Predators also decreased the prey populations ($F_{1, 20} = 411$, $p < 0.001$) and alone-grown prey populations were larger than prey populations exposed to predation within both resource levels ($F_{3, 20} = 251$, $p < 0.001$, Bonferroni adjusted multiple

comparisons: alone-grown prey vs. prey exposed to predation within low ($p < 0.001$) and high ($p < 0.001$) resource level). Also the population sizes of preys that were exposed to predators were larger in high than low resource level ($F_{3, 20} = 251$, $p < 0.001$, Bonferroni adjusted multiple comparisons: prey exposed to predation in low vs. prey exposed to predation in high resource level ($p = 0.002$).

3.2 Predator population size dynamics during the experiment

Predator population sizes decreased in all treatments during the experiment (Greenhouse-Geisser corrected $F_{2.9, 59.7} = 6.02$, $p < 0.001$, Figure 4.). In high resource level predator population sizes remained larger compared to low resource level treatments ($F_{1, 20} = 142$, $p < 0.001$). Also the predator with prey treatments had smaller population sizes than alone-grown predator treatments ($F_{1, 20} = 692$, $p < 0.001$) and this difference were seen within both resource levels ($F_{3, 20} = 308$, $p < 0.001$, Bonferroni adjusted multiple comparisons: alone-grown predator vs. predator grown in the presence of prey in low ($p < 0.001$) and high ($p < 0.001$) resource level). Interestingly, population sizes of predator with prey treatments did not differ between resource levels (Figure 4, picture B, Bonferroni adjusted multiple comparisons: $p = 0.632$).

3.3 Dynamics of pH

pH was measured as a background variable during the experiment because it is known to affect growth of bacteria (Neidhardt 1990) and protozoa (Prescott 1958). In the beginning, pH increased momentarily in predator alone and prey with predator treatments in low resource level but started to decrease after 5 weeks in all treatments (Greenhouse-Geisser corrected $F_{9.7, 146} = 6.03$, $p < 0.001$) approaching the mean value of 7.4 ($pH = 7.43$, $N = 36$, $SD = 0.35$). The experimental community ($F_{2, 30} = 721$, $p < 0.001$, Figure 5.) and resource level ($F_{1, 3} = 26.7$, $p < 0.001$) had effect on pH, predator alone treatments having the highest, prey with predator treatments intermediate and the prey alone treatments lowest pH value. The high pH values of alone-grown predator treatments are due to PPY-medium which did not include phosphate buffer.

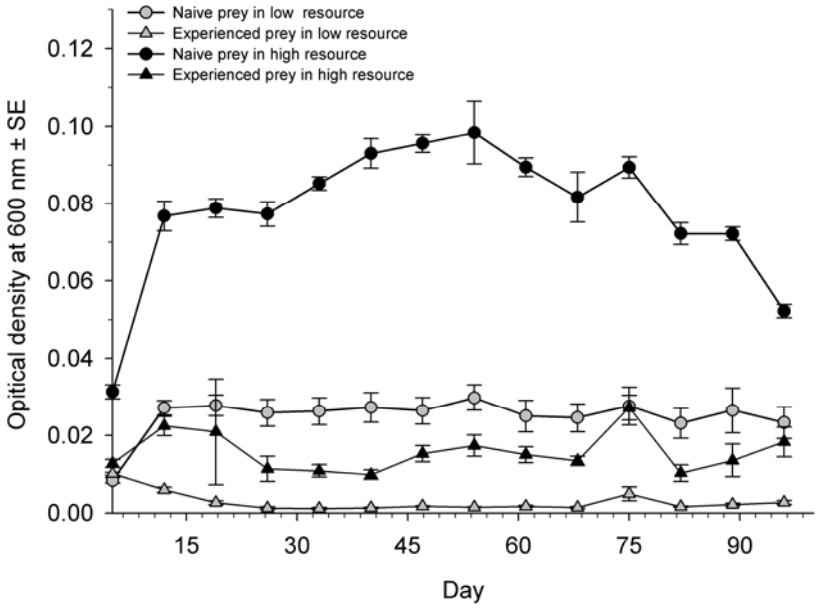

Figure 3. Mean (N = 6) population sizes of the prey *Serratia marcescens* in low and high resource concentrations in the absence or presence of the predator *Tetrahymena thermophila*

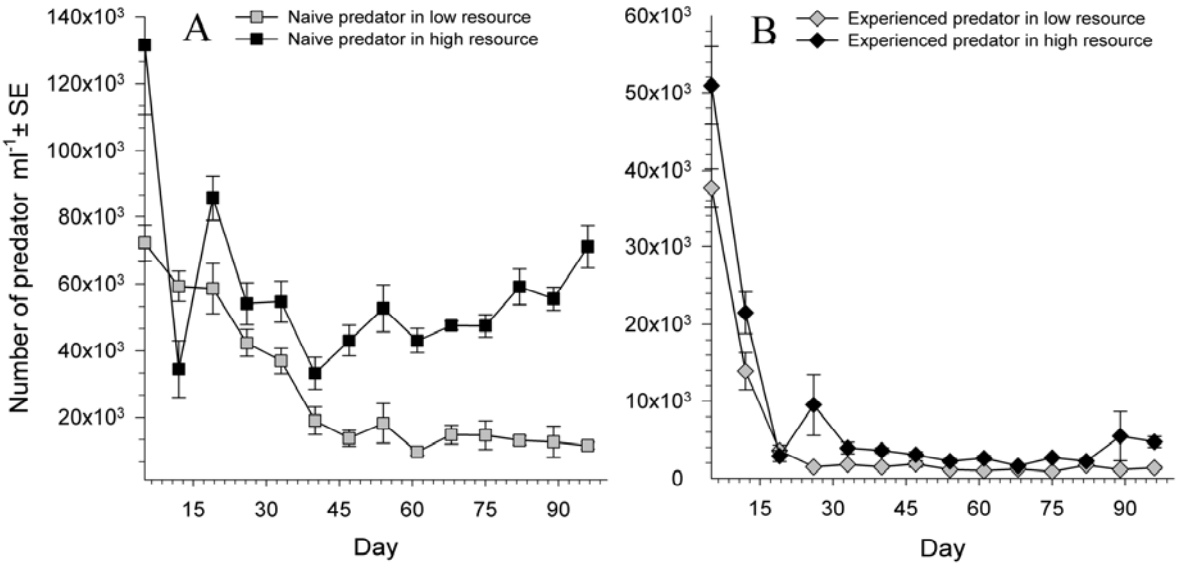

Figure 4. Mean (N = 6) population sizes of the naïve (feeding on PPY-medium in picture A.) and experienced (feeding on prey bacteria in picture B.) predator *Tetrahymena thermophila*.

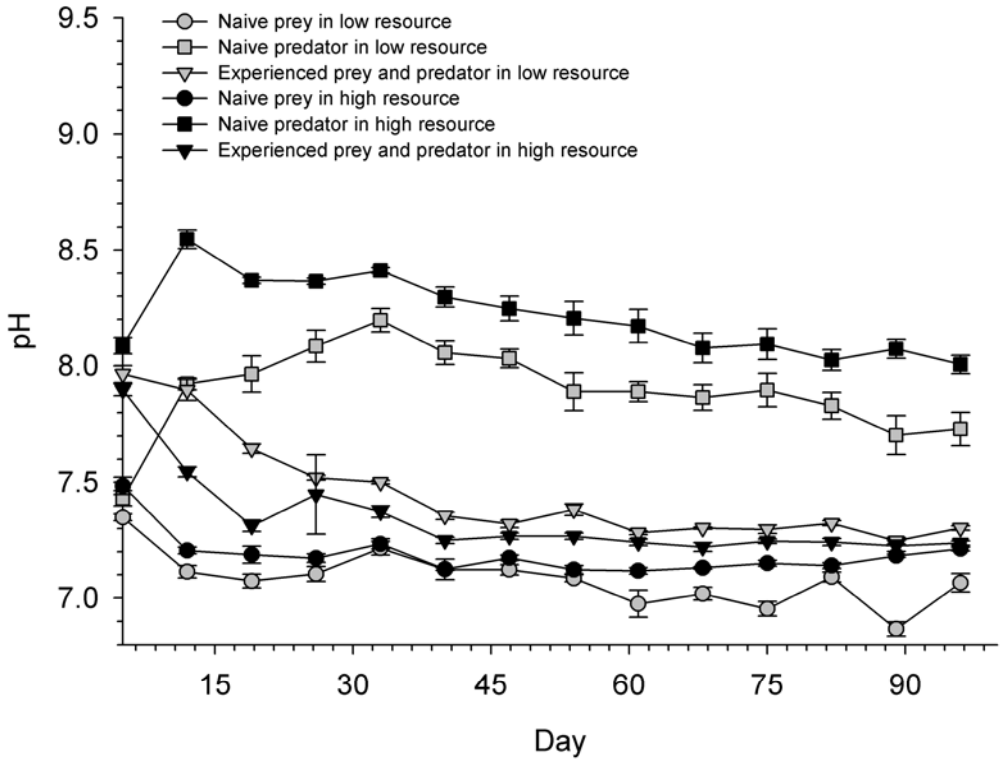

Figure 5. Mean (N = 6) pH in the different treatments. Growth medium was buffered in treatments which included the prey.

3.4 Evolutionary changes in K and r_{max} of prey

The interaction with time and resource level was significant in both carrying capacity (Greenhouse-Geisser corrected $F_{2.7,\ 54.6} = 12.4$, $p < 0.001$) and maximum growth rate (Greenhouse-Geisser corrected $F_{3.8,\ 76.8} = 9.7$, $p < 0.001$) of prey. In high resource level evolved preys had higher carrying capacity ($F_{1,\ 20} = 571$, $p < 0.001$) and maximum growth rate ($F_{1,\ 20} = 1142$, $p < 0.001$) when compared to preys evolved in low resource level (Figure 6 and 7). Interestingly, alone evolved preys had higher carrying capacity ($F_{1,\ 20} = 30.9$, $p < 0.001$) and maximum growth rate ($F_{1,\ 20} = 10.8$, $p = 0.004$) than preys that had been evolving with predators. In the case of carrying capacity, there was also an interaction between resource level and evolutionary experience ($F_{1,\ 20} = 5.7$, $p = 0.027$) and further analyses revealed that the evolutionary naïve and experienced preys differed in their carrying capacity within the low but not in the high resource level ($F_{3,\ 20} = 204$, $p < 0.001$, Bonferroni adjusted multiple comparisons: naïve vs. experienced prey in low ($p < 0.001$) and in high ($p = 0.96$) resource level). However, we did not find an interaction between evolutionary experience and resource level in the case of maximum growth rate ($F_{1,\ 20} = 1.13$, $p = 0.3$).

3.5 Defence mechanism of prey

The population sizes of *Tetrahymena* had to be counted manually already after one week of the beginning of the experiment because bacteria seemed to form aggregates in the prey with predator treatments (Figure 8.). No bacterial aggregations were however found

from the predator alone treatments. Forming aggregations has been reported earlier to be effective defence mechanism against particle-feeding protozoa (Salcher et al. 2005).

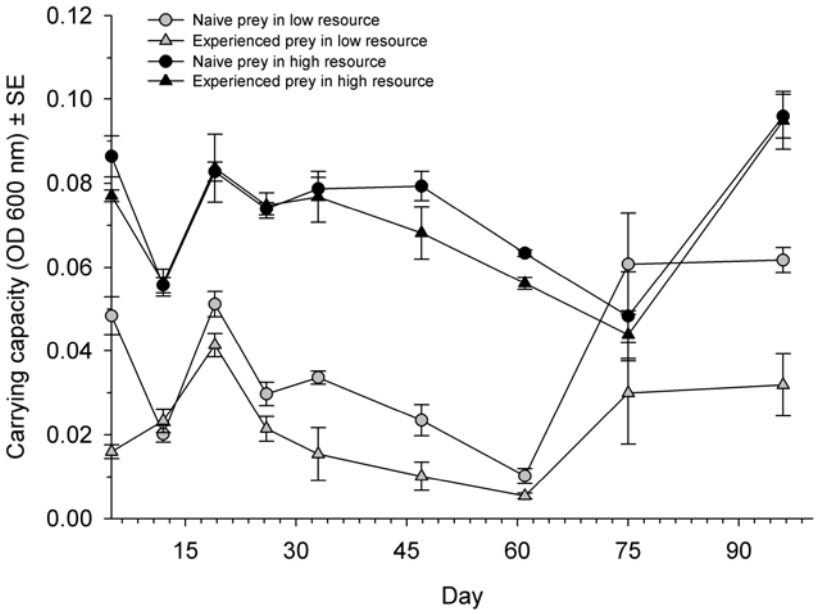

Figure 6. Mean (N = 6) carrying capacity of preys measured in separate experiments

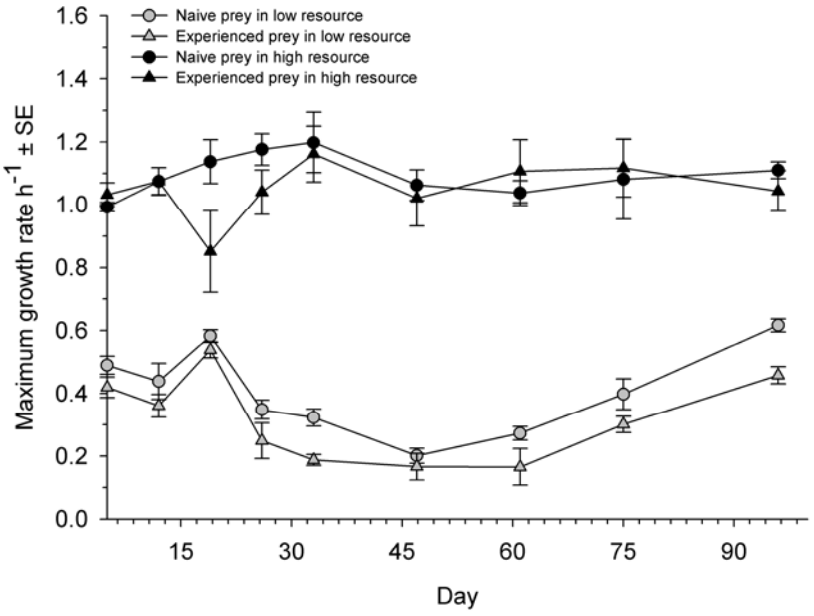

Figure 7. Mean (N = 6) maximum growth rate of preys measured in separate experiments

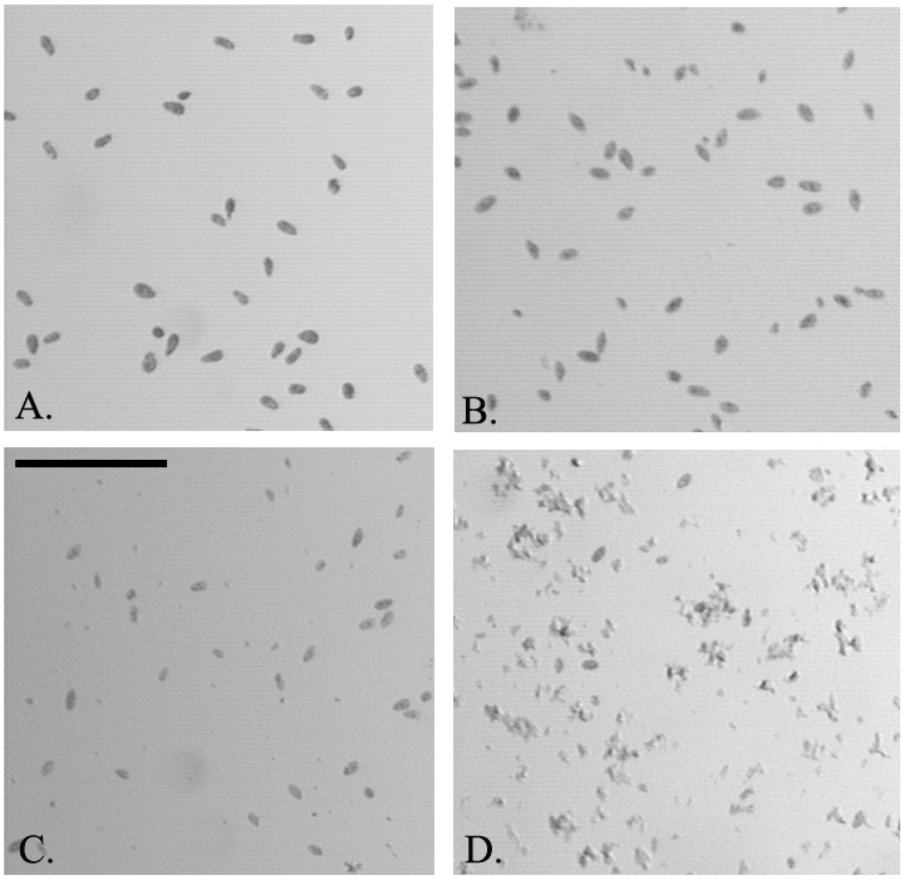

Figure 8. *Tetrahymena* living alone in high resource PPY-medium after 5 (picture A.) and 89 days (picture B.) and *Tetrahymena* living with prey *Serratia marcescens* after 5 (picture C.) and 89 days (picture D.) from the beginning of the experiment. Black bar in picture C. is 250 μm long and the scale is same in all pictures.

3.6 Evolutionary changes in K and r_{max} of predator

Predators had smaller carrying capacity in low than in high resource level (two-way ANOVA, $F_{1, 20} = 10.7$, $p = 0.004$) but the evolutionary naïve and experienced predators did not differ in carrying capacity ($F_{1, 20} = 2.49$, $p = 0.13$, Figure 9). However, an almost significant interaction with resource level and evolutionary experience was found ($F_{1, 20} = 3.7$, $p = 0.069$). Further analyses revealed that in the low resource level the carrying capacity of evolutionary experienced predators was smaller than carrying capacity of naïve predators (one-way ANOVA, pairwise comparisons, $p = 0.02$) but no differences were found in high resource level. The predators had larger maximum growth rate in high than in low resource level ($F_{1, 20} = 5.48$, $p = 0.03$) but statistical difference between the naïve and experienced predators was not found ($F_{1, 20} = 0.1$, $p = 0.75$, Figure 9.). The interaction between resource level and evolutionary experience was not either statistically significant ($F_{1, 20} = 0.003$, $p = 0.96$).

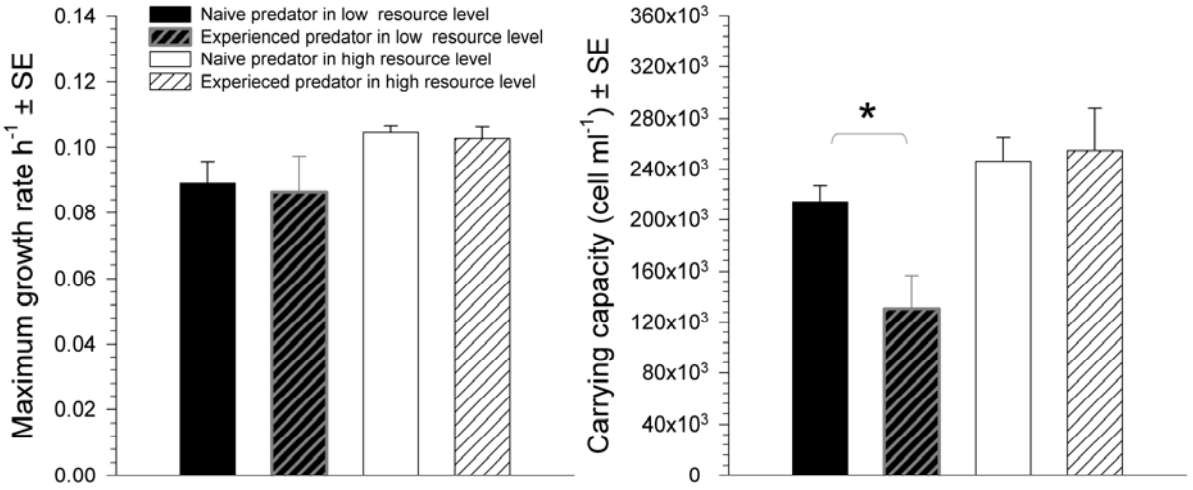

Figure 9. Mean (N = 6) maximum growth rate and carrying capacity of predators measured after 5 weeks from the beginning of the experiment.

4. DISCUSSION

4.1 The evolution of prey

This study demonstrates that resource environment affects to the evolutionary and ecological dynamics of predator-prey interaction. Predator and prey coexisted in this study system for 14 weeks. That the prey populations exposed to predation remained smaller than prey populations grown alone in both resource level treatments indicates a continuous predation pressure for the whole duration of the experiment. However, *Tetrahymena* did decrease to small numbers in prey with predator treatments which could indicate defensive adaptation of prey in response to predation. Even though pH differed slightly between experimental communities it stayed within the range of good growth conditions for *Tetrahymena thermophila* (Prescott 1958) and *Serratia marcescens* (Neidhardt et al. 1990) and unlikely affected this study's results.

According to the separate life history trait measurements preys that were exposed to predators had lower carrying capacity and maximum growth rate compared to preys that had been evolving alone. Interestingly, in the case of carrying capacity this difference was especially clear in the low resource level treatments. These results suggest a trade-off between defensive and competitive ability of prey meaning that in order to survive in the presence of predators, preys have to allocate energy to costly defence on the expense of other traits. Although the defence mechanism of prey was not assessed directly, the reduced competition ability of prey (e.g. reduction in carrying capacity) is thought to be a good indicator of costly defence (Bohannan & Lenski 2000, 2002) and this view is supported by previous predator-prey experiments done with microbes (Lenski 1988; Nakajima & Kurihara 1994; Yoshida et al. 2003, 2004). That we did not see differences between naïve and experienced preys competitive ability in the high resource level could be due to life history trait measurements which were done only in the same resource environment where preys had evolved previously during the evolution experiment. Therefore it is possible that the preys allocated energy to defensive ability in both resource levels but its associated costs were not detectable in the high resource level where preys had enough energy to afford both competitive and defensive ability. Further life history

trait measurements carried out from the frozen samples could reveal how the cost of defence of evolutionary experienced prey depends on the availability of resources.

The observed ecological dynamics also suggest that the experienced prey allocated energy to defence against predators in high resource level. Firstly, the population sizes of the predators feeding on the bacteria dropped steeper and faster than in the control treatments in both resource levels during the first three weeks of the experiment (Figure 4., picture B). Secondly, the population sizes of experienced predators dropped down to the same level in both resource levels. This means that the fourfold increase in the resources of prey did not transform to predator biomass but increased only population sizes of preys (Figure 3.). These results could be interpreted in several ways. It could be that the additional energy in prey with predator treatments increased mainly the frequency of defending prey clones over the non-defending prey clones (Bohannan & Lenski 1997, 1999). This would have led to the observed increase in the population size of preys but not to changes in predator population sizes as they were apparently unable to benefit from the defending prey clones. On the other hand, it could be that the additional energy uniformly increased the prey investment in defensive ability which could have improved their survival probability and increased their population size. A third explanation could be that the evolutionary outcome differs between the resource environments: high energy availability could allow other type of solutions to predator defence than the low energy environment. For example, there is consistent trend for the bacteria in oligotrophic environment to be smaller than the bacteria in eutrophic environments (Fuhman et al. 1989; Van Dyal et al. 1990; Moriarty & Bell 1993; Clements & Foster 1999). It has been also shown before that the bacterivory by protists can be size-selective (Lampert 1987) where small and big enough bacterial cells can survive protozoa's grazing (Nakajima & Kurihara 1994; Ammendola et al. 1998; Jürgens & Matz 2002). Therefore it is possible that the low energy environments could favour size-specific defence of prey through small and the high energy environments through big cell size. However, additional experiments from the frozen samples are needed to test above mentioned hypotheses.

Interestingly, the carrying capacity and maximum growth rate differed between the weekly measurements in both resource levels (Figures 6 & 7). This could be due to fluctuating selection where the direction and magnitude of selection changes temporally (Abrams 2000). In the case of naïve preys the amount of resources could have affected the strength of selection for competitive ability. In the prey with predator treatments selection for the defensive ability of prey could have fluctuated from seasons of strong directional selection (heavy predation) to stasis or even to periods when selection acts against defensive properties of prey depending on the density of predators. Fluctuating selection have been reported earlier elsewhere to be able to drive evolutionary and ecological dynamics of predator and prey (Gingerich 1983; Hairston 1988; Hairston & Dillon 1990; Yoshida et al. 2003). However, in this study the population sizes of preys and predators settled to quite stabile level after three weeks from the beginning of the experiment (Figure 3. and 4.). That the population sizes of predators dropped to very low levels support the idea that selection by predators was strongest in the beginning of the experiment and could have weakened thenceforth. We did not either detect clear cycles between the preys and predators during the experiment. Hence, it is possible that the evolution of prey took place during the first three weeks of the experiment and temporal variation in life history traits of prey is due to similar starting conditions of all treatments, cyclic changes (e.g. resource renewal in this study system) or unknown changes in the environmental conditions. Yet, the data from the population time series of prey and predator depicts the outcome of

predator-prey interaction only in the end of every renewal cycle and most probably the sampling interval of seven days was not frequent enough to detect the prey and predator cycles even if they occurred (compared to sampling interval of one day in the experiment of Yoshida et al. in 2003). Therefore it is possible that the ecological and evolutionary dynamics were continuous, but not detectable in our data, and the temporal variation in the life history traits of prey was produced by fluctuating selective forces.

4.2 Defence mechanism of prey

The strongest candidate for prey defence found in this experiment was their ability to form inedible aggregations. In this experiment this was detected from images where *Tetrahymena* population sizes were determined (Figure 8.). Bacterial aggregates can result from increased excretion of exopolymers of bacterial cells which can lead cells to attach each other and form long cell filaments or microcolonies (Jürgens et al. 1999; Hahn & Höfle 2001; Simek et al. 2001). Forming aggregations in the presence of predators could be highly advantageous because big prey assemblages can not be grazed by particle-feeding protozoa (Salcher et al. 2005). However, forming aggregates is also likely to be costly for the prey because of production of abundant extracellular matrix. Also the decrease in the nutrient absorbing cell surface area per bacterium could lead to weakened resource use efficiency and poor competitive ability. However, there are many other bacterial defences against predators (Matz & Kjelleberg 2005) and additional analyses from the frozen samples are needed to confirm the specific defence mechanism of prey.

4.3 The evolution of predator

Also tentative evidence from evolution of predator was detected in this experiment. The interaction with evolutionary experience and resource level was nearly significant (ANOVA, $p = 0.069$) and further examination of simple effects revealed that the experienced predators had smaller carrying capacity than naïve predators when grown in low resource level PPY-medium (Figure 9, pairwise comparison, $p = 0.002$). One explanation for the poorer growth of evolutionary experienced predators in PPY-medium is their costly adaptation to use living bacteria as a food source. This specialization could require modifications in food digestion of predators (Bukharin & Nemtseva 2001) leading to weakened PPY-medium use efficiency. Moreover, the experienced predators lowered carrying capacity could be indicative of costly adaptation to overcome defensive ability of prey, e.g. to improve predation ability or try to survive with less food. That the decrease in carrying capacity of experienced predators was observed only in the low resource level supports an idea that predators can overcome the costs induced by bacterial food in conditions where resources are abundant. Alternatively, bacterial prey might have adapted against predators more effectively in high resource level (e.g. forming bigger aggregates) to which predators could not simply respond with counter adaptation. It is also possible that the naïve predators actually adapted to use low resource level PPY-medium more efficiently during the experiment and the difference seen in carrying capacity could be due to the evolution of naïve predators. However, as the predators had been growing in PPY-stock medium for almost 6 months prior to the initiation of the experiment it is more likely that the selection for PPY-medium use efficiency had already taken place. Therefore the observed difference in carrying capacity is likely to be explained by the evolutionary response of experienced predators to their bacterial prey. During the experiment, the life history traits of predators were measured once whereas the life history traits of prey were measured 9 times. Also the interaction with resource level and evolutionary experience

was marginally significant. Thus, evolutionary change of predator should be considered as tentative and further work is needed to fully answer the questions concerning coevolution.

4.4 The interaction of rapid evolution and ecological dynamics

Thompson formulated in 1998 that the evolution should be considered as rapid, when the changes in phenotypes of organisms can simultaneously affect the ecological dynamics. In 2005 Hairston et al. refined Thompson's view in their article by stating that evolution is rapid in this ecological context only if the heritable phenotypic change occurs sufficiently quickly to alter the trajectory of an ecological process while it is still in progress. This view of rapid evolution was supported by their microcosm experiment where the period of oscillations and the phase relations between predatory rotifer (*Brachionus calyciflorus*) and prey algae (*Chlorella vulgaris*) were dramatically affected by whether or not the prey could evolve (Yoshida et al. 2003, 2004). The evolutionary responses found in my study can be considered rapid as they were noticeable within a week and affected simultaneously the ecological properties of the microbial community.

Philips proposed already in 1974 that densities of edible resources could be top-down regulated while the densities of inedible resources would be determined by the productivity of the environment. Bringing the rapid evolution in to the picture leads to the breakdown of this dichotomy of edibility as the "resources" are often living organisms capable of evolutionary change. In my study the fourfold increase in the resources of prey increased the prey population sizes both in the absence and in the presence of the predators (high resource level treatments in Figure 3.). However, only a small and statistically insignificant increase in the numbers of the predators was detected in high resource level (Figure 4.). This supports idea that bacteria were able to allocate resources to defence against predators in high resource level and restrict the energy to turn into predator biomass. This finding is consistent with earlier studies done by Bohannan and Lenski (1997 & 1999) in which the increase in the resources of prey bacteria (*Escherichia coli*) did not lead to an increase in the population size of predators (bacteriophage T4). This was due to defending prey clones which increased in frequency in response to enrichment on the expense of non-defending prey clones.

In natural aquatic and terrestrial communities energy is transferred to upper trophic levels through decomposer microbes and productivity have shown to control the food-chain properties in microbial communities (Kaunzinger & Morin 1998; Bohannan & Lenski 1999). Given the evolutionary potential of microbes they should not be considered as "black boxes" who's through the energy flows with constant rate. Instead, they are capable of allocating energy in different amounts to different traits maximising their fitness under changing selective pressures (Nakajima & Kurihara 1994; Rebound & bell 1997; Kassen & bell 1998; Yoshida et al. 2003, 2004). Usually trade-offs between different traits control these conflicting demands set upon organisms by the environment. In what degree the trade-offs will limit the adaptation of the organisms can depend ultimately on the availability of the energy. For example, the rapid evolution of prey in response to predation can significantly weaken the strength of the predator-prey interaction if the nutrient concentration of the environment can compensate the costs of prey's defence. Thus, the prey populations regulated previously by the top-down forces (regulated by predators) can turn to be regulated by bottom-up forces (regulated by resources of the environment) along with the emergence of defending prey clones and increase in energy availability (Bohannan & Lenski 1997, 1999). My results add more support to the idea that the changes in

productivity of the environment (e.g. eutrophication) can affect the trophic level dynamics of organisms through interaction of ecological and rapid evolutionary processes. As a result, it may not be possible to understand the trophic level dynamics only from the ecological perspective without considering the evolution in action.

4.5 Conclusions

In summary, we found that the prey bacteria *Serratia marcescens* evolved during the experiment and there was also tentative support for the evolution of predator. These results are consistent with present theory of evolution between predator and prey to according which traits of prey are more likely to evolve than traits of predator (Abrams 2000). Overall, the evolutionary changes were seen as poorer growth of evolutionary experienced prey and predator and more clearly within low resource level. This is presumably due to a trade-off between competitive ability and predator avoiding/predation related traits of prey and predator. Even though we did not observe prey bacteria's cost of defence in high resource level the population time series of predator suggest that preys invested in defence against predators also in the abundance of resources. Thus, environments rich in energy could promote the evolution of predator-prey interaction by allowing preys to allocate energy to costly defence without compromising other fitness-related traits. We also found evidence that rapid (less than one week time scale) evolutionary responses can feed back to the ecological interactions, affecting population dynamics and trophic level biomasses, in the simple two species model community. In the future more work is needed to assess how the ecological and evolutionary forces interact in more realistic model communities where e.g. multiple species interact simultaneously or resources fluctuate temporally.

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