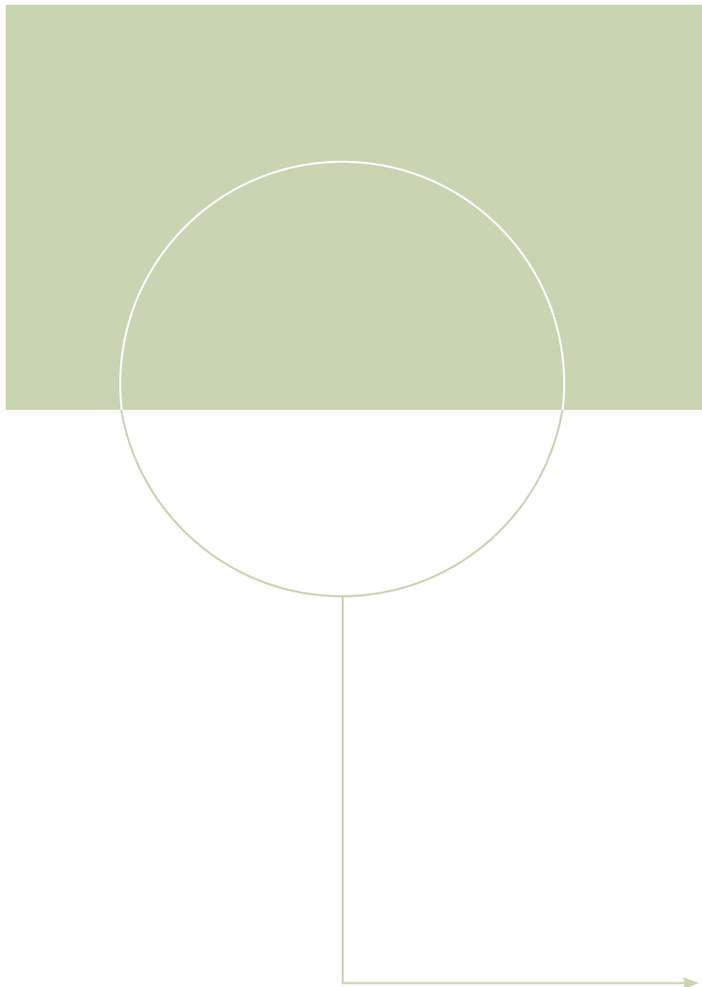


Master's thesis

2019

Madeleine Stenshorne Gundersen

NTNU
Norwegian University of
Science and Technology
Faculty of Natural Sciences
Department of Biotechnology and Food Science

Madeleine Stenshorne Gundersen

The effect of r- and K-selection on planktonic microbial community characteristics in lab-scale bioreactors

May 2019

NTNU
Norwegian University of
Science and Technology

NTNU
Norwegian University of
Science and Technology

NTNU
Norwegian University of
Science and Technology

The effect of r- and K-selection on planktonic microbial community characteristics in lab-scale bioreactors

Madeleine Stenshorne Gundersen

Biotechnology - MBIOT5
Submission date: May 2019
Supervisor: Olav Vadstein
Co-supervisor: Ingrid Bakke

Norwegian University of Science and Technology
Department of Biotechnology and Food Science

Acknowledgements

This project was performed at the Department of Biotechnology and Food Science (IBT) at the Norwegian University of Science and Technology (NTNU) in Trondheim.

First of all, I would like to thank Ian Moreland for collaboration on the experimental part of this project and for always urging me to question and think for myself. My supervisors Olav Vadstein and Ingrid Bakke have my deepest gratitude for the academic growth I have experienced the past one and a half years. Olav, you have helped me tremendously with your knowledge about ecological processes and statistical approaches. You motivated me to understand and explore topics I found hard, and I appreciate that you never underestimated me. Ingrid, thank you for always caring about my progress, and helping me throughout this project. Your competence in experimental and analytical methods has been essential. Most of all, thank you for motivating me throughout the sometimes troublesome experimental period. I can't imagine having better supervisors then you two.

I would also like to give my appreciation to Mia T. Mjøs and Randi Utgård for always helping me in the lab when I needed it. There are no ways I can repay Jakob Pettersen for all the time he has taken to help me with my data analysis, you have saved my day more times than I can count. My research group, Analysis and Control of Microbial Systems (ACMS), has inspired me throughout my master program with interesting presentations and discussions. Thank you all for your constructive critique.

The best study hall buddy in the world, Vetle Simensen, deserves my appreciation. You have been there throughout my project cheering me on. Thank you for all your feedback on this thesis. I would like to thank my mom Anne Stenshorne and Jonas Jam for listening to me talk for hours about topics you have no backgrounds for understanding. You have made me better at communicating my knowledge. Last, but not least, I would like to thank my friends and classmates for making my time at NTNU the best years of my life.

Trondheim, May 2019
Madeleine Gundersen

Abstract

Uncrowded environments with resources in surplus and empty niches select for r-strategic organisms that have high maximal growth rates. K-strategists, on the other hand, have higher substrate affinity and utilisation efficiency, making them better competitors in environments where the biomass is close to or at carrying capacity. Selection of K-strategists, or K-selection, has in land-based aquaculture systems yielded communities with higher diversity, increased temporal compositional stability and reductions in the abundance of possible pathogenic bacteria.

The objective of this thesis was to explore how r- and K-selection affected the community characteristics of planktonic seawater *Bacteria* at two different carrying capacities. A 2x2 factorial crossover experiment, with three replicates, was run for seven weeks with a switch in r- and K-selection after four weeks. The bacterial communities were analysed using flow cytometry and Illumina sequencing of the V3 and V4 variable regions of the 16S rRNA gene.

K-selected communities had higher bacterial densities and lower mean RNA content than r-selected ones. The differences in alpha-diversity of order one between the selection regimes were not substantial, although K-selection appeared to increase the evenness. *Alpha-* and *Gamma-proteobacteria* dominated the communities. During r-selection, *Gamma-proteobacteria* increased in abundance, while *Alpha-proteobacteria* increased during K-selection. A few genera made up the majority of the communities. *Vibrio* and *Colwellia* competed well during r-selection, while *Roseovarius* was the best competitor during K-selection. On the temporal scale, the community members became more phylogenetically similar, indicating an overall selection of bacteria with high fitness to the experimental situation. The temporal stability in community composition decreased during K-selection. However, the replicates within a selection regime developed in a similar way, indicating a strong deterministic succession pattern. An exciting observation was that *Vibrio*, a genus with many known marine pathogens, was out-competed in all reactors during K-selection. This supports the hypothesis that K-selection is a good strategy for the management of microbial communities in land-based aquaculture.

Sammendrag

Miljøer med tilgjengelige ressurser, tomme nisjer og mye plass selekterer for r-strategiske organismer som har høye maksimale vekstrater. K-strateger, derimot, har høyere substrat affinitet og utnyttelses effektivitet, som gjør de konkurransedyktige i miljøer der biomassen er nær eller ved miljøets bæreevne. I land-baserte oppdrettsanlegg har seleksjon for K-strateger, K-seleksjon, resultert i bakteriesamfunn med høyere diversitet, økt temporal sammensetnings stabilitet og reduksjon i mengden potensielle patogene bakterier.

Hensikten med denne oppgaven var å utforske hvordan r- og K-seleksjon påvirket det planktoniske bakteriesamfunnet i sjøvann, og om bæreevnen påvirket utfallet. Dette ble studert i et 2x2 faktorialt krysningsforsøk, med tre replikaer, over syv uker, med et bytte i r- og K-seleksjon etter fire uker. De bakterielle samfunnene ble analysert med flowcytometri og Illumina sekvensering av de variable regionene V3 og V4 i 16S rRNA genet.

K-selekterte samfunn hadde høyere bakteriell tetthet og lavere gjennomsnittlig RNA innhold enn r-selekterte samfunn. Det var ingen store forskjeller i alfa-diversitet av orden en over tid mellom seleksjonsregimene, selv om det var antydning til at K-seleksjon økte jevnheten i samfunnene. *Alpha-* og *Gamma-proteobacteria* dominerte i samfunnene. Mengden *Gamma-proteobacteria* økte under r-seleksjon, mens *Alpha-proteobacteria* økte under K-seleksjon. Noen få slekter utgjorte majoriteten av samfunnene, og seleksjonsregimene påvirket forekomsten til mange av disse. *Vibrio* og *Colwellia* konkurrerte godt under r-seleksjon, mens *Roseovarius* var den beste konkurrenten under K-seleksjon. Over tid ble bakteriene i samfunnene mer fylogenetisk like, som tydet på en konstant seleksjon for bakterier med høy fitness til den eksperimentelle situasjonen. Den temporale sammensetnings stabiliteten sank under K-seleksjon. Likevel utviklet replikaene innad i et seleksjonsregime seg relativt likt, som indikerte et sterkt deterministisk suksesjonsmønster. En interessant observasjon var at *Vibrio,* en slekt med mange marine patogene stammer, ble utkonkurrert i alle reaktorer under K-seleksjon. Dette støtter hypotesen om at K-seleksjon er en god strategi for styring og kontroll av bakterielle samfunn i land-baserte oppdrettsanlegg.

Table of contents

1 Introduction

1.1 Microbial ecology and community assembly processes

A community is the collection of potentially interacting species that occur in space and time. Microbial ecologists seek to answer which microbes that inhabit an environment, what their roles and interactions are and how the structure of the community changes over space and time (1). Such knowledge is valuable to purposefully steer the composition of a microbial community to optimise a particular functional role, like nutrient cycling or biological pathogenic-control (2).

Richness (i.e. the number of species) and evenness (i.e. the equability of the relative abundances) describes the diversity of a community, while four basic processes influence community assembly. Drift and selection result in species loss, while dispersal and speciation increase the number of species (3,4).

1. Drift, a stochastic process, occurs because there is a probability that an individual of a species dies before it has the chance to reproduce independently of its fitness (i.e. the expected quantity of offspring per time unit) to the environment. Drift, therefore, introduces stochasticity that results in a random fluctuation of species abundances (4). A species' abundance in the community determines how much it is affected by drift. Low abundance species are more likely to go extinct because the random fluctuation in abundance can bring the population size to zero. However, when the population size is large, drift is of minor significance (4,5).

2. Selection, a deterministic process, occurs when populations have differences in fitness; where the population with higher fitness is expected to have higher growth rates based on the biotic and abiotic conditions in the environment (4). Abiotic conditions such as nutrient availability, pH, salinity and temperature affect which species that have the genetic potential of inhabiting an environment (3). The biotic condition arises because all populations interact at some level. These interactions are categorised as neutral-, negative- and positive interactions for both populations or positive for one and negative for the other. Competition is a known negative-negative interaction, in which two species compete for the same resource. Both have decreased growth rates because neither monopolises the resource, which can be everything from carbon sources, essential minerals and physical space. Competition over an extended period can result in one species out-competing the other (6).

3. Dispersal is the movement of organisms across space, either through immigration or emigration (4). Microbes are easily transported passively through for example water currents,

wind or attached to an animal. Dispersal introduces new species to an environment; however, it only increases the richness if the species is established in the community which is governed by selection and drift.

4. Speciation is the generation of new species. However, diversification, the generation of new genetic material, might also play a notable role for microorganisms. Both processes are generally rapid for microorganisms, as they have short generation times, are prone to mutations and can perform horizontal gene transfer (3).

A question one asks when studying communities is; given the environmental conditions, is there a defined community that will inhabit it? Each population has a niche, or functional role, in the community, and competition arises to fill all niches. When each niche is filled, the ecosystem function is at its theoretical maximum and additions of new populations adds redundancy to the ecosystems. When an ecosystem has high redundancy, the loss of one population will not affect the functionality of the ecosystem greatly, as a new population takes over its role (7). The orderly sequential change in population presence and abundance in a community over time is termed succession (6). Succession continues until a relatively stable composition is achieved. As microbial communities regularly are exposed to disturbances (i.e. changes in the environment), they rarely reach this steady state. However, some communities have high stability, meaning they are better at withstanding disturbances thus keeping the composition more similar on the temporal scale (8,9).

The previous section described how microbial communities change over time and which processes that make communities different. Next, a brief description of how researchers gain information about them is presented.

1.2 How are microbial communities studied?

The goal when studying and describing microbial communities is to understand who the community members are and what they do. The first step is to sample the microbes present in the environment, before using culture-dependent or independent methods, or even a combination, to obtain information about them. Culture-dependent methods rely on the cultivation of community members to analyse and characterise them. The knowledge one gains by studying the microbes isolated from their environment is valuable, but these methods are limited as only 1% of bacteria are culturable on agar media, a phenomenon known as the “great

plate count anomaly". Culture-independent techniques, such as microscopy, activity measurements, fluorescence *in situ* hybridisation and sequencing, do not rely on the microbes ability to grow isolated on agar-plates (1). The development of these culture-independent methods has been essential for the studies of community composition and function because they yield a more representative and detailed overview of the communities than culture-dependent methods, and represents a huge reduction in workload.

High-throughput sequencing of marker genes in communities has been fundamental to answer "who are there?". The gene encoding the small ribosomal subunit RNA, 16S rRNA gene (or 18S rRNA for eukaryotes), is most commonly used to obtain taxonomic information. These data are further used to study community diversity and phylogeny. The 16S rRNA gene is necessary for life and is highly conserved in some regions and variable in others, which enables distant and close phylogenetic relationships to be determined (10). Polymerase chain reaction (PCR) targeting the conserved regions of the 16S rRNA gene in combination with massively parallel sequencing (termed amplicon sequencing) has enabled microbial ecologist to sequence all organisms that contain the 16S rRNA gene from a sample.

Bacterial communities can be studied in microcosms, which are simplified physical models of an ecosystem. A lab-scale bioreactor is an example of a microcosm. Studies using such systems aim to simplify the ecosystem to its fundamentals, reduce complexity to constrained variables and reduce heterogeneity. The goal is to detect general ecological patterns or test ecological theories that can be used to explain observations on large-scale ecosystems (7).

1.3 Life strategies, and r- and K-selection

Life strategies are categorical concepts that aim to explain an organism's behaviour and response to environmental conditions given its physiological traits. These traits have been selected for by biotic and abiotic selection pressures. A strategy concept based on either optimisation of rapid growth or competitive ability is the r- and K-life strategy, respectively (11).

These survival strategies were first defined for macroorganisms by MacArthur and Wilson (12) and proven applicable to explain different behaviours within *Bacteria* by Andrews and Harris (13). The basis of r- and K-selection theory is the logistic equation for growth per time unit;

$$\frac{dn}{dt} = rn\left(\frac{K - n}{K}\right) \quad [1]$$

where r is the maximum specific rate of increase, K the carrying capacity (i.e. the maximal biomass an environment can sustain) and n the population size (14). One of the postulates for the application of r/K-theory to microbial communities is that an environment select for either r- or K-strategist, termed r- and K-selection. Organisms with high maximal growth rates and quick responses to sudden increases in nutrient supply are characterised as r-strategists and thrive in uncrowded environments with available nutrients and empty niches. K-strategists, on the other hand, are characterised by high substrate affinity and utilisation efficiency, thus out-competing r-strategists when the community comes close to the carrying capacity and nutrients are limiting (11,13).

The rRNA gene is localised in the ribosomal RNA operon (rrn), and bacteria can contain between one to fifteen rrn copies. High maximal growth rates are correlated with the number of rrn copies (15); hence r-strategists contain more rrn copies than K-strategists and have a higher ribosomal concentration when actively dividing (15). The characteristic traits of the r- and K-strategies for microbes are summarised in Table 1 (13,16).

Table 1: Characteristic phenotypic traits for r- and K-strategist. Adapted from Andrews and Harris, 1986 and Vadstein et al. 1993

Trait	**r-strategist**	**K-strategist**
Maximum growth rate	High	Low
Substrate affinity	Low	High
Substrate utilisation efficiency	Low	High
Response to enrichment	Fast growth after variable lag	Slow growth after variable lag
Competitive ability at substrate limitation	Low	High
rrn copies	Many	Few
	r-selected environment	**K-selected environment**
Biomass density at carrying capacity	Unstable	Stable
Resistance to density-dependent (starvation) and independent (catastrophes) mortality	Low	High/variable

1.4 K-selection as a tool for biological control

K-selected communities have different characteristics than those r-selected. For example, r-selected communities are often dominated by a few species, with a higher probability for community structure change after a perturbation (6). K-selected communities, on the other hand, have displayed both higher diversity and stability in for example land-based aquaculture systems (17), which further is associated with increased resistance to invasion by opportunistic organisms (17,18). Moreover, most pathogens are categorised as r-strategists, as the opportunistic lifestyle of pathogens depends on their rapid growth during favourable conditions (19). The characteristics belonging to K-selected communities are desired in industries that manage microbial communities (e.g. land-based aquaculture- and wastewater treatment systems). K-selection has previously been proposed as a tool to increase the viability of marine larvae (16) and as a biological control tool in land-based aquaculture systems (20). In fact, the application of K-selection in such systems has reduced the abundance of possible pathogenic bacteria (21), increased the survival of larvae (22) and their performance (17). Even though K-selection generally yields communities with higher diversity, there are instances where the diversity is reduced even though other K-selection characteristics were present (personal communication Attramadal, K). This inconsistency emphasises that there are aspects of the effect of r- and K-selection we do not comprehend.

Concerning wastewater treatment, the stability of K-selected communities has an increasing interest in space technology. Nutrient recycling in space will require microbial communities that are reliable and steady over time. Indeed, increased richness has yielded communities with higher stability and function (18). However, maintaining high richness communities over time requires a better understanding of the successional patterns and community assembly processes that are characteristic of such stable communities.

Ecological theory as a tool to control and manipulate microbial communities has, as mentioned above, been used successfully. Nevertheless, there is a lack of knowledge to which community assembly processes that are important during r- and K-selection, and how selection affects the successional patterns over time. This knowledge is not only valuable for industrial applications but important to better understand the effect of human intervention to natural K-selected environments (e.g. pollution to oligotrophic waters (23) or refilling of aquifers (24)).

2 Project aim and research questions

The main goal of this project was to investigate the effect of r- and K-selection on the planktonic bacterial community characteristics at two different carrying capacities. r-selection was obtained by periodical strong (1:50) dilution, resulting in a cell density reduction and a pulse of nutrients, thereby yielding an uncompetitive environment which promotes fast growing r-strategists. K-strategists, on the other hand, are selected for in environments with strong competition and densities close to the carrying capacity. Cultivation was therefore done in chemostats, with a continuous supply of nutrients and biomass close to the carrying capacity. The r- and K-selection regimes were switched halfway in the experiment.

Based on the knowledge, and knowledge gaps, presented in the Introduction, the following questions and accompanied hypothesises were investigated;

• **Will r- and K-selection yield communities dominated with bacteria of the intended life strategy?** r- and K-selection is expected to yield communities dominated with bacteria best adapted to their environment. Hence, r-strategists should be abundant during r-selection, and K-strategists during K-selection. Switching the r- or K-selection regime should change the community composition.

• **Will possible pathogenic bacteria be out-competed during K-selection, and do they reappear during a switch to r-selection?** K-selection has been successful in reducing the abundance of possible pathogenic bacterial strains in land-based aquaculture systems (e.g. (21)). It was expected that in this constrained bioreactor environment, K-selection should still reflect previous findings if interactions between populations cause the removal of possible pathogenic bacteria. Further, if the opportunistic populations were reduced during K-selection, they were expected to bloom again when the selection was switched from K- to r.

• **Is there a clear separation between the four cultivation regimes over time with regards to density and mean RNA content?** Because higher carrying capacity can sustain a community with higher biomass, it was expected to observe higher densities in communities with high compared to low carrying capacity. The r-selected communities were expected to contain more RNA than K-selected because 1) r-strategists have more rrn operons (15) and 2) r-selected communities had higher minimum growth rates (i.e. 2/day vs 1/day).

• **Is diversity affected by r- and K-selection and carrying capacity?** Because K-strategists are better competitors than r-strategists, it was expected that K-selection would yield higher

diversity. Increasing the carrying capacity should not create new niches, and therefore not induce changes in the diversity between communities with the same r- or K-selection regime.

• **Is there distinct community patterns related to selection regime and time?** Selection of bacteria able to grow in the experimental situation was expected to be the main driver for community assembly during the first couple of days in all selection regimes. After that, competition was expected to be more substantial in the K-selected reactors, whereas drift should be of higher relevance in the r-selected as population sizes were periodically diluted. Thus, replicas during K-selection in the first selection period were expected to present similar successional patterns, while replicas during r-selection were expected to have higher in-group variance.

3 Materials and Methods

3.1 Experimental design and setup

3.1.1 Experimental design

The purpose of this project was to investigate the effect of r- and K-selection on the planktonic bacterial community characteristics at two different carrying capacities. A 2x2 factorial crossover experiment, with r- or K-selection and carrying capacity as the two factors, was carried out over 50 days. Half of the bioreactors were K-selected and half r-selected the first 28 days (Figure 1). K-selection was achieved by a continuous supply of media to the reactors, whereas r-selection was obtained by manually diluting the culture 1:50 every second day. After 28 days the r- or K-selection was switched while carrying capacity was kept constant (either high or low) throughout the experiment. Each cultivation regimes had three biological replicates. The four groups are referred to as RKH, RKL, KRL and KRH. RK indicates r-selection the first 28 days, following K-selection the last 22, whereas KR refers to K-selection in the first selection period, before r-selection in the second. H and L indicates high and low carrying capacity respectively.

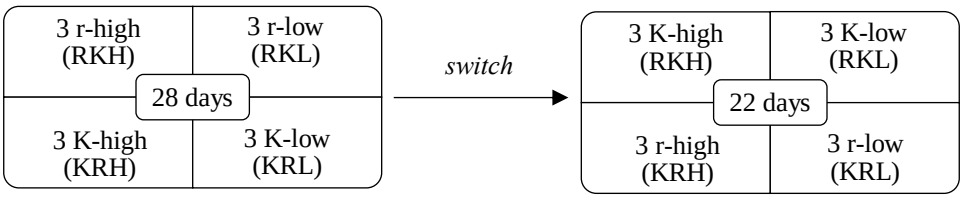


Figure 1: Schematic overview of the experimental design. A total of 12 bioreactors, three biological replicates for each cultivation regime, were maintained for 50 days. RK: r-selection from day 0-28, K-selection from day 28-50. KR: K-selection from day 0-28, r-selection from day 28-50. H: high carrying capacity, L: low carrying capacity.

This experiment was part of a collaboration with the PhD candidate Ian Moreland, Oregon University. The workload was divided equally for maintenance and sample collection during the time the bioreactors were operated. For some procedures and methods, work was only performed by one person to minimise variability, and for practical reasons, indicated in the following description of methods.

3.1.2 Bioreactor setup

Each bioreactor consisted of a 500 mL glass bottle (Duran) with a five-port connection lid (Duran). All reactors contained 250 mL culture which was kept homogenous with a magnetic stirrer (MIX 6, 2mag AG). Hydrated air was filtered at 0.2 μm (Millipore), and delivered to all

reactors. Media was pumped by a peristaltic pump (Watson Marlow 520S) during K-selection, calibrated to continuous flow at 250 mL/day, and connected to the air-supply through Y-shaped connectors. During K-selection, the reactors had one inlet and outlet, while all other ports were sealed with silicone (732 multi-purpose sealant, Dow Corning) to establish a positive air pressure pushing excess culture to the waste tank. The temperature was controlled by cooling generators (A MX07R-20V12V cooling generators, VWR International) that delivered water of 15°C to two water baths (custom made plexiglass). The bioreactors were divided, so each water bath contained one or two of the biological replicates. The splitting of reactors of the same regime was performed to account for possible temperature defaults in one water bath. The setup is presented in Figure 2.

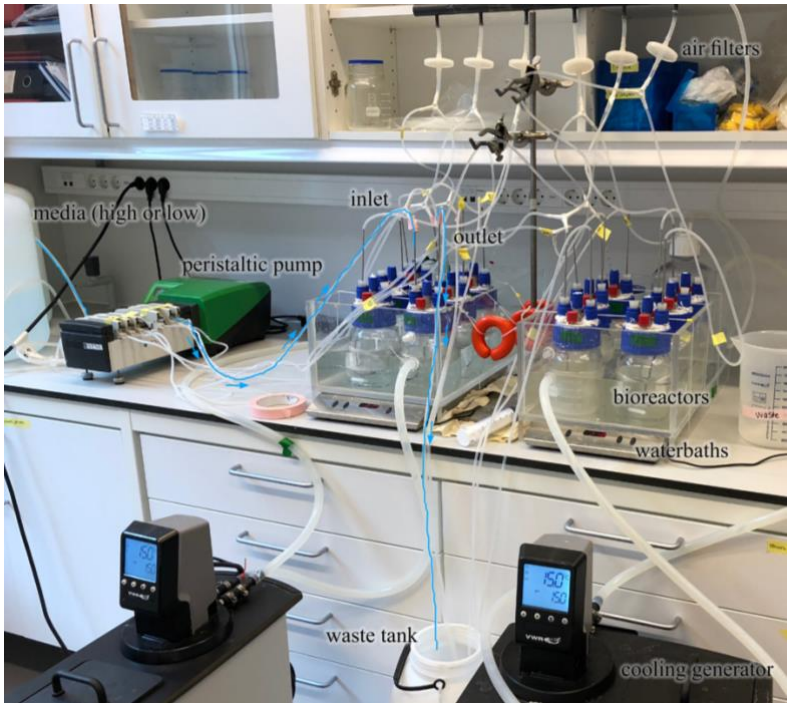


Figure 2: Experimental setup. Media was mixed with hydrated air and delivered to the bioreactors through the silicone tubes. A path for media delivery to a K-selected reactor is presented in blue.

3.1.3 Inoculum and cultivation regimes

The initial microbial community (natural seawater) was collected from 70 meters depth from the Trondheimsfjord, Brattøra, at SINTEF Sealab (sand filtered), and was assumed to be K-selected. For the reactors starting with K-selection as cultivation regime, 250 mL of the natural seawater was used as inoculum, and M65-media was delivered continuously to the reactors (flow rate 250 mL/day). Initial r-selection was performed by diluting natural seawater 1:50 with M65 media, and further diluted 1:50 with M65 media every second day. After 28 days, the r-

and K-selection was switched, so previous K-selected reactors were diluted, and r-selected ones received continuous media. M65 media-, M65 stock solution-, vitamin solution- and trace metal solution recipes are presented in Appendix A. Half of the reactors received media with a low concentration of M65 (1 mg/L), while the other half had high (5 mg/L). Prior to making media, the interior of the large plastic containers was acid washed to remove biofilm. Acid washing was performed by swirling 10% HCl carefully around the entire interior of the container and left overnight. The container was then rinsed with distilled water about 20 times and autoclaved before filtration.

3.1.4 Maintenance of bioreactors

The bioreactors were manually inspected and maintained every sampling day. The temperature in the water baths was checked, and all tubes inspected to ensure everything was working. Every eight days, the reactor bottles were exchanged by new sterile bottles to minimise biofilm growth. Water community samples were taken every day for the first four days after the initial inoculation and the switch and otherwise every second day. The water community samples were used to determine bacterial density, mean RNA content, quantify the r- and K-strategist fraction and investigate community composition and diversity, described in more detail below.

3.2 Flow cytometry as a tool to determine bacterial density and mean RNA content

3.2.1 The principles behind flow cytometry

Cytometry is the measurement and characterisation of cells and cellular components. The instrument consists of fluidics (sheath fluid), optics (light and fluorescent lasers) and electronics (detectors and computer). The differences in pressure between the sheath fluid and sample ensure that cells are aligned in a single file, as illustrated in Figure 3 (25). The single cells are then illuminated by a laser and detectors record light scattering and fluorescent emission. The detectors are placed to record forward scatter (FSC), side scatter (SSC) or fluorescence (FL). The FSC detector is located at the same axis as the light source. FSC is proportional to the cell size and is mainly used to differentiate between different types of particles, for example, viruses and bacteria, or single cells and aggregates. The SSC detector is positioned at a 90 degrees angle to the light source and is used to analyse the granularity or internal complexity of a single cell (25). Fluorescence occurs when an excited electron returns to its ground state and emits excess energy as light waves of specific wavelengths. Cellular components can either

fluorescent naturally (e.g. chlorophyll) or be stained so the fluorescent lasers can detect them. (25). Light scattering and fluorescent emission can thus be used to measure and analyse specific cell characterisations, such as RNA content, cell cycle phase, live-dead analysis and specific protein density, to mention a few application areas.

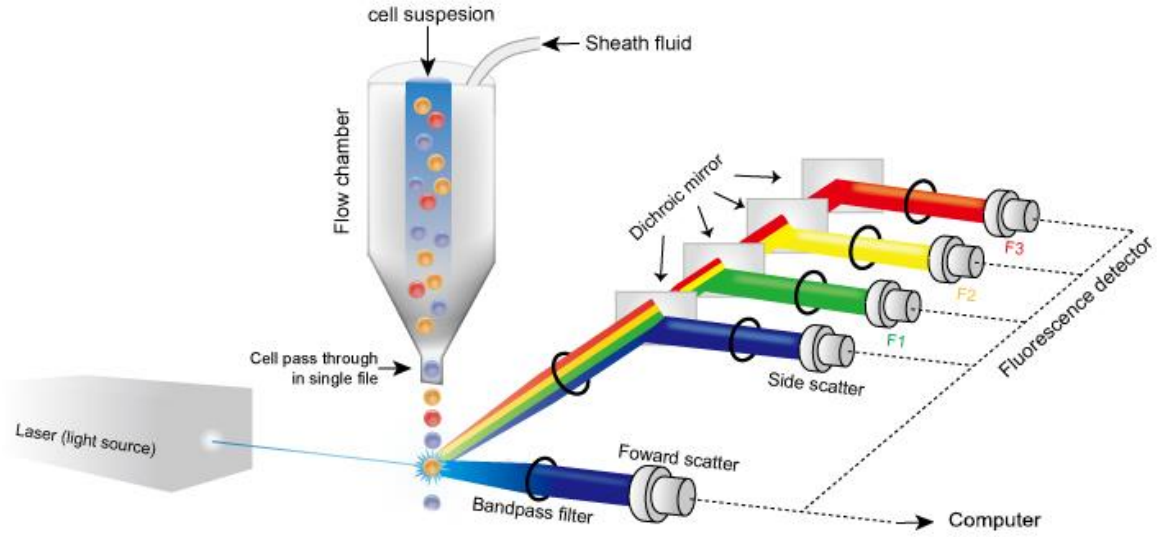


Figure 3: Schematic overview of the principles of flow cytometry. Cells are passed as a single file through a laser, and detectors record the data. *Adapted from creative-diagnostics.com/flow-cytometry-guide.htm*

Flow cytometry has proven to be a valuable tool to use for microbial community analysis as it measures many cells per second, thus giving applicable statistical data. In comparison to bigger particles, such as human cells, data collection of bacterial cells is more dependent on staining and the use of thresholds. Different fluorescent dyes are commonly used to differentiate between subpopulation containing specific cellular components (26), while thresholds are set for a specific parameter and are the lowest signal intensity value the instrument collects (27).

3.2.2 Bacterial density and RNA content of water community samples

A flow cytometer (BD Accuri C6) was used to quantify cell densities (cells/mL) and RNA content per cell as fluorescent signal intensity. The flow cytometer was regularly calibrated with calibration beads (6- and 8-Peak Validation Beads, 3 mm, BD Accuri Cytometers) to ensure accurate sampling. The C6 flow cytometer used has six lasers and detectors. The instrument indicated whether the value it presented was the sum of all events measured (-A, area) or the intensity of the signal from a single event (-H, height).

The dye SYBR Green II RNA gel stain (ThermoFisher Scientific) was used to stain bacteria in the community samples. SYBR Green II binds to RNA and forms a SYBR Green II/RNA complex. The dye is excited at 254 nm and emits fluorescence between 497 to 520 nm (28), within the FL1 detector range at 533±30 nm (29). SYBR Green II stock solution (10 000x) was diluted 1:50 in 0.2 μm filtered 1/10 TE-buffer (Appendix B), and 10 μL of this working solution was added to 1 mL of each community sample. The samples were then incubated for a minimum of 15 minutes and run for 2.5 minutes at medium fluidics (35 μL/minute) with a FL1-H threshold at 3000. The sample fluid collector was washed, and the tube rack agitated between each sample. After every fourth sample, 0.2 μm filtered Milli-Q water was run for 3 minutes to neutralise the instrument. If recorded events/μL were above 10000, the community sample was diluted before staining.

Data acquired was analysed using the BD Accuri C6 Software. The first step of the analysis was to isolate events assumed to represent the bacterial population. Based on experience in the ACMS group (NTNU), events with a FSC-A signal above 10^5 were considered aggregates and FL1-A signals below 10^4 to be noise from smaller particles such as viruses. These events were consequently not included in further analysis. For each sample, the cell density, mean RNA content (as mean FL1-A signal) and mean cell size (as mean FSC-A signal) were determined.
To test for statistical significance between groups, data were first tested for normal distribution with the Shapiro-Wilk test of normality (30). When data were normally distributed, a two-sided paired sample t-test was used to test for statistical significance between groups, while pairwise Mann-Whitney-Wilcoxon test (non-parametric) was used when data was not (31). There was assumed dependency based on sampling time. All statistical tests in this thesis were performed at a significance level of $\alpha = 0.05$.

3.3 Quantification of the fraction of r- and K-strategists in samples

Both culture-dependent and semi-independent methods can be used to estimate the fraction of r- and K-strategists. In this project, nutrient pulsing was performed as the partly culture-independent method and plate assay as the culture-dependent.

3.3.1 Estimation of r- and K-strategists fraction through nutrient pulsing

Ribosomal RNA (rRNA) make up around 82-90% of the total RNA in bacterial cells (32). As r-strategists generally have more rrn copies and higher maximal growth rates than K-strategists

they are able to upregulate their growth faster. This shift in RNA content can be recorded with flow cytometry after a nutrient pulse, and further used to estimate the fraction of r- and K-strategists in microbial communities. The key to the method is to measure the cells' RNA content before they divide. A protocol developed by Hege Brandsegg was used to estimate the fraction of r- and K-strategists (33).

Nutrient pulsing was performed a total of five times (day 0, 12, 28, 36 and 50). A nutrient pulse working solution was prepared for all the sample days; as described in Appendix C. Three technical replicates were run per biological replicate in the following way: 6 mL of the reactor community was aseptically transferred to a sterile 15 mL cultivation tube and mixed with 2.8 µL of the nutrient pulse solution. The cultivation tubes were incubated vertically for 7.5 hours in darkness at 15°C (Minitron, Infors-HT), with a backup sample taken after approximately 2.5 hours. Samples were stained with SYBR Green II and analysed on the flow cytometer. The overall goal was to achieve an increase in RNA content (FL1-A signal) without an increase in cell density. Therefore, when samples had an increase in density over 20% the backup sample was used for analysis. Data were analysed in the same way as for regular flow cytometer but with the additional estimation of K- and r-strategist fractions. Events on the FL1-A histogram from 10 000 to 27 440 were categorised as K-strategists, events over 53 782 as r-strategist, and in-between as bacteria with an undefined life strategy.

3.3.2 Estimation of r- and K-strategists fraction through a plate assay

A plate assay was used as the culture-dependent method for estimating the r- and K-strategist fractions on day 0, 14, 28 and 50. The author diluted community samples in 0.2 µm filtered Milli-Q water to 10^{-3}, 10^{-4} and 10^{-5} before 70 µL was spread out on M65 agar plates, Appendix D, in a sterile hood (SAFE2020, VWR). The plates were incubated in the dark at 15 °C (Minitron, Infors-HT). Moreland counted colonies when there was a sufficient amount and again after some time. The difference in colonies between the first and second count was supposed to be used to calculate the proportion of fast-growing bacteria. Unfortunately, counting of colonies was not performed on a regular scheme, defying the assumption of the method, which is counting after three days and again after three weeks (34). As a consequence, no results could be generated from this method.

3.4 Sequencing of bacterial communities

3.4.1 Sampling of bioreactor communities and extraction of DNA

Bacterial cells from the bioreactors were collected at 18 time points throughout the experimental period (day 0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 28, 29, 30, 32, 36, 40, 44 and 50). Samples were taken more frequently in the initial selection phase and after the switch (between day 28 and 29) to observe changes in community composition better. Bacterial cells were collected by pushing approximately 30 mL of the water community samples through a 0.2 μm filter, before storage at -20 °C until DNA extraction.

Extraction of DNA was performed by Moreland using the Qiagen DNeasy PowerSoil DNA extraction kit. The protocol from the supplier (Qiagen) was used (Appendix E). Samples were randomly extracted to reduce bias and ensure that not all samples from a sampling day had to be disregarded in case of poor extraction efficiency or contamination. Extracted DNA was stored at -20 °C until PCR amplification.

3.4.2 The principle behind Illumina sequencing

Illumina sequencing is composed of four steps; sample preparation, cluster generation, sequencing and data analysis. During sample preparation indexes and adapters are attached to the DNA to be sequenced. The indexes are used to tag the DNA for origin backtracking, allowing multiple samples to be run on the same flow cell. DNA to be sequenced are injected to the flow cell and bind to the lawn of oligonucleotides that are complementary to the adaptor. Bridge amplification is then performed on the bound DNA strand. The double-stranded DNA is denatured, and the original DNA washed away before cluster generation. During cluster generation, the DNA stretch is replicated up to 1000 times. Before sequencing, the reverse strands are cleaved (Figure 4). The nucleotides used are fluorescently tagged with a terminator for polymerisation, facilitating sequencing by synthesis. After each addition of a nucleotide, a light source excites the clusters, and a detector measures the fluorescent signal before the terminator is cleaved. This process continues until the DNA is sequenced. The generated sequence read is removed before sequencing of the index codes. Illumina sequencing first performs sequencing on the forward strand, before sequencing the reverse strand, a process known as paired-end sequencing (35).

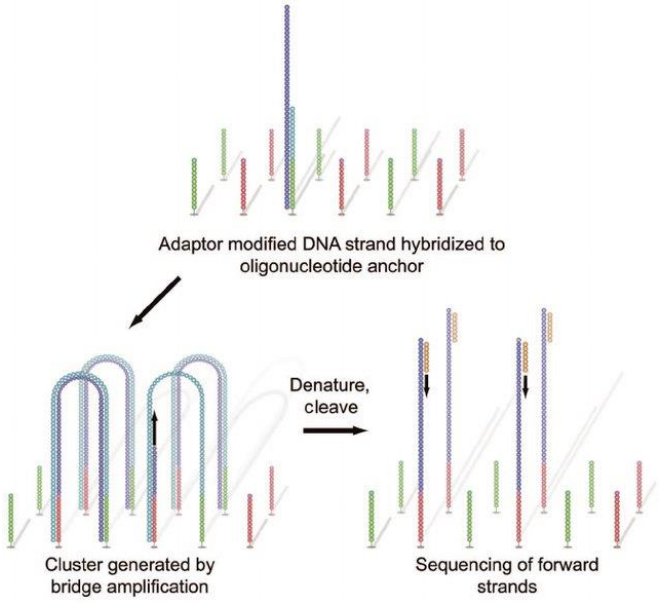


Figure 4: Principle of Illumina sequencing, illustrating hybridisation, bridge amplification and forward strand synthesis. Adapted from Voelkerding et al. (36).

3.4.3 16S rDNA amplicon sequencing as a tool to describe microbial communities

The functional role of rRNA is to associate with proteins to assemble ribosomes that translate mRNA to proteins. Protein synthesis is essential for life, and rRNA is, therefore, one of the most conserved genes (6). The 16S rRNA gene is the most used marker gene related to the studies of microbial diversity, taxonomy and phylogeny (10). The gene has many characteristics that make it useful for examining community composition and phylogeny. It contains conserved regions that make it possible to design broad range PCR primers that can target all bacteria on a high taxonomic level. The nine variable regions in the gene can be used to investigate close relationships as these are more susceptible to mutations. As the gene is not prone to horizontal gene transfer, it is assumed to transfer vertically (6,10). Using rRNA to investigate phylogenetic relationships and microbial diversity has been performed since 1977, and extensive databases and tools based on the gene have been developed. The main drawback to studying communities based on the 16S rRNA-gene is that different taxa contain different copy numbers of the rrn operon, as mentioned previously (37). Bacteria with many rrn operons are therefore overrepresented when the 16S rRNA gene is sequenced. Illumina sequencing is a popular sequencing platform to use for microbial community sequencing. Sample preparation is accomplished through PCR, and has two main goals; 1) to incorporate Illumina adapters and indexes to the DNA fragments to be analysed and 2) amplify target DNA to a sufficient amount (26,38). This method generates PCR amplicons and is accordingly termed amplicon sequencing.

3.4.4 16S rDNA amplicon library preparation

The library preparation consisted of PCR to attach Illumina adapters and amplify the 16S rRNA gene region V3 and V4, normalisation of amplicon products, indexing to tag samples and finally normalisation and pooling of samples. The author performed all steps described below.

3.4.4.1 Amplification of the 16S rRNA gene region V3 and V4

PCR was used to amplify the V3 and V4 region of the 16S rRNA gene of *Bacteria*, with the primers Ill338F and Ill805R (Sigma). Primer sequences are presented in Table 2. Before extracted DNA from the reactor samples were amplified, the PCR protocol was optimised with regards to the number of cycles and magnesium concentration.

Table 2: Primer sequences used to amplify the V3 and V4 region of the bacterial 16S rRNA gene. Sequences in bold represent the Illumina adapters. A = adenine, T = thymine, C = cytosine, G = guanine, N = A, T, C or G, W = A or T, K = G or T and V = G, C or A.

Primer	Nucleotide sequence
Ill338F	5'-**TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG NNNN** CCT ACG GGW GGC AGC AG-3'
Ill805R	5'- **GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G NNNN** GAC TAC NVG GGT ATC TAA KCC-3'

The amount of reagents added to each PCR reaction is presented in Table 3. The master mix was prepared for 24 samples at a time in a PCR workstation (VWR) that was UV and ethanol treaded before use. A negative control was included for each PCR run to ensure that reagents were uncontaminated. The program used in the thermocycler (Bio-Rad t100 Thermal cycler) is given in Table 4. For samples with low PCR amplification yield, the number of cycles was increased.

Table 3: Concentrations and amounts of reagents added per sample to amplify the 16S rDNA of *Bacteria*.

Reagent	Distributor	Concentration	Amount per sample
PCR gradient dH_2O	Rouche	-	16.563 µL
5x Phusion buffer HF (7.5 mM MgCl2)	ThermoFisher	10 µM	5.0 µL
Primer Ill338F	Sigma	10 µM	0.75 µL
Primer Ill805R	Sigma	10 µM	0.75 µL
dNTP	VWR	10 mM per dNTP	0.625 µL
$MgCl_2$	ThermoFisher	50 mM	0.5 µL
Phusion Hot Start II DNA polymerase	ThermoFisher	2 units/µL	0.1875 µL
DNA template	-	-	1.0 µL

Table 4: Cycling conditions for PCR amplification of the 16S rDNA of bacterial cells.

Temperature	Time	
98 °C	1 min	
98 °C	15 seconds	28 cycles
55 °C	20 seconds	
72 °C	20 seconds	
72 °C	5 min	
4 °C	1 min	
10 °C	∞	

Gel electrophoresis was performed to ensure correct amplicon product size and amount on a 1% agarose gel in 1x TAE buffer by adding 3 μL sample and 1 μL loading dye to each well. The gel was run for 1 hour at 140 V and visualised with 300 ms exposure on a transilluminator (G:BOX Syngene, GeneSnap). The concentration of the amplified PCR product was determined by fluorometric quantification (Qubit 4, Invitrogen) for a set of random samples to ensure adequate DNA concentration. The protocol is attached in Appendix F.

3.4.4.2 Indexing of samples

Two amplicon libraries were prepared, consisting of 144 and 142 samples, respectively. Normalisation was performed to ensure that all samples contained the same amount of DNA. The SequalPrep Normalization plate (96) kit (Invitrogen), protocol in Appendix G, was used for normalisation. The kit requires a minimum of 250 ng DNA per well and yields 25 ng DNA per amplicon after normalisation. Next, all amplicons were indexed, in which short oligonucleotides with known sequences were added to the Illumina adapter. Indexing secures backtracking of amplicons to a particular sample. The amounts of reagents added to each amplicon is presented in Table 5, which was amplified by the cycling conditions in Table 6.

Table 5: Concentrations and amounts of reagents needed to index one community sample.

Reagent	Distributor	Concentration	Amount per sample
PCR gradient dH_2O	Rouche	-	11.687 µL
5x Phusion buffer HF (7.5 mM MgCl2)	ThermoFisher	10 mM	5.0 µL
dNTP	VWR	10 mM per dDNT	0.625 µL
Phusion Hot Start DNA polymerase	ThermoFisher	2 units/µL	0.188 µL
Index 1 (N-series)	Illumina	-	2.5 µL
Index 2 (S-series)	Illumina	-	2.5 µL
Normalised template	-	-	2.5 µL

Table 6: Cycling conditions for indexing of amplicons.

Temperature	Time	
98 °C	1 min	
98 °C	15 seconds	8 cycles
50 °C	20 seconds	
72 °C	20 seconds	
72 °C	5 min	
4 °C	1 min	
10 °C	∞	

After PCR, gel electrophoresis was performed to confirm that the amplicons were indexed. The indexed amplicons were normalised as previously described before they were pooled and concentrated (AmiconUltra 0.5 centrifugal filter devices- 30K membrane, Merck Millipore). The concentrations of the pooled amplicons were measured on a NanoDrop device to be 10.2 and 11.0 ng/µl for library 1 and 2, respectively. The two amplicon libraries were sequenced with V3 reagents by 300 base pair paired-end reads on two MiSeq Illumina lanes at the Norwegian Sequencing Centre (NSC).

3.5 Processing and analysis of sequencing data

3.5.1 Quality filtering, OTU clustering and taxonomic assignment

The USEARCH pipeline was used to process the sequencing data (39). First, paired reads were merged simultaneously as primers and reads shorter than 400 base pairs were removed. The reads were then quality filtered to an error rate of 1%. Third, unique sequences (singletons) were disregarded. As there is no universally accepted species concept for *Bacteria* (40), operational taxonomic units (OTUs) are used to study bacterial diversity. OTUs are clustered based on DNA sequence similarity. Here, OTUs were clustered at a 97% similarity level at the

same time as chimeras were removed. Lastly, unique OTUs were assigned taxonomy by comparing their sequences to the RDP reference data set (version 16) at a 80% confidence threshold. The output of the pipeline was an OTU table containing the number of sequence reads per OTU for each sample and the taxonomic assignment of each OTU. The dataset was further processed and analysed in R studio (version 3.5.1).

3.5.2 Sub-setting of the dataset

The samples sequenced of interest in this project were; 204 bioreactor community samples, 2 seawater inoculum samples and 4 samples of two mock communities (replicas run on the two Illumina lanes). The mock communities consisted of nine different bacteria with known abundances and were used to determine that the reproducibility between the two lanes was satisfactory. Further analysis was only carried out with sequence data from the bioreactors and inoculum. The sample from KRL, replicate 1, day 32, was lost during library preparation and was therefore not included in the analysis. One bioreactor sample (RKH replicate 3 day 16) was of insufficient quality and consequently removed from the dataset, resulting in a total of 204 samples (202 bioreactors + 2 inoculum).

3.5.3 The normalisation of sequence depth and estimation of coverage

Sequencing effort describes the increase in the number of OTUs as a function of sequencing depth and was determined with the function rarecurve() in the vegan package (version 2.5.3). Many statistical approaches are sensitive to varying sequencing depth, and for this reason, samples were normalised to 10 000 sequence reads. Normalisation was performed by randomly sub-sampling 10 000 reads per sample without replacement 1000 times with the function phyloseq_mult_raref() from the metagMisc package (version 0.0.4). This generated 1000 datasets, of which the mean was taken, and again sub-sampled to 10 000 reads per sample. Prior to the normalisation, four samples were upscaled to 10 000 reads, as their slopes on the rarefaction curve were close to zero.

The current methods used in microbial ecology are not able to describe the communities in totality, and it is important to note that the data is relative, not absolute. This relativity is caused by bias that can arise during sampling (e.g. small sampling volume, here 30 mL from 250 mL), DNA extraction (e.g. some cells are harder to lyse) and PCR (e.g. randomness of the polymerase) to mention a few (10,41). Chao1 is a non-parametric species estimator that

estimates the true richness based on the observed richness (42) and is used to determine the datasets coverages of the actual community. The function estimate_richness()in the phyloseq package (version 1.26.0) was used to calculate Chao1 (43). Coverage was calculated by dividing the observed richness over the estimated richness and multiplying by one hundred.

3.5.4 Diversity analysis

Diversity is described by richness and evenness (44). Diversity was evaluated and described on three different levels: alpha, beta and gamma diversity. Alpha diversity reflects the diversity within a sample, differences in diversity between samples are described by beta diversity (10), while gamma diversity quantifies the total diversity in a defined region or set of samples (45). Together alpha, beta and gamma diversity were used to describe patterns in diversity and to investigate changes in microbial composition spatially and over time.

How diversity should be calculated is an ongoing discussion in the field of ecology, and many diversity indices exist. The current discussion is leaning towards using 'diversity of order q' (^{q}D), also termed Hill numbers, true diversity and the effective number of types (44,46–48), as this index satisfies the eight diversity indices criteria for microbial community analysis, defined by Lucas et al. (46). One compelling criterion is that the diversity values should be intuitive. For example, if the number of OTUs in the dataset doubles, the richness should double (i.e. the replication principle). Moreover, it should be easy to understand how rare and abundant OTUs are weighted in the index. Another criterion, essential in microbial ecology, is that the index should be insensitive to datasets containing many zeros. Based on the criteria defined, Lucas et al. stated that ^{q}D is recommended in diversity analysis of microbial datasets, and this concept was therefore used in this thesis. The equation for the diversity of order q is given in equation 2 where ^{q}D denotes the diversity number, q the diversity order $(0,1,..,\infty)$, p_i the relative abundance of species i of a total of R species, and $^{q}\overline{p_i}$ the q-weighted generalised mean (44).

$$^{q}D=\frac{1}{^{q}\overline{p_i}}=\left(\sum_{i=1}^{R} p_i^{q}\right)^{1/(1-q)} \qquad [2]$$

The equation is intuitive because it is understandable for the reader to know the weight of abundance; with increasing q, the weight of the abundant OTUs increases. In the simplest case, were q=0, no abundance is taken into account, and richness, the observed number of OTUs, is calculated. Diversity of order one, ^{1}D, also known as exponential Shannon, is calculated based on the abundance of each OTU and with no weight of rare or common species. ^{1}D values,

therefore, incorporate both richness and evenness and takes the maximum value of ${}^{0}D$.${}^{2}D$ is the equivalent of the inverse Simpson, and ${}^{\infty}D$ of the inverse Berger-Parker, two other well-known diversity indices, both with increasing weight on the most abundant OTUs (46). ${}^{\infty}D$ takes the value of $1/p_{max}$, which can be used to quantify the abundance of the most abundant OTU. Throughout the thesis, ${}^{1}D$, ${}^{2}D$ and ${}^{\infty}D$ will be used instead of exponential Shannon, inverse Simpson and inverse Berger-Parker, respectively.

Evenness describes the equitability of the proportional abundances of the OTUs in a community. Because diversity can be described by richness multiplied by evenness, the formula for evenness can be expressed as ${}^{q}E={}^{q}D/{}^{0}D$ (44). Evenness then has an intuitive maximum value of 1. The lower the value of evenness, the more uneven the community is. In this thesis, ${}^{1}E$ was calculated to evaluate evenness.

Alpha diversity was calculated with the renyi() function in vegan for different diversity orders (49). To test whether the diversity numbers were normally distributed, the Shapiro-Wilk test of normality was performed (30). Statistical significance between groups was determined with a two-sided paired sample t-test when the data were normally distributed and with pairwise Mann-Whitney-Wilcoxon test when it was not (31). There was assumed dependency based on sampling time. Gamma diversity was estimated by pooling all samples for a defined set of samples (e.g. all samples within a cultivation regime or the whole dataset) before using the same diversity measurements as for alpha diversity (45). Beta diversity can be assessed by several methods and should reflect the variation of species composition among sites. Here, the multiplicative and distance-based approaches were applied.

In the multiplicative approach, beta diversity describes the difference between the average sample and the total diversity and is estimated from alpha and gamma diversity as presented in equation 3 (45). The mean alpha diversity ($\bar{\alpha}$) was calculated by taking the average of the alpha diversity measurements for the set of samples chosen.

$$\beta = \gamma/\bar{\alpha} \qquad [3]$$

In the distance-based approach, the compositional dissimilarity between two samples is quantified and used to analyse the variation of dissimilarity. This approach is independent of gamma and alpha diversity. Dissimilarity indices calculate the distance between two samples based on the OTU-matrix and are either abundance or incidence based (50). Examples of

abundance-based indices are the Hellinger distance and Bray-Curtis dissimilarity, while Sørensen is an incidence-based index. The maximum value of the Hellinger distance is $\sqrt{2}$, while both Bray-Curtis and Sørensen take the maximum value of 1, indicating that two samples are completely different (50). The total beta diversity is the variation in the OTU-matrix (Y), which can be decomposed as presented in equation 4.

$$\beta_{tot} = \text{Var(Y)} = \text{SCBD} + \text{LCBD} = \text{Replacement}_{total} + \text{Richness difference}_{total} \quad [4]$$

Another distance index, UniFrac, calculates the distance between two communities based on phylogenetic information. This index is either abundance-based (i.e. unweighted UniFrac) or incidence-based (i.e. weighted UniFrac). MEGA7 was used to construct a phylogenetic tree of the OTUs in the dataset (51).

3.5.5 Species contribution to dissimilarities between communities

Species contribution to beta diversity (SCBD) and local contribution to beta diversity (LCBD) quantify how individual OTUs or samples affect the variation in the OTU matrix, respectively. SCBD and LCBD were estimated for the dataset using the Hellinger distance with the function beta.div() in adespatial (version 0.3.2). The SCBD identifies the variation each OTUs contributes to the Hellinger distance matrix.

Analysis of similarity percentages (SIMPER), on the other hand, quantifies the contribution of the *i*-th species to the variation between sample groups in the Bray-Curtis distance matrix (52). This method differentiates from SCBD as it uses Bray-Curtis and not Hellinger distances, and is calculated based on between-group variance, not in-group variance. SIMPER was calculated using simper() in vegan.

A weakness with SIMPER is that it identifies OTUs with a large in-group variance to be significant to differences between groups, a problem highlighted by Warton et al. (53). This problem arises because SIMPER does not account for the mean-variance relationship in the dataset. As a solution to this, generalised linear models (GLMs) can be used instead of SIMPER to identify OTUs that contribute most to variation between groups. The mvabund package (version 4.0.1) with the functions manyglm() and anova.manyglm() generates GLMs of community datasets. Unfortunately, the code was not optimised for large datasets, and after running for approximately ten weeks, the calculation was terminated.

3.5.6 Quantifying the contribution of replacement and richness differences

The total beta diversity can be partitioned into replacement and richness difference, see equation 4. Replacement, or turnover, describes the gain and loss of OTUs, while richness difference reflects the difference in the number of OTUs (45,50,54). The contribution of replacement and richness difference to the compositional difference between two consecutive sampling days was calculated based on the Sørensen index. The formulas for the calculations are presented in Table 7 (54). The functions beta.div() and beta.div.comp() in the adespatial package (version 0.3.2) was used for this purpose (55).

Table 7: The formulas for calculating the dissimilarity between two samples based on Sørensen and the contribution of replacement and richness difference where a is the number of shared OTUs, b the OTUs unique to sample 1 and c the OTUs unique to sample 2. Adapted from Legendre 2014 (54).

	Formula
Sørensen dissimilarity	$(b + c)/(2a + b + c)$
Replacement	$2\ \min(b,c)/(2a + b + c)$
Richness/abundance difference	$\lvert b - c \rvert/(2a + b + c)$

3.5.7 Multivariate analysis of the dataset

In addition to analysing the variance quantitively, it was visualised and modelled using multivariate analysis. Principal coordinate analysis (PCoA) was used to ordinate the variance to a two-dimensional plot (56). PCoA ordinations were achieved through the function ordinate() and plot_ordination() in phyloseq with the Bray-Curtis, Sørensen and UniFrac (both weighted and unweighted) distance indices. Permutational multivariate analysis of variance (PERMANOVA) on the Bray-Curtis and Sørensen dissimilarity matrix was used to test for statistically significant differences between groups. In short, PERMANOVA tests whether the central location and dispersion of one group is significantly different from another. As the data is non-parametric, permutations are performed to increase the precision of the p-value (57). The PERMANOVA test was performed with the function adonis() in the vegan package, with 999 permutations.

Distance-based redundancy analysis (db-RDA) was used to model the variation as a function of the experimental variables (58). As it is not recommended to perform db-RDA on non-Euclidian distances, the Bray-Curtis distance matrix was square root transformed, making the matrix Euclidian, before used in the function dbrda() from vegan (52). Both forward and reverse

selection of variables, and a mixture of the two, were used to model the variation in the distance matrix. The model selection was based on the adjusted coefficient of multiple determination (R_a^2) values (59). In the first selection period (day 0-28), a K-selected community (natural seawater) was subjected to either r- or K-selection. However, in the second selection period (day 28-50), K-selected communities were exposed to r-selection and r-selected communities to K-selection. Because the starting conditions of the two selection regimes were different, the variance was modelled independently for the two periods when the communities were assumed to be acclimated to the selection (day 6-28 for period one, and day 36 to 50 for period two).

3.5.8 Taxonomic composition of samples

The taxonomic composition was plotted for each selection regime over time to inspect the development in the communities. The consistency in composition between replicas was determined, and composition investigated on all taxonomic levels: phylum, class, order, family, genus and OTU. To filter out low abundance OTUs, causing noise in the compositional plots, OTUs had to have an abundance of a minimum of 1% in at least one sample at the class, order and family level, three samples at the genus level and six samples at the OTU level.

3.5.9 Analysing similarity through time

A community's temporal stability can be investigated by determining the compositional similarity over time. In this thesis, similarity through time was observed through moving window analysis (22,60,61), accomplished by comparing the Bray-Curtis or Sørensen dissimilarity between two consecutive sampling days and plotting them as a function of time.

4 Results

The objective of this thesis was to determine how r- and K-selection affected the community characteristics of planktonic seawater *Bacteria*. Four cultivation regimes, each with three replicates, were maintained for 50 days. Half of the reactors (RK) were r-selected the first 28 days before the selection was switched from r-to-K-selection. The other half were K-selected in the first selection period (KR), before a switch to r-selection in the second selection period. In each selection regime group, half of the reactors were run at high (H) carrying capacity, and the other half at low (L).

4.1 The fraction of r- and K-strategists over time

The fraction of r- and K-strategists were estimated as described in 3.3.1, and changed over time and according to the r- and K-selection regime (Figure 5). Throughout the experiment, K-selection decreased the fraction of r-strategists. This development is easily visualised for the KR reactors comparing day 12 and 28, where the fraction of r-strategists decreased from 74% to 50% r-strategists. The same is true for the RK reactors from day 28 to 36 to 50, where the fraction of r-strategists decreased from 89% to 47% (Figure 5 a). The seawater inoculum contained the highest fraction of K-strategists at 70%.

Carrying capacity also affected the fraction of r-strategists. During K-selection in the RK reactors (day 36 and 50), the fraction of r-strategists was 7% lower on day 36 and 21% lower on day 50 in the reactors with low carrying capacity (RKL) compared to the ones with high. The same was not observed in the KR reactors during K-selection, however, probably because KRH is only represented by one replica because of experimental errors. The similarity between replicates is presented in Figure S1.

At the end of the first selection period, the fraction of r-strategists was 44% lower in the KR-reactors (K-selected) than in RK-reactors (r-selected). Further, at the end of the second period, the RK reactors (K-selected) had 74% less r-strategist than the KR-reactors (r-selected) (Figure 5 b). Thus, the r- and K-selection regimes were successful in increasing the fraction of bacteria with the life strategy selected for.

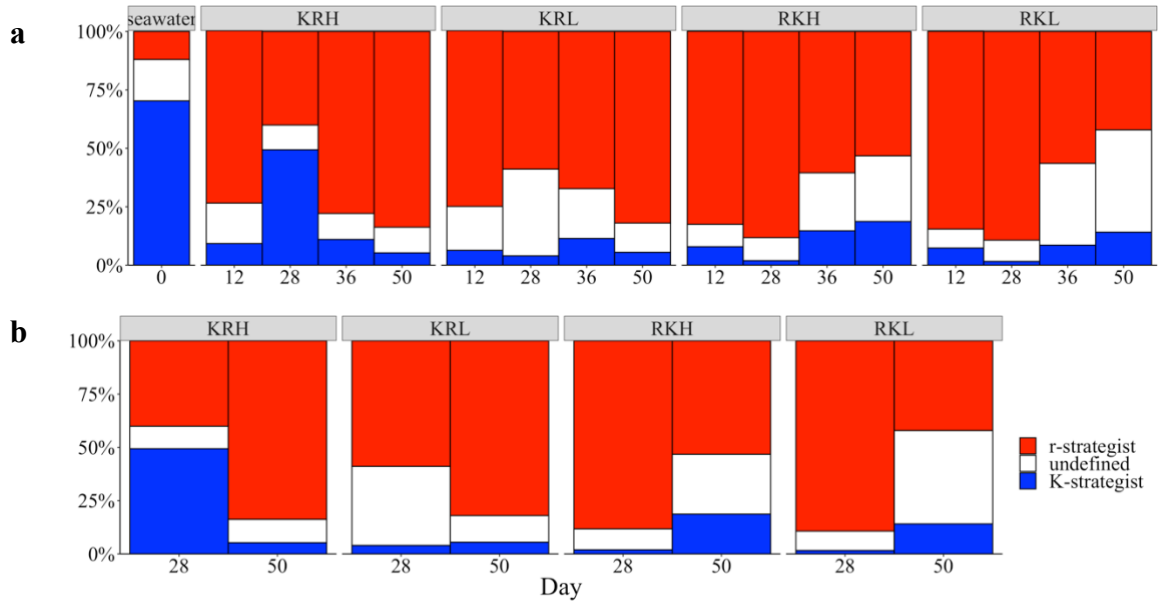


Figure 5: The fraction of r- and K-strategists in community samples determined by nutrient pulsing. Undefined is categorised as neither r- nor K-strategists. KRH and RKL on day 28 are only represented by one replicate. a) The development of the fractions over time, b) comparing the end of the two selection periods. RK: r-selection from day 0-28, K-selection from day 28-50. KR: K-selection from day 0-28, r-selection from day 28-50. H: high carrying capacity, L: low carrying capacity.

4.2 The effect of cultivation regimes on bacterial cell density

The bacterial cell density changed over time as a result of the cultivation regimes (Figure 6). In general, K-selection resulted in higher cell densities. During the first selection period, KRH had an average of 13.8 million cells more per millilitre than the RKH group ($p = 4.3 \times 10^{-8}$, Wilcoxon signed rank test). KRL had 3.7 million more cells per millilitre on average than RKL ($p = 0.002$, Wilcoxon signed rank test). In the second selection period, all reactors increased in cell density until day 34, after that they decreased and stabilised around 10-20 million cells per millilitre. During the stable period (day 42-50), there were no significant differences between the selection regime ($p = 0.4$, Paired t-test). However, before the stabilisation, RKL and RKH (K-selected) had an average of 24.0 and 15.1 million cells more per millilitre than the KRL and KRH (r-selected) respectively (L: $p = 0.0003$ and H: $p = 3.6 \times 10^{-7}$, Wilcoxon signed rank test). Carrying capacity affected the mean density significantly only between KRH and KRL in the first period, with a density difference of 12.9 million cells per millilitre ($p = 3.5 \times 10^{-5}$, Wilcoxon rank sum test). The mean cell size (Figure S2) was larger in r-selected bioreactors, on average 26% higher in the first selection period (day 3-28, $p = 1.3 \times 10^{-7}$) and 13% in the second (day 29-50, $p = 0.06$).

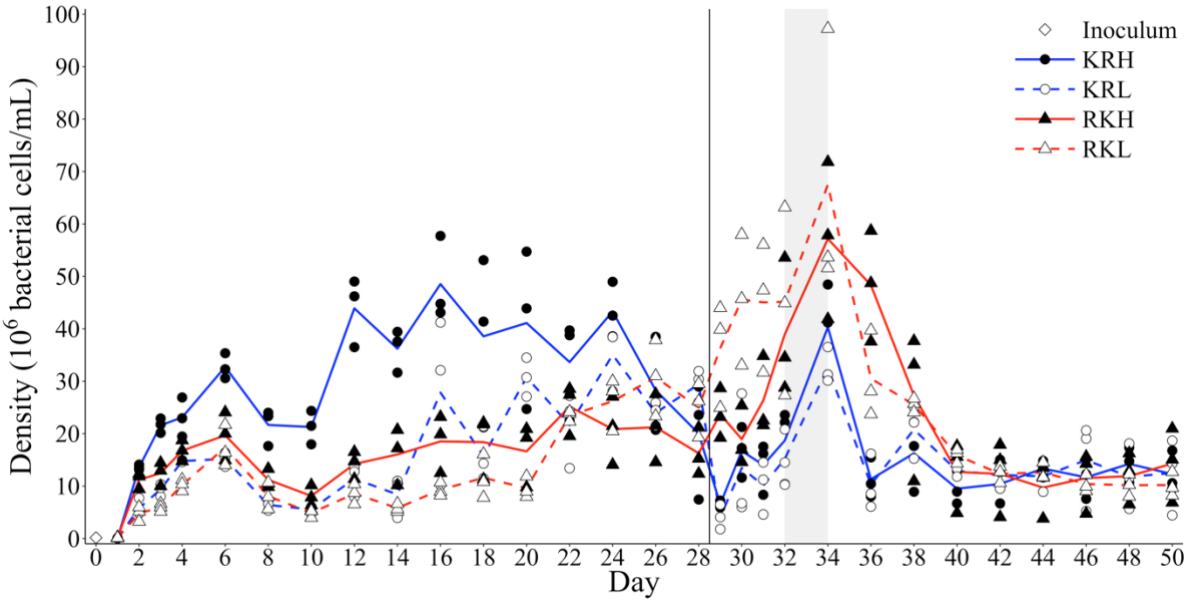


Figure 6: Bacterial cell density (10^6 cells per mL) for the four cultivation regimes over time. The lines indicate the mean of biological replicates within the cultivation regimes (n=3) at each time point. The solid vertical line indicates the r and K-selection regime switch. The temperature was at 20 °C during the area highlighted in grey. RK(△ and ▲): r-selection from day 0-28, K-selection from day 28-50. KR(○ and ●): K-selection from day 0-28, r-selection from day 28-50. H (filled): high carrying capacity, L (blank): low carrying capacity.

4.3 The effect of cultivation regimes on mean RNA content

The mean RNA content changed over time within a cultivation regime and was generally higher during r-selection (Figure 7). The communities started to separate with regards to RNA content on day 4. Before this day, it was a peak in mean RNA content (day 2) followed by a drop (day 3). From day 4 to 29, r-selected bioreactors had on average 20% higher mean RNA content than K-selected (2.0×10^{-16}, Paired t-test). In the second selection period, the difference in mean RNA content between the r- and K-selected reactors was smaller, at 10%, however, still significant (p = 0.001, Wilcoxon signed rank test). The difference in mean RNA content was insignificant between reactors with the same selection regime but different carrying capacities (i.e. KRH vs KRL and RKH vs RKL) in the first period. However, in the second selection period, KRH had on average 16% more RNA than the KRL group (p= 0.0002, Wilcoxon rank sum test) but on day 50 there was no statistical significance between KRH and KRL (p = 0.4, Paired t-test).

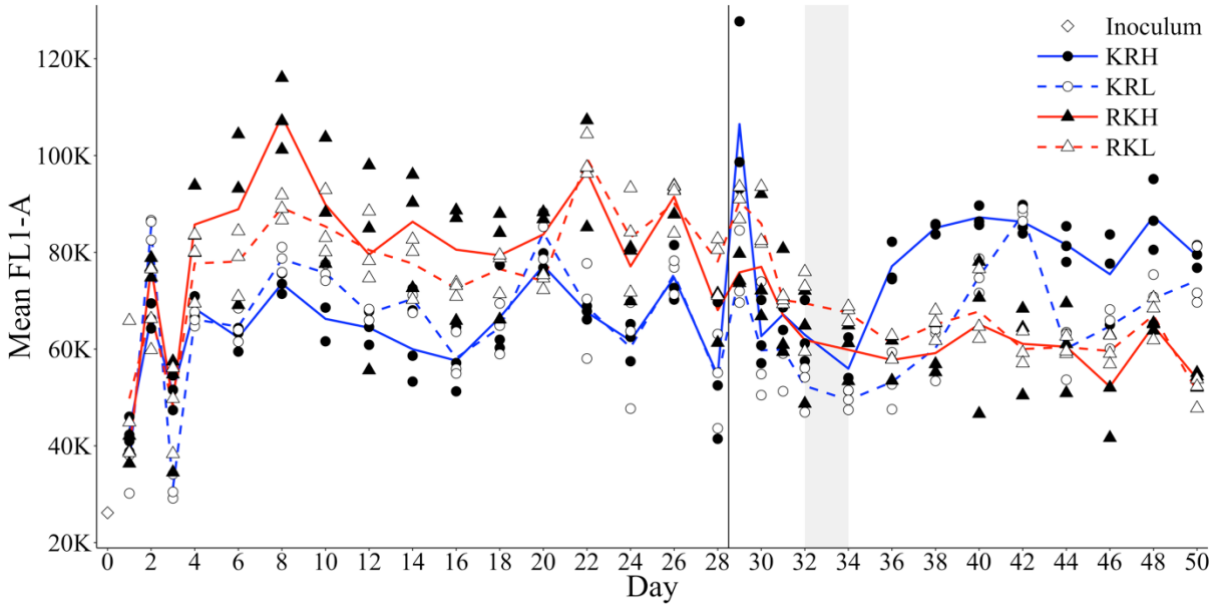


Figure 7: Mean RNA content, measured as mean FL1-A, for the four cultivation regimes over time. The lines indicate the mean of biological replicates within the cultivation regimes (n=3) at each time point. The solid vertical line indicates the r and K-selection regime switch. The temperature was at 20 °C during the area highlighted in grey. RK(△ and ▲): r-selection from day 0-28, K-selection from day 28-50. KR(○ and ●): K-selection from day 0-28, r-selection from day 28-50. H (filled): high carrying capacity, L (blank): low carrying capacity.

4.4 The diversity of the reactor communities

The raw dataset contained a total of 12 945 783 sequence reads and 204 samples, varying from 1781 to 204 465 reads per sample and a mean of 63 460 reads (± 31 411 SD). A total of 1551 OTUs were identified in the dataset, which was estimated with Chao1 to cover 47 to 100% of the actual OTU-inventory in the samples with a mean of 82% (± 8% SD). The dataset was sub-sampled to 10 000 sequences per sample. In the normalised dataset, a total of 1543 OTUs were detected with a mean loss of 0.4 OTUs per sample. This indicated that the normalised dataset represented the non-normalised dataset well.

4.4.1 Diversity within the reactor communities (alpha diversity)

Diversity within the communities, i.e. alpha diversity, was calculated for each biological replicate at each time point (see 3.5.4 for details). Diversity dropped drastically from day 0-2 (Figure 8). Overall, the reactors with KR-selection regime had 30% higher richness (^0D) compared to the RK-group ($p=1.5 \times 10^{-13}$, Wilcoxon signed rank test) (Figure 8 a). The KRL community had a reduction from 125 to 77 OTUs from day 20 to 24; however, gradually increased back to 131 OTUs on day 30. This reduction in richness was not observed for the KRH reactors. Moreover, during the second selection period, there was no significant difference as a result of carrying capacity (KRH vs KRL $p = 0.08$, RKH vs RKL $p = 0.4$, Paired t-test). For the RK group, the richness was reduced the first six days, before it steadily increased until day 20. After that, the richness stayed relatively stable.

Compared to the richness, diversity of order one was not as affected by the selection regime (Figure 8 b). This indicates that the difference between KR and RK was mainly due to OTUs with low abundance. KRH was the only selection regime that was significantly different ($p<0.05$, Wilcoxon rank sum test) from the others in the first selection period. However, the means often overlapped and did not present a detectable pattern, indicating that there might not be any biological relevance. In general, the communities had low evenness, as the maximum evenness that can be obtained is one (Figure 8 c). This indicated that some OTUs were very abundant and dominated in the communities. The mean evenness was typically between 0.05 and 0.13. The KRH reactors evenness dropped from 0.1 on day 29 to 0.04 on day 40, likely as a result of the r-selection. The evenness increased gradually towards the end of the experiment to 0.06. At the end of the experiment, the RK group was 48% more even than the KR group ($p = 9.0 \times 10^{-6}$, pairwise Wilcoxon rank sum test).

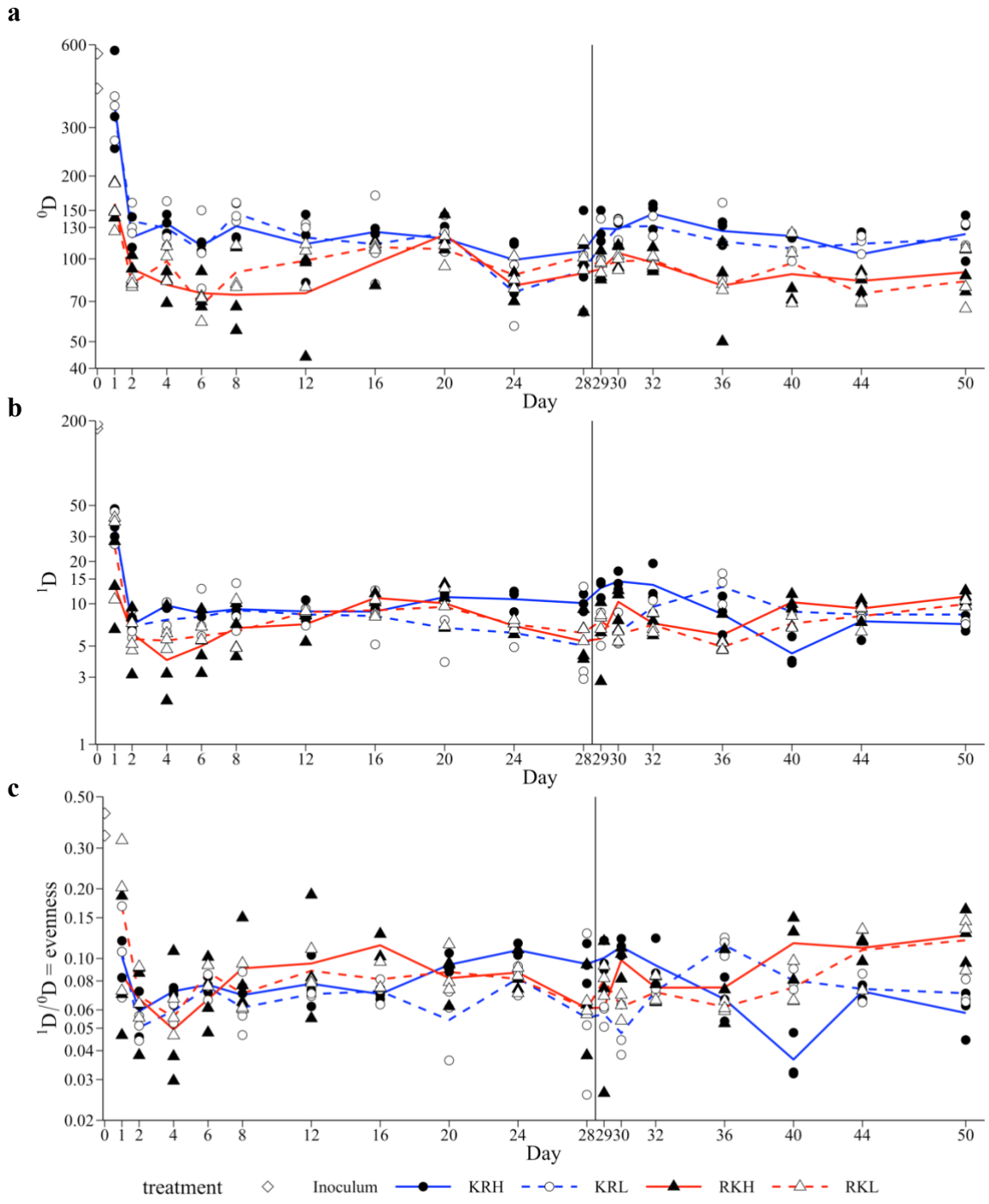


Figure 8: Diversity of order a) zero and b) one, and c) evenness for each cultivation regime as a function of time. The lines indicate the mean of biological replicates within the cultivation regimes (n=3) at each time point. The solid vertical line indicates the r- and K-selection regime switch. Notice, the y-axis is log-scale. Inoculum (◇): natural seawater. RK(△ and ▲): r-selection from day 0-28, K-selection from day 28-50. KR(○ and ●): K-selection from day 0-28, r-selection from day 28-50. H (filled): high carrying capacity, L (blank): low carrying capacity.

4.4.2 Total diversity in the whole dataset and the selection groups (gamma diversity)

The total diversity, i.e. gamma diversity, serves as a reference to alpha diversity. Comparisons of the mean alpha diversity and total diversity in a specific set of samples (e.g. all reactors in the KRH group) indicates if there is variation within the set of samples chosen, and further, on which diversity order the variation arises.

A loss of over 50% of the OTUs was observed in the first three days, as reflected by gamma ^0D in Table 8. The ^0D beta diversity excluding day 0-2 was seven, indicating a huge variation in the OUT inventory in different reactor communities. The evenness indicated that there were some OTUs that dominated the whole dataset (evenness 0.033), but that different OTUs dominated in different communities (average evenness 0.11). This indicated that the selection regimes affected community composition.

Table 8: Gamma, mean alpha and multiplicative beta diversity values for the diversity of order 0 and 1 and their respective evenness with the sample set as the whole dataset and excluding day 0-2. Beta diversity was calculated as gamma/mean alpha.

	All days			**Without day 0-2**		
	^0D	^1D	evenness	^0D	^1D	evenness
γ	1543	23	0.02	714	19	0.03
$\bar{\alpha}$	119	11	0.09	106	8	0.08
β	13	2	0.17	7	2	0.33

As it was hypothesised that there should be differences in community composition as a result of the selection regime, the total diversity within each cultivation regime group was determined for the first and second selection period (Table 9). The period before the r- or K-selection switch was defined as day 6-28, and the period after from 36-50. This sub-setting was based on the assumption that took some time for the communities to acclimate to the selection.

In the KR regime, the KRL group had 11% more OTUs than KRH in the first period, while the difference was only 0.4% in the second period. Within the RK group, RKH had 2% more OTUs than RKL in the first period and 17% more during the second (gamma ^0D, Table 9). The richness difference between the first and second period was of greater magnitude in the KR-selection groups where it dropped 27% in KRH and 35% in KRL, compared to 9% and 23% in RKH and RKL, respectively (gamma ^0D, Table 9). Despite this observation, the mean alpha ^0D

was reasonably consistent in the first and second selection period within the cultivation regimes, however around 20% lower in the RK compared to the KR reactors.

In the KRH selection group, r-selection led to a decrease in gamma ^{1}D, ^{2}D and $^{\infty}D$, while it led to an increase in the KRL group. The most dominant OTU decreased in abundance, represented by gamma $^{\infty}D$, during r-selection in the KRL group (from 40% to 20%), but increased in the KRH group (from 25% to 50%). K-selection in the RKH selection group resulted in an increase in gamma diversity, while RKL stayed relatively constant. K-selection decreased the average richness in the reactors in the RKL group. The RKH group became more even during K-selection (from 0.04 to 0.07) , and the most abundant OTU decreased from 40% to 14%.

Table 9: Gamma, mean alpha and beta diversity of order 0, 1 and ∞, and evenness in the four cultivation regimes before and after r- and K-selection switch. The first selection period was defined from day 6 to 28, and the second, after the switch, from day 36 to 50 (^{0}D = richness, ^{1}D = exponential Shannon, $^{\infty}D$ = inverse Berger-Parker). RK: r-selection from day 0-28, K-selection from day 28-50. KR: K-selection from day 0-28, r-selection from day 28-50- H: high carrying capacity, L: low carrying capacity.

Cultivation regime	**KRH**				**KRL**			
Selection period	**First**		**Second**		**First**		**Second**	
r/K-selection	**K**		**r**		**K**		**r**	
	γ	**$\bar{\alpha}$**	**γ**	**$\bar{\alpha}$**	**γ**	**$\bar{\alpha}$**	**γ**	**$\bar{\alpha}$**
^{0}D	339	116	247	120	383	113	248	115
^{1}D	14.5	9.7	9.3	7.0	10.9	7.9	14.5	9.8
$^{\infty}D$	3.8	3.3	2.0	2.2	2.6	2.8	5.0	3.3
Evenness ($^{1}D/^{0}D$)	0.04	0.1	0.04	0.06	0.03	0.07	0.06	0.09

Cultivation regime	**RKH**				**RKL**			
Selection period	**First**		**Second**		**First**		**Second**	
r/K-selection	**r**		**K**		**r**		**K**	
	γ	**$\bar{\alpha}$**	**γ**	**$\bar{\alpha}$**	**γ**	**$\bar{\alpha}$**	**γ**	**$\bar{\alpha}$**
^{0}D	283	89	257	87	277	95.5	213	85
^{1}D	11.6	7.6	18.2	9.3	10.7	7.6	9.8	7.6
$^{\infty}D$	2.4	2.4	6.9	3.5	2.8	2.7	2.8	3.2
Evenness ($^{1}D/^{0}D$)	0.04	0.09	0.07	0.1	0.04	0.08	0.05	0.09

Based on gamma diversity, it can be concluded that there were differences in community structure based on as a result of the cultivation regimes. Even though the differences between communities were smaller within the cultivation regimes compared to the whole dataset, it was evident that there were differences in community structure for the individual reactors.

4.5 Effect of cultivation regimes on community composition and differences between communities

In this section, the effect of r- and K-selection on the community composition and an investigation into what caused the variation between communities is presented.

4.5.1 Taxonomic composition over time

Four classes made up the majority of the taxa present (Figure 9 a). *Gamma-proteobacteria* and *Alpha-proteobacteria* were most abundant, with *Flavobacteria* and *Bacilli* following. The switch between r- and K-selection, between day 28 and 29, resulted in a change of the composition at the class level. *Gamma-proteobacteria* dominated more during r-selection, while *Alpha-proteobacteria* made up more of the community during K-selection (comparison of these two classes in Figure S3). During the first days, *Gamma-proteobacteria* dominated all communities and had an abundance of 90-95% in the KR reactors (day 2) and RK reactors (day 4). At day 6, this class continued to dominate in the RK group (r-selection) but was reduced in the KR group (K-selection). Further, *Gamma-proteobacteria* dominated more in reactors with high nutrient supply (H groups). For example, from day 12 to 28, the abundance of *Gamma-proteobacteria* was around 20-25% for the KRL reactors, 30-35% for the KRH reactors, and 70-75% in the RKH and RKL reactors.

In the inoculum samples, 38% of the OTUs were unclassified at the class level, while 25% of the OTUs in the reactor communities were unclassified at the class level. When day 1 and 2 were excluded, this un-classification was further reduced to only 13%. Only 122 OTUs reached an abundance ≥1% in any sample, and a few of these dominated the community composition (in alphabetic order; Figure 9 b).

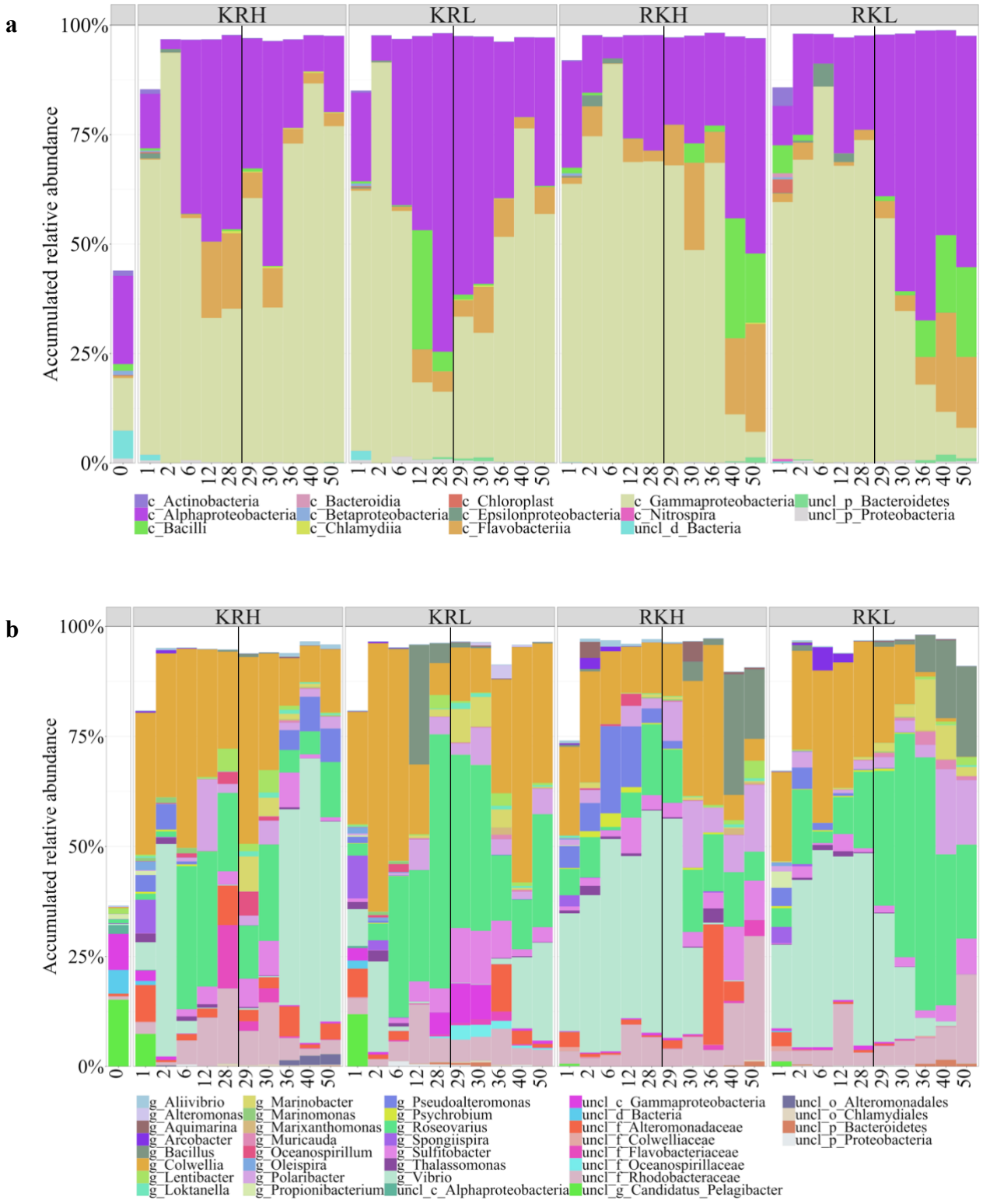


Figure 9: The accumulated relative abundance of bacterial a) classes and b) genera for reactor samples (n=3) observed over time for the four cultivation regimes and the inoculum at day 0. Only classes and genera represented by a proportion of ≥1% in at least one (for class) or three (for genera) of the samples are presented. The solid vertical line indicates when the regime switched between r- and K-selection. The x-axis is sampling day and white space filtered out OTUs. RK: r-selection from day 0-28, K-selection from day 28-50. KR: K-selection from day 0-28, r-selection from day 28-50- H: high carrying capacity, L: low carrying capacity.

Bacillus, Colwellia, Marinobacter, Polaribacter, Roseovarius, Sulfitobacter and *Vibrio* made up the majority of the communities over time (Figure 10, stacked barplot Figure S4). They often made up over 70% of the relative abundance. Most of these genera thrived under different selection regimes. *Vibrio* and *Colwellia* decreased in abundance during K-selection and increased during r-selection for all selection regime groups. *Vibrio* was undetected in all reactors at the end of the K-selection regime but had abundances up to 50% during r-selection. *Roseovarius*, *Bacillus and Polaribacter,* on the other hand, increased under K-selection and decreased during r-selection. Moreover, high carrying capacity (H) was accompanied by higher abundances of the genera that increased during r-selection, while low carrying capacity (L) had higher abundances of genera that thrived during K-selection.

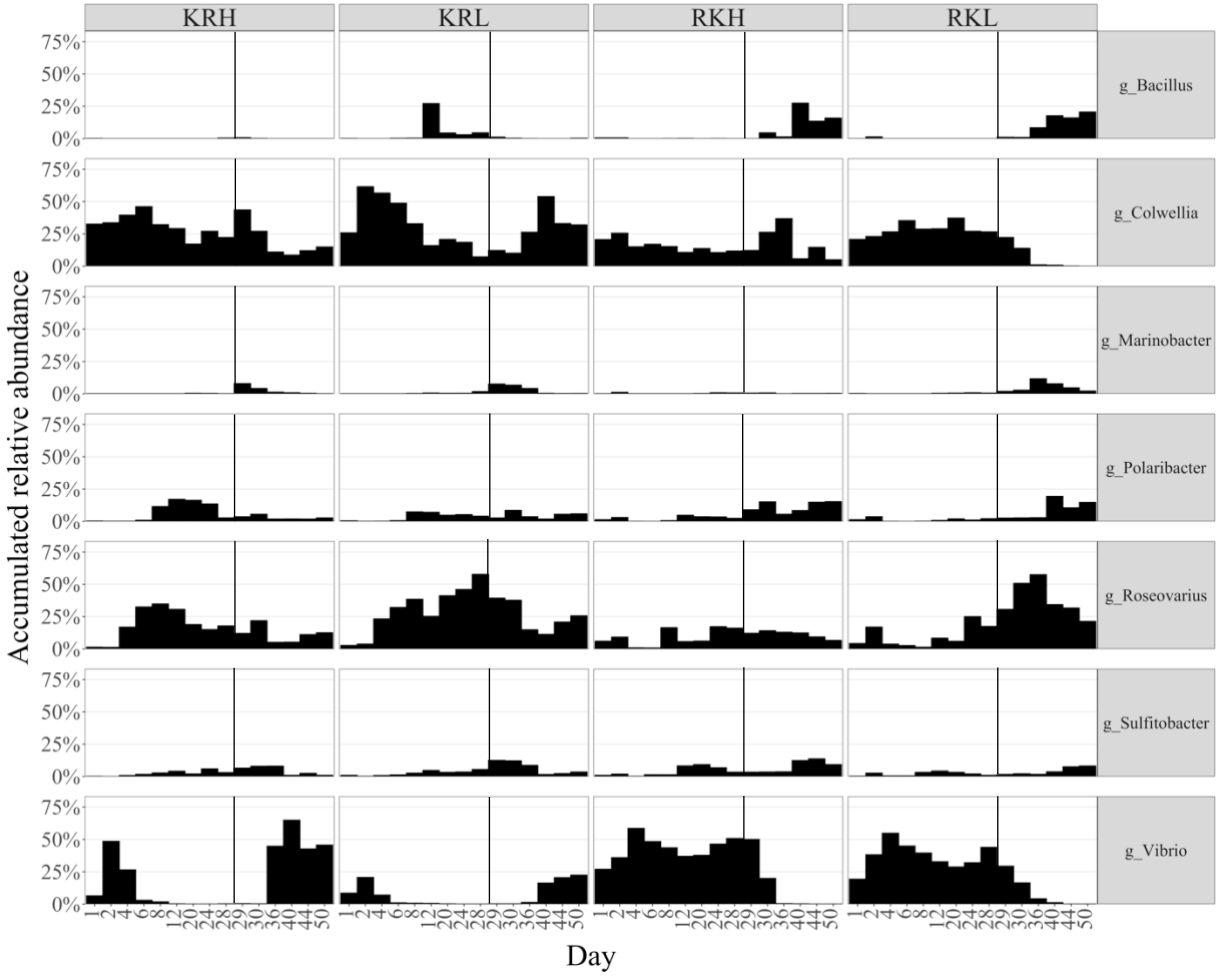


Figure 10: The six major genera observed in the dataset and their accumulated relative abundance over time for the four cultivation regimes (n = 3). The solid vertical line indicates the r- and K-selection regime switch. The x-axis indicates the experimental day. RK: r-selection from day 0-28, K-selection from day 28-50. KR: K-selection from day 0-28, r-selection from day 28-50. H: high carrying capacity, L: low carrying capacity.

Five OTUs belonging to the genera presented above contributed 51% to the variation in the Hellinger distance matrix (Table 10, see 3.5.5 for details).

Table 10: OTUs, and their respective genus classification, contributing over 5% to the variation in the Hellinger distance matrix based on day 6-28 and 36-50. SCBD: species contribution to beta diversity.

OTU	Genus	SCBD
OTU_1	Vibrio	21.0%
OTU_8	Colwellia	7.6%
OTU_6	Colwellia	7.8%
OTU_7	Bacillus	6.4%
OTU_2	Roseovarius	8.8%

Only 6 and 11 OTUs for the KR and RK regimes, respectively, contributed more than 1% to the variation between r- and K-selection along with p-values < 0.1, as determined by SIMPER analysis (Table 11). The comparison of r- and K-selection for all cultivation regimes is presented in Appendix I, Table S8, where it was identified 7, 4, 9 and 8 OTUs for KRH, KRL, RKH and RKL, respectively, that contributed more than 1% to the variation, and had a p-value < 0.1.

Table 11: The OTUs that contributed more than 1% to the variation between r- and K-selection in the KR and RK group, and had a p-value <0.1, determined by SIMPER analysis. The mean abundances are based on day 6-28 in the first selection period and day 36-50 for the second period. RK: r-selection from day 0-28, K-selection from day 28-50. KR: K-selection from day 0-28, r-selection from day 28-50.

OTU	genus	KR			RK			favoured during
		variation contribution	mean abundance during		variation contribution	mean abundance during		
			r-selection	K-selection		r-selection	K-selection	
OTU_1	Vibrio	16 %**	32 %	0.4 %	19 %**	38 %	1 %	r-selection
OTU_2	Roseovarius	11 %**	13 %	32 %	9 %**	10 %	23 %	K-selection
OTU_6	Colwellia	6 %	13 %	9 %	7 %**	14 %	4 %	r-selection
OTU_9	f_Alteromonadaceae	3 %	5 %	2 %	2 %*	1 %	4 %	none
OTU_11	Pseudoalteromonas	2 %*	4 %	0 %	-	-	-	r-selection
OTU_8	Colwellia	6 %	8 %	14 %	-	-	-	K-selection
OTU_5	Polaribacter	-	-	-	5 %	2 %	11 %	K-selection
OTU_10	f_Rhodobacteraceae	-	-	-	2 %**	0.1 %	4 %	K-selection
OTU_14	f_Rhodobacteraceae	-	-	-	2 %**	0.2 %	4 %	K-selection
OTU_16	f_Rhodobacteraceae	-	-	-	2 %	1 %	3 %	K-selection
OTU_3	Sulfitobacter	-	-	-	3 %*	4 %	7 %	K-selection
OTU_4	Marinobacter	-	-	-	2 %**	0,4 %	3 %	K-selection
OTU_7	Bacillus	-	-	-	8 %**	0 %	15 %	K-selection

* $p \leq 0.05$, ** $p \leq 0.01$

4.5.2 r- and K-selection contributed to differences between the communities

The community dissimilarity over time was ordinated with PCoA using a) Bray-Curtis and b) Sørensen dissimilarity (Figure 11). The variation explained by the first 50 axis's is presented in Figure S5. The Bray-Curtis ordination (Figure 11 a), explained 50% of the variation in the dissimilarity matrix by the first two coordinates (35.6%+14.8%). The cultivation groups (KRL, KRH, RKL and RKH) clustered tighter over time and based on r- and K-selection. The selection appeared to create a succession that resulted in a trajectory in the ordination with loading either decreasing on the axis 1 for K-selection (i.e. points move to the left with time) or increasing for r-selection (i.e. points move to the right with time). A comparison of the ordination on week 4 and 7 illustrates this. PERMANOVA analysis performed separately for the data within week 3, 4, 6 and 7 revealed that the community composition was significantly different between the four cultivation groups ($p = 0.001$ within each week). Within the weeks, the effect of r- and K-selection contributed to more than 50% of the compositional variation between the cultivation regimes ($p = 0.001$ within each week). The carrying capacity had no significant effect on community composition in the first selection period and only accounted for 7% of the variation between cultivation regimes within week 3 ($p = 0.156$) and week 4 ($p = 0.153$). However, during the second selection period, the contribution of carrying capacity was significant and increased to 12% within week 6 ($p = 0.009$) and 11% on week 7 ($p = 0.034$).

The points clustered tighter based on selection regime in the Sørensen ordination (Figure 11 b). This tight clustering indicated that the selection affected the OTU-inventory. The differences between cultivation regimes were significant and accounted for about 50% of the variation within week 3, 4, 6 and 7 ($p=0.001$ within each week), determined by PERMANOVA analysis. Moreover, the effect of carrying capacity on the variation was lower in the Sørensen dissimilarity compared to in the Bray-Curtis dissimilarity, and only accounted for 3-7% ($p > 0.1$ within each week).

Ordinations were also done using both weighted and unweighted UniFrac dissimilarities (Figure S6). For the unweighted UniFrac ordination, the cultivation regimes did not separate as well as they did in the Sørensen ordination. This interblending of points indicate that all communities contained OTUs with close phylogenetic relationships. The ordination based on weighted UniFrac displayed the same pattern as the ordination based on Bray-Curtis, that is, a separation on axis 1 based on selection regime. Thus, r- and K-selection affected the abundance of OTUs within the communities.

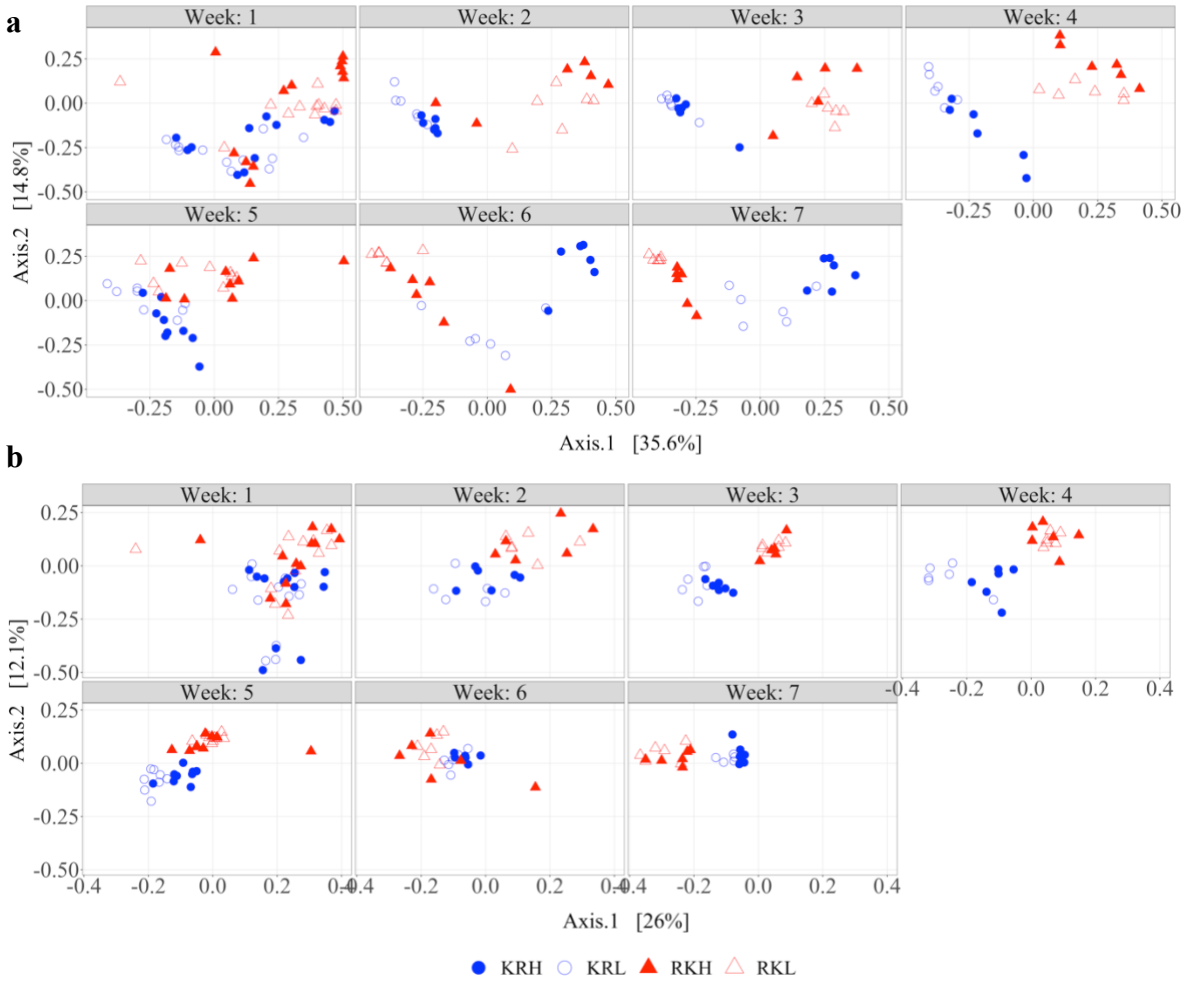


Figure 11: PCoA ordination based on a) Bray-Curtis and b) Sørensen dissimilarity. The switch in r- and K-selection was between week 4 and 5. RK: r-selection from week 1-4, K-selection from week 5-7. KR: K-selection from week 1-4, r-selection from week 5-7. H: high carrying capacity, L: low carrying capacity.

That r- and K-selection was the main contributor to the differences between the cultivation regime groups was also apparent when comparing the mean Bray-Curtis dissimilarities over time within and between cultivation regimes (Figure 12). Overall the communities within a cultivation regime were less dissimilar than when comparing communities with different carrying capacity (i.e. KRH vs KRL and RKH vs RKL). Cultivation regimes with the same carrying capacity but different selection were most dissimilar (i.e. KRL vs RKL and KRH vs RKH). The dissimilarity within cultivation regimes was around 0.3-0.5, between groups with different carrying capacity 0.4-0.6 and between groups with different selection 0.6-0.8. There were little differences in the within-group dissimilarity based on the selection regime, i.e. K-selection did not make the communities more similar.

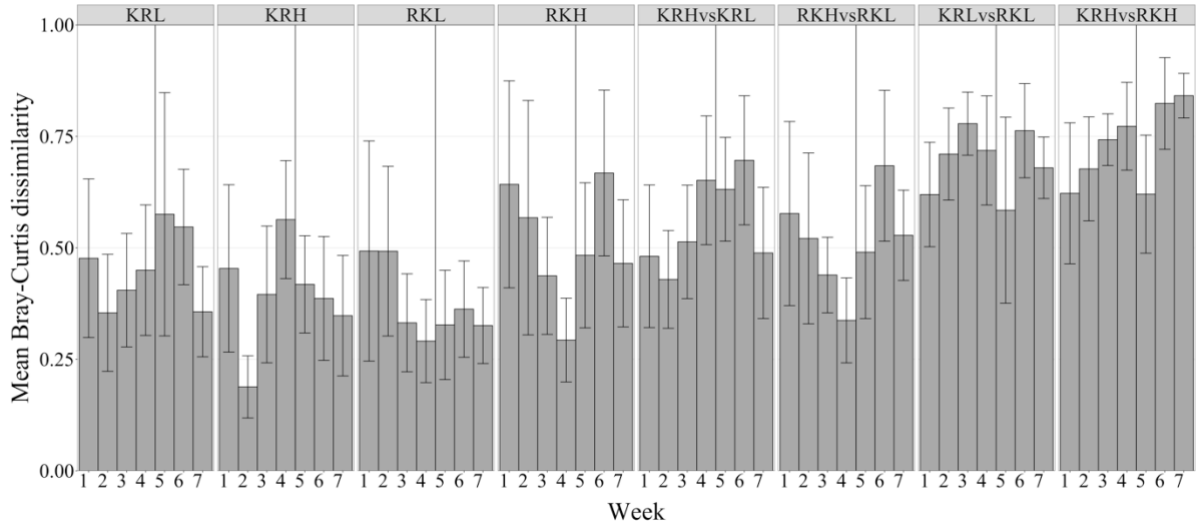


Figure 12: Within-group and between-group mean Bray-Curtis dissimilarity over time. Error bars indicate standard deviation. The switch in r- and K-selection was between week 4 and 5 and is indicated by the solid vertical line. RK: r-selection from week 1-4, K-selection from week 5-7. KR: K-selection from week 1-4, r-selection from week 5-7. H: high carrying capacity, L: low carrying capacity.

4.5.3 Quantifying variation linked to the experimental variables

Distance-based redundancy analysis, db-RDA, was used to quantify the variation explained by the experimental variables (day, selection regime and carrying capacity) in the Bray-Curtis dissimilarity matrix. Week 3-4 and 6-7 were chosen to represent the first and second selection period, respectively, and was modelled separately. In both cases, the model was significant ($p = 0.001$), but the experimental variables explained only 29% (first period) and 25% (second period) of the variation. The model for the first period incorporated day, selection regime and carrying capacity, while the model for the second period removed day when the forward/combined selection was used. Nevertheless, the selection regime was identified to contribute 86% and 72% to the model in the first and second selection period, respectively.

4.6 Temporal stability of the communities

As the experiment was conducted over seven weeks, temporal stability was of interest. High stability was here defined as communities having low dissimilarity from one day to the next. First, the similarity between biological replicates was determined, before investigating the community composition change between two adjacent sampling days. Lastly, I analysed whether community dissimilarity was caused by the replacement of OTUs or richness differences.

4.6.1 Successional patterns between the replicates

A Bray-Curtis PCoA ordination was performed for each selection regime separately, to investigate the similarity between biological replicas (Figure 13).

Replicas were more similar during the first week when exposed to K-selection compared to r-selection, as the points cluster tighter in the KRL and KRH groups. However, the average dissimilarity between replicas increased from around 20% on day 8 to 60% in the KRH and 50% in the KRL groups towards the switch (Figure S7). After the K-to-r-selection switch, the replicates became more similar again and stabilised around 40%. For the RKH group, the replicates were about 60% dissimilar the first two weeks, before they became less dissimilar towards the switch to around 40%. The dissimilarity between replicates in the RKL group varied during the first week, from 20% to 60%, before they stabilised around 40%. After the r-to-K-selection switch, the RKL replicates clustered tightly for each sampling day. The RKH group were more scattered at the beginning of the K-selection period but became more similar over time from day 40.

Although there was some dissimilarity between replicates, they were located relatively similar in the ordination plot based on the sampling day which indicates a defined successional pattern as a consequence of the selection regime.

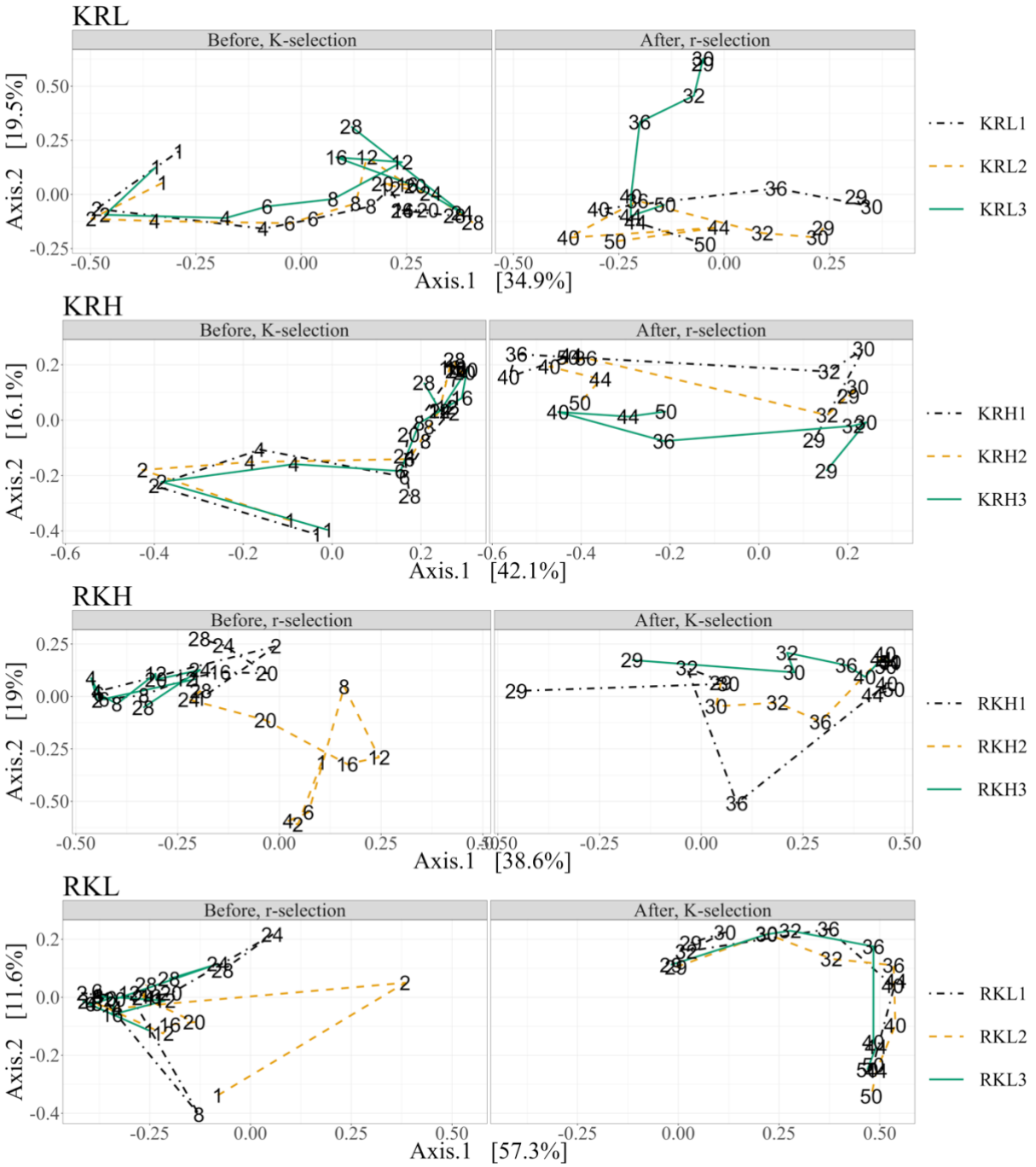


Figure 13: Similarity of biological replicates represented by Bray-Curtis PCoA ordination. Numbers indicate sampling day, and the colours represent the biological replicate. Before and after designate the r- and K-selection switch between day 28 and 29. RK: r-selection from day 0-28, K-selection from day 28-50. KR: K-selection from day 0-28, r-selection from day 28-50- H: high carrying capacity, L: low carrying capacity.

4.6.2 Temporal similarity for the selection regimes

The Bray-Curtis dissimilarity between biological replicates was compared between two adjacent sampling days to determine the temporal stability (Figure 15). For the KR group, the samples became more similar during the first eight days, before the dissimilarity increased. This pattern indicates that 1) the replicates became more compositionally different on each sampling day (see the previous section) and 2) that the communities within a replicate changed faster on the temporal scale from week 2 towards the end of week 4.

After the K-to-r-selection switch, the KRL group changed less between two sampling days, from about 45% between day 24 and 28 to 15% between day 29 and 30. However, after day 30, the compositional change over time increased, up to 50% dissimilarity between day 36 and 40. Nevertheless, towards the end of the experiment, the compositional change between sampling days decreased again, to around 30%. The KRH group followed the same pattern as the KRL group, but had higher compositional change between day 29 and 36, up to 75%. However, at the end of the experiment, the reactors in KRH changed less between two sampling days than in the KRL reactors, at around 20%. The RK group had lower compositional change compared to the KR group during day 12 to 28. The temporal dissimilarity increased in RKH up to about 90 % for one replica between day 36 to 40. The RKL group was relatively stable at around 25%.

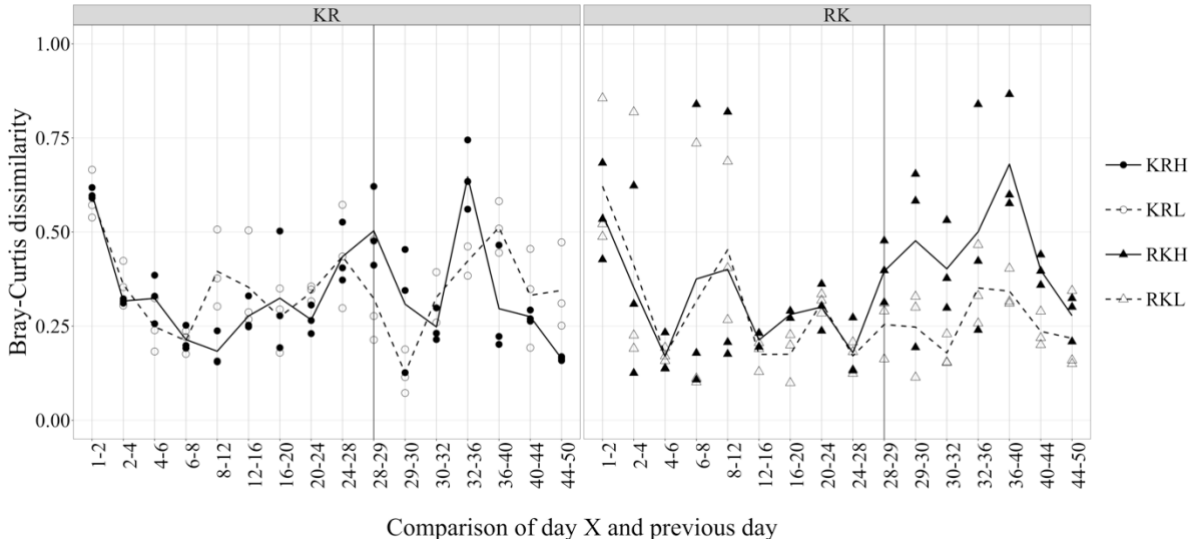


Figure 14: Dissimilarity over time for the four cultivation regimes, represented by moving window analysis of the Bray-Curtis dissimilatory matrix. Points indicate the comparison of biological replicates between two sampling days, and lines represent the mean within the selection regime (n=3). The solid vertical line indicates the r- and K-selection regime switch.

The RK group had 1.6 times higher variation between the replicates compared to the KR group during the first two weeks (Figure 15). During week three and four, the variation was equal regardless of cultivation regime. After the selection switch, the variance between replicates increased in all cultivation regime groups, except for RKL. The lowest variation was observed in week seven for the RKH reactors.

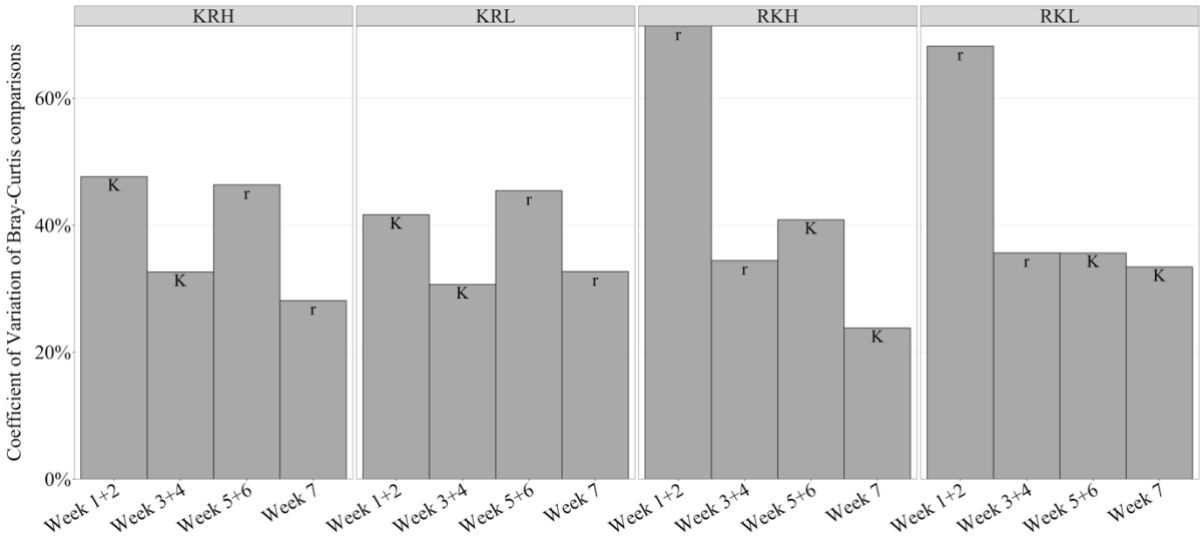


Figure 15: Coefficient of variation of the Bray-Curtis dissimilarity comparisons between two sampling days for different groups. RK: r-selection from day 0-28, K-selection from day 28-50. KR: K-selection from day 0-28, r-selection from day 28-50. H: high carrying capacity, L: low carrying capacity.

4.6.3 The contribution of replacement and richness differences

The contribution of replacement of OTUs and richness differences to the temporal Sørensen dissimilarity was determined (see 3.5.4 for details). Compared to the Bray-Curtis dissimilarity over time (Figure 14) the communities in the reactors were more similar between two sampling days when using Sørensen dissimilarity (Figure 16). For all reactors, the compositional change between day 1 and 2 was high, with richness difference being the primary cause of differences in the KR reactors, while both richness and replacement contributed in the RK reactors. Both richness differences and replacement contributed to differences between sampling days and did not appear to be linked to a selection regime. Most of the reactors had relatively low dissimilarity between sampling days over time (around 20-25%), except for RKH1, in which the compositional change was high for most sampling days (around 40%). The switch in r- and K-selection did not appear to increase the dissimilarity in any reactors.

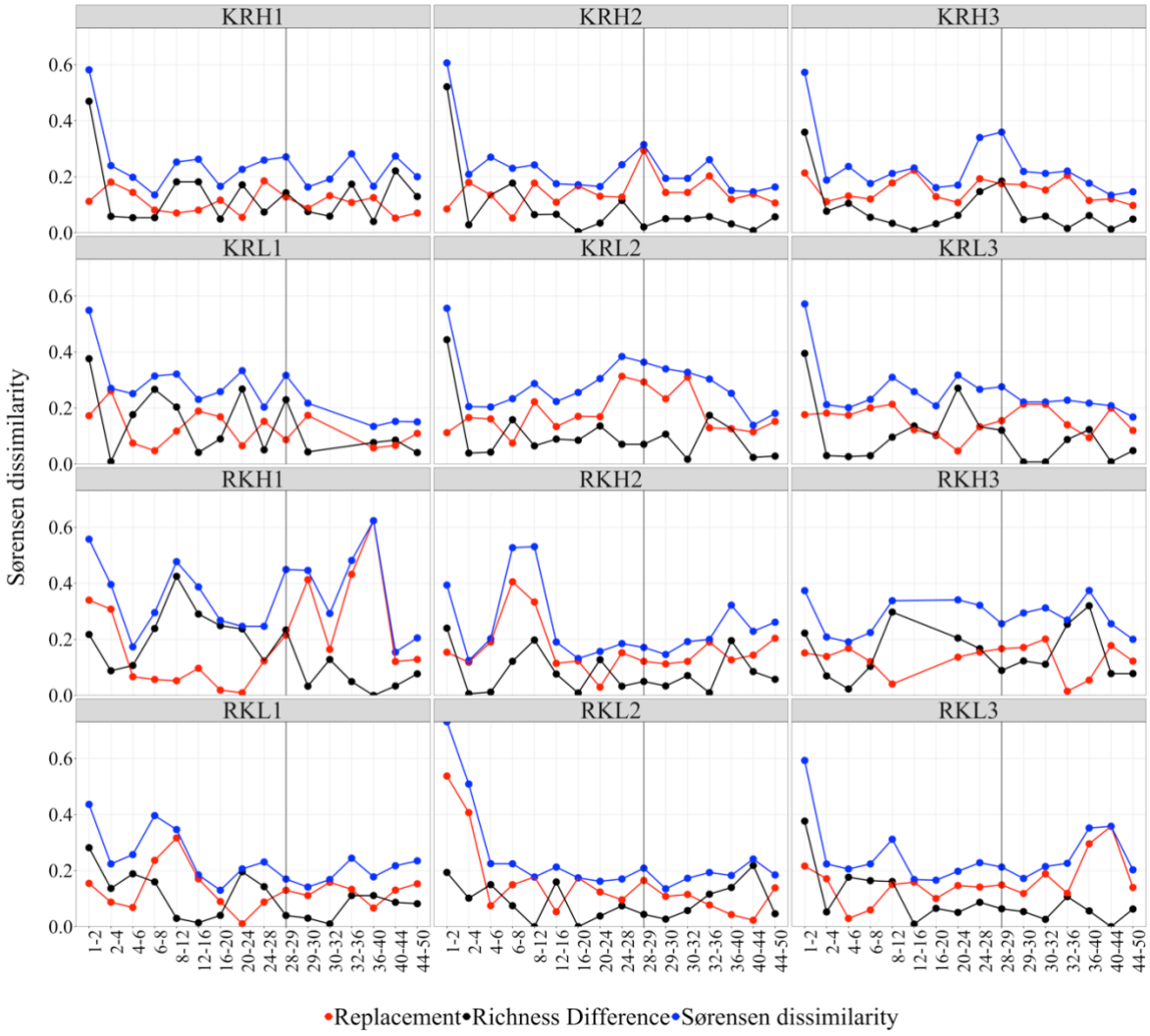


Figure 16: Contribution of replacement of OTUs and richness difference to the Sørensen dissimilarity for each reactor. The solid vertical line indicates the r- and K-selection regime switch. RK: r-selection from day 0-28, K-selection from day 28-50. KR: K-selection from day 0-28, r-selection from day 28-50. H: high carrying capacity, L: low carrying capacity.

5 Discussion

5.1 Initial selection drastically changed the community characteristics

One of the most substantial differences in community characteristics were between the inoculum and the reactors. It is not unreasonable to assume that the reactor environment contained fewer and different niches than the seawater collected from 70-meters depth. Because individual bacteria have defined abiotic and biotic conditions in which they are viable (62), changing the environmental conditions were expected to affect the community composition.

The initial cultivation resulted in all communities being r-selected the first couple of days, as 1) the carrying capacity was higher in the media than in the seawater and 2) the growth rates were set to a minimum of 1/day (K-selection) and 2/day (r-selection). During the initial days, these two factors resulted in a huge loss of diversity in the reactors compared to the inoculum (Figure 8). For example, on day 4 the average richness had dropped from 486 OTUs in the inoculum to 132 and 89 OTUs in the KR and RK groups, respectively. This reduction was likely caused by a washout of populations not able to divide above the minimum growth rate.

The media was filtered and had increased nutrient concentrations compared to the natural seawater. It was constructed to be carbon limiting, resulting in a surplus of both nitrate and phosphate, two components often causing nutrient limitation in seawater (63). Moreover, all micronutrients needed to sustain growth were present in the media. Hence, bacteria specialised in better utilisation and capturing of these elements (i.e. K-strategists) no longer had this advantage. Inoculation of planktonic bacteria in filtered seawater or enriched media is known to boost the population size of r-strategists, mainly from the class *Gamma-* and *Alpha-proteobacteria* (64–68). During the first couple of days, this shift in composition at the class level was observed; the abundance of *Gamma-proteobacteria* increased from approximately 10% in the inoculum to 70-90% in the reactor communities (Figure 9 a). Further, K-strategists generally have smaller cells than r-strategists (69), so the substantial increase in mean community cell size (Figure S2) is a clear indication of r-strategic dominance. The larger cell sizes were accompanied by increased mean RNA content (Figure 7), further demonstrating strong r-selection in all bioreactors the first couple of days.

Even though the richness dropped as a result of the inoculation, the overall richness in the reactors was higher, containing 876 OTUs not detected in the inoculum. This could be an

indication of populations exiting dormancy, which is an adaption many bacteria are capable of to decrease their metabolic requirements and survive in an unfavourable environment (70). It has been estimated that about 35% of marine aquatic bacteria are dormant at any time, as this adaption is beneficial in oligotrophic environments (70). It should be noted that some bias can arise prior to or during sequencing. It has previously been displayed that between replicates (same DNA extract, three different amplicon preparations), the low abundance OTUs changed (71). In the non-normalized dataset, 971 OTUs (of 1543 OTUs) had below 100 sequence reads. This high fraction of low-read-sequences could either indicate that these were erroneous sequences generated during library preparation or that they were part of the rare biosphere.

Natural seawater was used as inoculum as it was desired to perform the experiment on a diverse microbial community; which was accomplished. However, the observed community changes between the inoculum and reactor communities indicate that all reactors were r-selected during the first couple of days. Nevertheless, the K-selection regime was strong enough to create patterns that made the RK and KR groups different.

5.2 Communities were at carrying capacity in the first selection period, but not in the second

The bioreactors were operated at two different carrying capacities (high and low), in which the density should be higher for communities with high carrying capacity. It was expected to see a more defined difference between communities undergoing K-selection with different carrying capacity, as these had continuous dilution, and should, therefore, have relatively stable biomass over time. K-strategists have lower maintenance requirements, higher substrate affinity (72), and smaller cells (69) compared to r-strategists, which further enables them to sustain a higher population density at the same carrying capacity. As biomass is the density of individuals multiplied by their size (73), biomass is inadequately represented by bacterial density. To determine if the density was a good representation of the biomass the communities mean size (mean FSC) was quantified. The mean cell size (Figure S2) was higher in reactors undergoing r-selection, and thus the density does not fully represent the biomass. Biomass was estimated by multiplying the mean cell size with density and had the same development as density. A better representation of the biomass can be gained by determining the real cell sizes.

In the first selection period, KRH had on average 44% higher density than the KRL group ($p = 3.5 \times 10^{-5}$). Although not statistically significant, RKH had higher densities for the majority of the period compared to RKL. However, in the second selection period carrying capacity did not have an impact on density, and there were unexpected responses in bacterial density. Firstly, there was a peak in density in all reactors on day 34. Between day 32 and 34 there were difficulties with the cooling system, resulting in a 5 °C temperature increase. As growth rates increase with temperature (74), the density peak was likely due to the temperature increase.

Secondly, from day 40 all the communities stabilised around 10-20 million bacteria per millilitre. Natural seawater was used to make media, which was stored in the dark at room temperature. At day 30, the tank was refilled with fresh seawater, and new media made on day 32. Over time, the fraction of dissolved organic carbon in the seawater tank should decrease, as bacteria continue to respire and grow. As no other experimental changes were made this stabilisation remains unexplainable. If the media was the cause of the patterns observed in density, it is hard to determine how this affected the individual community. It does, however, especially in combination with the temperature problems, weaken the experimental control during the second period. Overall, it is hypothesised that the biomass of the K-selected communities was below carrying capacity in the second selection period. Nevertheless, there were patterns in community characteristics that appear unaffected by the possible media issue.

5.3 Community mean RNA content increased during r-selection

It was expected that r-selected communities contained more RNA on average than K-selected communities. This assumption was based on that 1) r-strategists contain more rrn-copies (69) and 2) the populations had a higher minimum growth rate (2/day vs 1/day). Carrying capacity, however, was not expected to affect the mean RNA, as populations received the same resources and had the same dilution method within the selection regime.

The data supported the hypothesis, and r-selected bioreactors contained 20% more RNA on average (Figure 7). An interesting observation was the peak in RNA content for the KR-reactors on day 29. The strong dilution increased the nutrients supplied per bacterium compared to when cultured under K-selection. Accompanied by the decrease in density and increase in cell size, these findings indicate that r-strategic populations in the community rapidly exploit their environment when conditions allow for it (e.g. dilution).

5.4 The diversity was affected by the selection regimes

K-selected communities displayed higher diversity and stability compared to r-selected ones in land-based aquaculture systems (17). Communities with high evenness have demonstrated increased resistance to stresses (i.e. more stable) (75), and therefore one can argue that K-selected communities should have higher evenness than r-selected ones. Overall, the effect of the selection did not cause clear differences in diversity between the RK and KR groups, except for richness. Further, the r- or K-selection switch did not alter the diversity within the KR and RK groups greatly. The diversity quite similar within the cultivation regimes over time (Figure 8).

Richness was the only diversity order in which there were considerable differences between the RK and KR groups. The mean richness in the KR groups for the entire experiment was 30% higher than in the RK group (Figure 8 a and Table 9), although at some time points (e.g. day 20) there were no differences between the groups. During r-selection, it was observed an increase in richness for both groups. The way r-selection was performed in this experiment could explain this increase. Right after the dilution, there were plenty of nutrients available to all bacteria in the community, regardless of whether the bacteria were K- or r-strategists. This relates to the intermediate disturbance hypothesis, that postulates that an ecosystem has maximal richness at intermediate disturbances (76). The increased richness could, therefore, be because more bacteria were able to divide faster than the dilution rate and reach abundances above a detectable level. The selection pressure was stronger during K-selection since the populations with the best fitness to the media gained higher abundances. It is hypothesised that the inoculation method affected the richness throughout the experiment. The 1:50 dilution of the inoculum, for the RK reactors, likely resulted in a lower potential OTU-inventory, while the continuous dilution of the inoculum, in the KR reactors, gave the populations more time to adapt to the media.

It has been shown in multiple studies of water microbiota in the rearing of marine larvae that richness is reduced as a consequence of r-selection (reviewed in (17)). As far as I know, no other studies have investigated the community dynamics when there is a rapid switch from r-to-K-selection or vice versa. As observed here, r-selection did not always decrease the richness. After the r-to-K-selection switch in the RK reactors, the richness remained lower than the r-selected communities (i.e. KR reactors). This observation contradicts the expected outcome of r- and K-selection based on previous studies but was likely caused by the constrained

environment in the reactors and a reduced initial OTU-inventory. In a land-based aquaculture system, the environment is more heterogeneous compared to the reactor environments. When such a system is K-selected, the diversity can be higher as more populations are able to exploit the niches they have high fitness to. However, here the main selection was for populations with high fitness to the restricted niches. There was observed a gradual increase in diversity of order one in the RK groups as a result of K-selection, and the group had 27% higher diversity of order one at day 50 compared to the KR group; however, the differences were non-significant. Therefore, based on these results, it cannot be concluded that the present K-selection yields higher diversity communities than r-selection. More defined patterns were discovered when investigating compositional changes in the communities.

5.5 The cultivation regimes structured community composition

The estimation of r- and K-strategists fractions in the communities indicated that r- and K-selection was successful (Figure 5). The fraction of K-strategists never reached the same magnitude as in the inoculum seawater, probably caused by the foothold the r-strategists got in the initial selection period. Nevertheless, the fraction of r-strategists decreased over time during K-selection and increased during r-selection. The undefined fraction of the communities often constituted a higher fraction than K-strategists, highlighting that the r- and K-strategy is a continuum, and not two absolute extremes (11,72).

The selection regimes affected the community composition the most as verified by all statistical tests and exploratory ordinations. In both the Bray-Curtis (abundance) and Sørensen (presence-absence) based plots, the communities became more similar within the r- or K-selection regimes (i.e. RK vs KR) over time (Figure 11 a and b). The samples clustered tighter for Sørensen similarity compared to Bray-Curtis, indicating that the same OTUs inhabited the communities within the same selection regime, but in different abundances (see for example week 4 and 6 in Figure 11 a and b). Both PERMANOVA and db-RDA analysis identified selection regime as the main factor affecting community structure. Even though the variation in community structure explained by the two methods were different, they have the same conclusion, emphasising the robustness of the conclusion.

On the class level, the general trend was that *Gamma-proteobacteria* increased in abundance during r-selection, and decreased during K-selection. *Alpha-proteobacteria* appeared to

compete with *Gamma-proteobacteria* because when one increased in abundance the other decreased. Some genera of *Alpha-proteobacteria* are classified as K-strategists, while others are classified as r-strategists (69,77).

A few genera dominated the communities (Figure 10). *Bacillus, Polaribacter, Roseovarius* and *Sulfitobacter* had higher abundances during K-selection within some, or all, of the selection regimes (Table 11 and Table S8). However, they are not all classified as K-strategists based on knowledge about the genera. For example, many marine *Bacillus* stains contain between six to fifteen copies of the rrn operon and have high growth rates (78). Further, *Roseovarius* and *Sulfitobacter* are members of the *Roseobacter* clade, which is found exclusively in saline environments (79,80). Members of this clade have large cells and genomes, versatile metabolic pathways and many respond rapidly to increased nutrient supply (81,82). Because of these characteristics, *Bacillus*, *Roseovarius* and *Sulfitobacter* are more likely r-strategists, even though they had higher abundances during K-selection.

The *Polaribacter* genus contains strains with different life strategies. Some are characterised as r-strategists (83), while others have a mixed lifestyle switching from an r- to K-strategy. This classification is on the basis that they grow fast during algal blooms and that they have highly specialised genomes (84). Because some *Polaribacter* strains contain 2-6 rrn copies, it is possible that this strain was, in fact, a K-strategist (85). Because OTU 5 belonging to *Polaribacter* increased in abundance in the second selection period in the RK group, it is hypothesised that this strain was more towards the r-strategic side on the r/K-continuum. This positioning is because it had relatively high abundance during r-selection and therefore needed to have a growth rate above 2/day.

To dominating genera that had overall higher abundances during r-selection were *Colwellia* and *Vibrio*. Two *Colwellia* OTUs (OTU 8 and 6) contributed with 15% to the variation in the whole Hellinger distance matrix (Table 10). OTU 6 had higher average abundance during r-selection in all cultivation regimes compared to during K-selection. On the contrary, OTU 8 had on average higher abundance during K-selection in all selection regimes (Table 11 and Table S8). The abundance of OTU 8 was only high for one replicate within RKH and RKL. This illustrates a case where SIMPER has identified an OTU with a large in-group variance to be significant for differences between groups. A GLM that accounted for the mean-variance relationship would likely not have identified OTU 8 to be significant for differences between

r- and K-selection within the RK group. This OTU was likely not a K-strategist even though its average abundance was higher during K-selection. Indeed, the *Colwellia* genus contains many strains that have nine rrn operons, indicating that the OTUs likely were r-strategists (15). *Vibrio* was identified as the genus that contributed the most to the variation of the whole dataset, Table 10, and also to the variation between r- and K-selection within cultivation regimes (Table 11). *Vibrio* is known to respond rapidly to increased nutrient supply and is characterised as an r-strategist (66,69).

Although some of the genera with high abundance during K-selection could be K-strategists, it is more likely that they were more towards the r-side of the r/K-continuum based on their ability to be at relatively high abundance during r-selection. The hypothesis that K-selection would yield communities dominated by K-strategists was therefore not supported. Even though, K-selection reduced the abundance of known r-strategists (e.g. *Vibrio*), indicating that there was more resource competition during K-selection.

5.6 Elimination of *Vibrio* supports that K-selection is favourable for raring of marine larvae

There was a burst in well-known r-strategists (e.g. *Vibrio*) when the natural seawater community was cultured in enriched media. During both selection periods, K-selection resulted in a strong reduction in the abundance of *Vibrio*. Many strains of *Vibrio* are known to be pathogenic (e.g. *V. anguillarum* and *V. ordalii*), and cause *Vibriosis* (87). It is therefore important to keep the abundance of *Vibrio* low in aquaculture facilities. During K-selection *Vibrio* decreased in abundance while *Roseovarius* increased (Figure 10). The competition between members of the *Roseobacter* clade and *Vibrio* has been described previously (88,89), and members of *Roseobacter* have been claimed to have probiotic properties in the rearing of marine larvae. On the other side, the strain *Roseovarius crassostreae* causes *Roseovarius* oyster disease (90,91). Rearing of shellfish, especially oysters, should therefore be performed with care and high pathogenic control in K-selected water, as it might potentially select for this bacterium.

Moreover, even though possible pathogenic bacteria were undetectable (e.g. *Vibrio* day 24 and 28 KRL), they can still be part of the rare biosphere and bloom when the conditions allow for it (e.g. increased carrying capacity). Such a bloom was observed and was higher in the KR

reactors with high carrying capacity. This difference highlights the importance of keeping carrying capacity stable throughout an aquaculture system to minimise r-selection, as previously highlighted by Attramadal et al. (92). The results in the present experiment support the claim that K-selected water has a protective effect in the rearing of marine larvae in land-based aquaculture facilities.

5.7 Successional patterns were related to cultivation media and selection regime

In this experiment, dispersal was assumed to be excluded as an assembly process, and speciation a more long-term process. Hence, drift and selection were expected to be the important processes for community assembly, and further for creating successional patterns. It is believed that there were two major selection drivers; the constant selection of populations able to survive in the media and competition.

The OTU inventory in the reactors were more similar than that of the inoculum (Figure S8) and gradually became more similar over time, as seen in the Sørensen (Figure 11 b) and UniFrac similarity based ordinations (Figure S6 b). This development indicated a continuous selection for populations best adapted to the experimental situation, resulting in an overall deterministic successional pattern related to the cultivation in enriched media rather than to the selection. However, the selection regimes further shaped the successional pattern, as the selection resulted in temporal differences in community composition (Figure 9). All replicates, except RKH1, were around 25% dissimilar when comparing adjacent sampling days, which appeared to be the successional rate in the communities. The selection regimes did not appear to affect the replacement of OTUs nor richness differences, as both contributed to the Sørensen dissimilarity (Figure 16). It was hypothesised that drift resulting in population loss should be of higher importance during r-selection. However, as the richness difference was unaffected by the selection, it appears that this process affected all reactors in the same way. This could be because the volume diluted during K-selection, although continuous, was twice as high than during r-selection. The only period where drift appeared to be a dominating process was during day 1 and 2. Between these sampling days, almost every reactor had richness difference as the primary contributor. Moreover, the richness was lower in the RK groups compared to the KR group (Figure 8 a). When OTUs are present at very low abundances (as in the inoculum), drift becomes a more important process for community assembly. The strong dilution likely washed out many OTUs or kept them at an undebatable abundance (4).

Further, drift resulting in abundance differences appeared to affect the reactors during r-selection more than during K-selection the first two weeks. This is supported by:

1) The compositional differences between replicates within a sampling day were more scattered during r-selection compared to K-selection (Figure 13). The periodical dilution, combined with a reduced richness, caused different OTUs to increase in abundance between the replicates. For example, two of the replicates in RKL had about 50% of OTU 1 (*Vibrio*) on day 2 while the third replica only had about 5%. On day 4, they had the same abundance again. This further resulted in;

2) That the variation in temporal stability was higher in the RK group (Figure 15).

It was hypothesised that K-selection would yield communities with higher temporal compositional stability compared to those r-selected, as previously reported (17). However, neither the Bray-Curtis nor the Sørensen dissimilarity between sampling days indicated this (Figure 14 and Figure 16). Whereas the stability for presence-absence (Sørensen) was unaffected by the selection regime, K-selection appeared to decrease the stability over time when abundance was incorporated (Bray-Curtis). The replicates of the KR reactors became more and more dissimilar from day 12 and towards day 28 (Figure S7), indicating that the populations were competing. This is further supported by that K-selection over time increased the diversity of order one and the evenness (Figure 8 b and c), and that there was a successional change in which OTUs that dominated.

Although drift and competition introduced some variation between the replicates, there was an overall deterministic successional pattern in the community composition related to the cultivation regimes.

5.8 Experimental improvements to the nutrient pulsing method

A novel methodology to quantify the fraction of r- and K-strategists was used in this thesis. The only divergence from the protocol was that we chose not to fixate the community samples with glyceraldehyde prior to performing flow cytometry. We run a preliminary test to determine the effect of fixation and found that it reduced bacterial counts (-9%) and increased the RNA content (FL1-A) (5%). This choice led to difficulty when 36 samples were processed during the nutrient pulsing (compared to the twelve samples usually run). During the first reactor

nutrient pulse (day 12), the last twelve samples were processed approximately 1.5 hours after the first twelve. Consequently, we observed an increase in cell numbers based on the time samples were processed. Therefore at the next nutrient pulsing, day 28, samples were taken out simultaneously and placed in the refrigerator with the idea that decreasing the temperature would decrease metabolism and hence growth. This action was an uninformed and unwise decision, as placing the bacteria in the fridge also induces cold shock responses, which are termination and inhibition of transcription and translation, increased folding of the DNA and alteration of the cell-membrane to mention a few (93). This resulted in a substantial decrease in density based on when the samples were measured, likely caused by a reduction in RNA content below the FL1-H threshold. Data strongly exhibiting low density or a high reduction in RNA content compared to the non-pulsed sample were removed from the dataset.

Further, it is regrettable that no preliminary tests were performed with our communities. During the method development, batch-culture communities were used to quantify the r- and K-strategists (33). In batch-culture, no nutrients are added and as a consequence growth rate is reduced (94). When starving cells are given a nutrient pulse, they upregulate their replication system, and r-strategists are expected to perform this upregulation faster (33,72). Measuring the RNA content before the cells divide will then indicate the fraction of r-strategists. As our experimental parameters (15 °C and seawater) were the same as in the protocol, sampling was performed after 2.5 and 7 hours. However, the communities in this project were actively dividing, and the response time to nutrient supply was therefore much faster. After 7 hours we observed up to 500% increase in cell density, and therefore the backup samples had to be used to quantify the strategists' fractions. After 2.5 hours, the density increase was not as substantial, usually below 20%, although an increase of 70% was observed for one sample.

Even though the experimental execution of the nutrient pulsing could have been improved, the data presented in this thesis were applicable (i.e. density increase was below 20%). Nutrient pulsing is a promising method to use for quantifications of a communities r- and K-strategist fractions, given that the samples are fixated and preliminary tests are performed.

5.9 Proposed new research questions and projects

That the differences in diversity between the cultivation regimes were so low was an unexpected observation, and it is clear that we do not fully comprehend the processes determining community assembly. First, to better determine the effect of drift and competition some of the communities could be kept at the same selection regime throughout the experiment, while others perform the switch. Because using a high diversity community introduces some uncertainty to the effect of drift (i.e. it can either cause population loss or reduction in abundance), the effect of drift is probably better investigated with mock communities of strains with known abundances. Second, to determine the processes that generate community patterns observed in aquaculture facilities, the effect of dispersal has to be determined. A similar design with a switch in r- and K-selection could be performed in lab-scale bioreactors but with the addition of particle-associated bacteria (e.g. by introducing Kaldnes carriers). In this way, dispersal between particle-associated and planktonic communities will take place. This kind of design allows for different questions than investigated here. For example; Does the particle-associated community function as a reservoir of K-strategists? Is the particle-associated community less affected by selection than the planktonic? Are there differences in the overall diversity between r- and K-selected reactors?

Thirdly, from my experiments, there is an indication for competition between *Colwellia*, *Vibrio* and *Roseovarius*. It would be interesting to determine how these three genera interact with each other. *Phaeobacter,* a close relative of *Roseovarius*, is known to produce a substance inhibiting strains of *Vibrio* (88,89). However, it is not known if *Roseovarius* also produces this substance and if it affects *Colwellia*. Further, is the competition density dependent? Determining which conditions that promote competition between them would be an interesting research project.

Lastly, is a K-selected community better at resisting invasion? During this experiment, *Vibrio* decreased in abundance during K-selection, but would the community be able to keep its abundance low with multiple exposures of *Vibrio?* If so, is the resistance dependent on the abundance of the invader? It has recently been shown that invasibility is determined by the phylogenetic relationship between the community members and the invader (95). Therefore, investigating the invasibility of r- and K-selected communities by different invaders (e.g. characteristic r- and K-strategists or pathogens) can be valuable to understand the conditions that facilitate invasion success and further to improve ecosystem management.

Conclusions

The temporal effect of r- and K-selection on bacterial community characteristics were investigated by flow cytometry and sequencing of the V3 and V4 region of the 16S-rRNA gene at two different carrying capacities over seven weeks. After four weeks, the selection regime was switched.

Over time, K-selection reduced the fraction of r-strategists and resulted in communities with higher densities and lower RNA content compared to those r-selected. The richness was highest in the communities that were K-selected in the first selection period. However, the difference between the two selection groups was mainly caused by OTUs with low abundance and is suspected to be because of the strong dilution of r-selected communities during the inoculation. The difference in diversity of order one between the selection groups was insignificant, although K-selection appeared to increase the evenness gradually. The *a priori* hypothesis that K-selection results in communities with higher diversity was therefore not supported in this experiment. However, the selection regimes affected community composition. K-selection gradually increased the abundance of *Alpha-proteobacteria*, while *Gamma-proteobacteria* was favoured during r-selection. All communities were dominated by a few genera, not uncommon when cultivating communities in enriched media. Genera increasing in abundance during r-selection responded stronger with high compared to low carrying capacity. *Roseovarius*, *Vibrio* and *Colwellia* appeared to compete with each other, with *Roseovarius* winning during K-selection at low carrying capacity. The constant selection of populations best adapted to the media resulted in an overall deterministic successional pattern that further was shaped based on the selection regimes. Drift affected the r-selected reactors more than K-selected during the first two weeks and resulted in more compositional differences between replicates. K-selection however, facilitated for competition that over time decreased the temporal stability.

Vibrio, which contains strains that are pathogenic to fish, was eliminated in all communities during K-selection. This reduction supports that biological control through K-selection is a valuable tool in land-based aquaculture systems.

References

1. Barton LL, Northup DE, Northup DE. Microbial Ecology. Hoboken, United States: Wiley; 2011. Available from: http://ebookcentral.proquest.com/lib/ntnu/detail.action?docID=697468
2. Verstraete W, Wittebolle L, Heylen K, Vanparys B, de Vos P, van de Wiele T, et al. Microbial Resource Management: The Road To Go for Environmental Biotechnology. Engineering in Life Sciences. John Wiley & Sons, Ltd; 2007;7:117–26. Available from: https://doi.org/10.1002/elsc.200620176
3. Nemergut DR, Schmidt SK, Fukami T, O'Neill SP, Bilinski TM, Stanish LF, et al. Patterns and processes of microbial community assembly. Microbiology and molecular biology reviews : MMBR. American Society for Microbiology; 2013;77:342–56. Available from: https://www.ncbi.nlm.nih.gov/pubmed/24006468
4. Vellend M. The theory of ecological communities. Princeton, New Jersey : Princeton University Press; 2016.
5. Vellend M. Conceptual synthesis in community ecology. The Quarterly review of biology. United States; 2010;85:183–206.
6. Atlas RM. Microbial ecology : fundamentals and applications. 4th ed. Bartha R, editor. Menlo Park, Calif: Benjamin Cummings; 1998.
7. Konopka A. What is microbial community ecology? The ISME Journal. International Society for Microbial Ecology; 2009;3:1223. Available from: http://dx.doi.org/10.1038/ismej.2009.88
8. Shade A, Peter H, Allison SD, Baho DL, Berga M, Burgmann H, et al. Fundamentals of microbial community resistance and resilience. Frontiers in Microbiology. 2012;3.
9. McCann KS. The diversity–stability debate. Nature. Macmillan Magazines Ltd.; 2000;405:228. Available from: http://dx.doi.org/10.1038/35012234
10. Di Bella JM, Bao Y, Gloor GB, Burton JP, Reid G. High throughput sequencing methods and analysis for microbiome research. Journal of microbiological methods. Netherlands; 2013;95:401–14.
11. Ho A, Di Lonardo DP, Bodelier PLE. Revisiting life strategy concepts in environmental microbial ecology. FEMS Microbiology Ecology. 2017;93:fix006–fix006.
Available from: http://dx.doi.org/10.1093/femsec/fix006
12. MacArthur RH. The theory of island biogeography. Wilson EO, editor. Princeton, N.J: Princeton University Press; 1967.
13. Andrews JH, Harris RF. r- and K-Selection and Microbial Ecology. In: Marshall KC, editor. Advances in Microbial Ecology. Boston, MA: Springer US; 1986. p. 99–147.
Available from: https://doi.org/10.1007/978-1-4757-0611-6_3
14. McArthur JV. Microbial Ecology: An Evolutionary Approach. Academic Press; 2006.
15. Roller BRK, Stoddard SF, Schmidt TM. Exploiting rRNA operon copy number to investigate bacterial reproductive strategies. Nature Microbiology. Macmillan Publishers Limited; 2016;1:16160. Available from: https://doi.org/10.1038/nmicrobiol.2016.160
16. Vadstein O. A strategy to obtain microbial control during larval development of marine fish. Fish Farmig Technology. A.A.Balkema Publishers; 1993;69–75.
Available from: https://ci.nii.ac.jp/naid/10004771809/en/
17. Vadstein O, Attramadal KJK, Bakke I, Olsen Y. K-Selection as Microbial Community Management Strategy: A Method for Improved Viability of Larvae in Aquaculture. Frontiers in Microbiology. 2018. p. 2730. Available from: https://www.frontiersin.org/article/10.3389/fmicb.2018.02730

18. Cook KL, Garland JL, Layton AC, Dionisi HM, Levine LH, Sayler GS. Effect of microbial species richness on community stability and community function in a model plant-based wastewater processing system. Microbial ecology. United States; 2006;52:725–37.
19. Andrews JH, Rouse DI. Plant Pathogens and the Theory of r- and K-Selection. The American Naturalist. [University of Chicago Press, American Society of Naturalists]; 1982;120:283–96. Available from: http://www.jstor.org/stable/2461055
20. De Schryver P, Vadstein O. Ecological theory as a foundation to control pathogenic invasion in aquaculture. The ISME Journal. 2014/06/03. Nature Publishing Group; 2014;8:2360–8. Available from: https://www.ncbi.nlm.nih.gov/pubmed/24892581
21. Vestrum RI, Attramadal KJK, Winge P, Li K, Olsen Y, Bones AM, et al. Rearing Water Treatment Induces Microbial Selection Influencing the Microbiota and Pathogen Associated Transcripts of Cod (Gadus morhua) Larvae. Frontiers in Microbiology. 2018. p. 851. Available from: https://www.frontiersin.org/article/10.3389/fmicb.2018.00851
22. Attramadal KJK, Truong TMH, Bakke I, Skjermo J, Olsen Y, Vadstein O. RAS and microbial maturation as tools for K-selection of microbial communities improve survival in cod larvae. Aquaculture. Elsevier; 2014;432:483–90.
Available from: https://www.sciencedirect.com/science/article/abs/pii/S004484861400283X
23. Guo C, Xia X, Pitta P, Herut B, Rahav E, Berman-Frank I, et al. Shifts in Microbial Community Structure and Activity in the Ultra-Oligotrophic Eastern Mediterranean Sea Driven by the Deposition of Saharan Dust and European Aerosols. Frontiers in Marine Science. 2016. p. 170.
Available from: https://www.frontiersin.org/article/10.3389/fmars.2016.00170
24. Barba C, Folch A, Gaju N, Sanchez-Vila X, Carrasquilla M, Grau-Martínez A, et al. Microbial community changes induced by Managed Aquifer Recharge activities: linking hydrogeological and biological processes. Hydrology and Earth System Sciences. Copernicus Publications; 2019;23:139–54. Available from: https://www.hydrol-earth-syst-sci.net/23/139/2019/
25. Adan A, Alizada G, Kiraz Y, Baran Y, Nalbant A. Flow cytometry: basic principles and applications. Critical Reviews in Biotechnology. Taylor & Francis; 2017;37:163–76.
Available from: https://doi.org/10.3109/07388551.2015.1128876
26. BD Accuri C6 plus optics. BD Biosciences. Available from: http://www.bdbiosciences.com/us/instruments/research/cell-analyzers/bd-accuri/m/1294932/features/optics
27. Biosciences B. Threshold and Analysis of Small Particles on the BD Accuri™ C6 Cytometer. 2012. Available from:http://static.bdbiosciences.com/documents/accuri/Accuri-TB-Threshold-and-Analysis-of-Particles.pdf
28. SYBR® Green II RNA Gel Stain. Molecular Probes. Available from: https://assets.thermofisher.com/TFS-Assets/LSG/manuals/mp07568.pdf
29. BD Accuri™ C6 Plus System User's Guide. BD Biosciences. 2016.
Available from: https://static.bdbiosciences.com/documents/BD-Accuri-C6-Plus-Users-Guide.pdf
30. Wilk MB, Sharipo SS. An analysis of variance test for normality (complete samples). Biometrika. 1965;52:591–611. Available from: https://doi.org/10.1093/biomet/52.3-4.591
31. Løvås GG. Sammenlikning av grupper. Statistikk for universiteter og høgskoler. 3. utg. Oslo: Universitetsforl;

2013.

32. Blazewicz SJ, Barnard RL, Daly RA, Firestone MK. Evaluating rRNA as an indicator of microbial activity in environmental communities: limitations and uses. The ISME Journal. 2013/07/04. Nature Publishing Group; 2013;7:2061–8. Available from: https://www.ncbi.nlm.nih.gov/pubmed/23823491
33. Brandsegg H. Evaluation of Two Culture-Independent Methods for Quantification of the Fraction of K-and r-Strategists within Water Microbial Communities. 2015.
34. Skjermo J, Salvesen I, Øie G, Olsen Y, Vadstein O. Microbially matured water: a technique for selection of a non-opportunistic bacterial flora in water that may improve performance of marine larvae. Aquaculture International. 1997;5:13–28.
35. Illumina Sequencing Technology. Illumina. 2010. Available from: https://www.illumina.com/documents /products/techspotlights/techspotlight_sequencing.pdf
36. Voelkerding K V, Dames SA, Durtschi JD. Next-Generation Sequencing: From Basic Research to Diagnostics. Clinical Chemistry. 2009;55:641 LP – 658.
Available from: http://clinchem.aaccjnls.org/content/55/4/641.abstract
37. Farrelly V, Rainey FA, Stackebrandt E. Effect of genome size and rrn gene copy number on PCR amplification of 16S rRNA genes from a mixture of bacterial species. Applied and Environmental Microbiology. 1995;61:2798 LP – 2801. Available from: http://aem.asm.org/content/61/7/2798.abstract
38. Yates M V, Nakatsu CH, Miller R V, Pillai SD. Manual of Environmental Microbiology. Washington, DC: ASM Press; 2016.
Available from: https://ebookcentral.proquest.com/lib/ntnu/reader.action?docID=353553&query=
39. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 2010;26:2460–1. Available from: http://dx.doi.org/10.1093/bioinformatics/btq461
40. Madigan MT, Martinko JM, Brock TD, Bender KS, Buckley DH, Stahl DA. Microbial evolution and systematics. Brock biology of microorganisms. 14th ed. Harlow: Pearson; 2015.
41. von Wintzingerode F, Gobel UB, Stackebrandt E. Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. FEMS microbiology reviews. England; 1997;21:213–29.
42. Chao A. Nonparametric Estimation of the Number of Classes in a Population. Scandinavian Journal of Statistics. [Board of the Foundation of the Scandinavian Journal of Statistics, Wiley]; 1984;11:265–70. Available from: http://www.jstor.org/stable/4615964
43. McMurdie PJ, Holmes S. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLOS ONE. Public Library of Science; 2013;8:e61217. Available from: https://doi.org/10.1371/journal.pone.0061217
44. Tuomisto H. An updated consumers guide to evenness and related indices. Oikos. 2012;121:1203–18. Available from: http://dx.doi.org/10.1111/j.1600-0706.2011.19897.x
45. Legendre P, Borcard D, Peres-Neto PR. Analysing Beta Diveristy: Partitioning the Spatial Variaton of Community Composition Data. Ecological Monographs. John Wiley & Sons, Ltd; 2005;75:435–50. Available from: https://doi.org/10.1890/05-0549
46. Lucas R, Groeneveld J, Harms H, Johst K, Frank K, Kleinsteuber S. A critical evaluation of ecological indices for the comparative analysis of microbial communities based on molecular datasets. FEMS Microbiology Ecology. England; 2017;93.

47. Tuomisto H. Commentary: do we have a consistent terminology for species diversity? Yes, if we choose to use it. Oecologia. Berlin/Heidelberg; 2011;167:903–11.

48. Tuomisto H. A consistent terminology for quantifying species diversity? Yes, it does exist. Oecologia. Berlin/Heidelberg; 2010;164:853–60.

49. Dixon P. VEGAN, a package of R functions for community ecology. Journal of Vegetation Science. John Wiley & Sons, Ltd (10.1111); 2003;14:927–30. Available from: https://doi.org/10.1111/j.1654-1103.2003.tb02228.x

50. Legendre P, De Cáceres M. Beta diversity as the variance of community data: dissimilarity coefficients and partitioning. Ecology Letters. John Wiley & Sons, Ltd (10.1111); 2013;16:951–63. Available from: https://doi.org/10.1111/ele.12141

51. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular biology and evolution. United States; 2016;33:1870–4.

52. Legendre P, Gallagher ED. Ecologically meaningful transformations for ordination of species data. Oecologia. 2001;129:271–80. Available from: https://doi.org/10.1007/s004420100716

53. Warton DI, Wright ST, Wang Y. Distance-based multivariate analyses confound location and dispersion effects. Methods in Ecology and Evolution. John Wiley & Sons, Ltd (10.1111); 2012;3:89–101. Available from: https://doi.org/10.1111/j.2041-210X.2011.00127.x

54. Legendre P. Interpreting the replacement and richness difference components of beta diversity. Global Ecology and Biogeography. John Wiley & Sons, Ltd (10.1111); 2014;23:1324–34. Available from: https://doi.org/10.1111/geb.12207

55. Stéphane D, David B, Guillaume B, Daniel B, Sylvie C, Guillaume G, et al. adespatial: Multivariate Multiscale Spatial Analysis. 2018.

56. Ramette A. Multivariate analyses in microbial ecology. Head I, editor. FEMS Microbiology Ecology. Blackwell Publishing Ltd; 2007;62:142–60.
Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2121141/

57. Anderson MJ. A new method for non-parametric multivariate analysis of variance. Austral Ecology. John Wiley & Sons, Ltd (10.1111); 2001;26:32–46.
Available from: https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

58. Borcard D, Gillet F, Legendre P. Numerical Ecology with R. Cham: Springer International Publishing, Cham; 2018.

59. Blanchet FG, Legendre P, Borcard D. Forward selection of explanatory variables. Ecology. Wiley Online Library; 2008;89:2623–32. Available from: https://doi.org/10.1890/07-0986.1

60. Shade A, Read JS, Welkie DG, Kratz TK, Wu CH, McMahon KD. Resistance, resilience and recovery: aquatic bacterial dynamics after water column disturbance. Environmental microbiology. England; 2011;13:2752–67.

61. Marzorati M, Wittebolle L, Boon N, Daffonchio D, Verstraete W. How to get more out of molecular fingerprints: practical tools for microbial ecology. Environmental microbiology. England; 2008;10:1571–81.

62. Pocheville A. The Ecological Niche: History and Recent Controversies. In: Heams T, Huneman P, Lecointre G, Silberstein M, editors. Handbook of Evolutionary Thinking in the Sciences. Dordrecht: Springer

Netherlands; 2015. p. 547–86. Available from: https://doi.org/10.1007/978-94-017-9014-7_26

63. Bristow LA, Mohr W, Ahmerkamp S, Kuypers MMM. Nutrients that limit growth in the ocean. Current Biology. 2017;27:R474–8.
Available from: http://www.sciencedirect.com/science/article/pii/S0960982217303287

64. Beardsley C, Pernthaler J, Wosniok W, Amann R. Are readily culturable bacteria in coastal North Sea waters suppressed by selective grazing mortality? Applied and Environmental Microbiology. American Society for Microbiology; 2003;69:2624–30. Available from: https://www.ncbi.nlm.nih.gov/pubmed/12732530

65. Fuchs BM, Zubkov M V, Sahm K, Burkill PH, Amann R. Changes in community composition during dilution cultures of marine bacterioplankton as assessed by flow cytometric and molecular biological techniques. Environmental Microbiology. Wiley Online Library; 2000;2:191–201.
Available from: https://doi.org/10.1046/j.1462-2920.2000.00092.x

66. Eilers H, Pernthaler J, Amann R. Succession of pelagic marine bacteria during enrichment: a close look at cultivation-induced shifts. Applied and Environmental Microbiology. 2000;66:4634–40.

67. Stevenson LH. A Case for Bacterial Dormancy in Aquatic Systems. Microbial Ecology. Springer; 1977;4:127–33. Available from: http://www.jstor.org/stable/4250517

68. Williams PJLB, Gray RW. Heterotrophic Utilization of Dissolved Organic Compounds in the Sea II. Observations on the Responses of Heterotrophic Marine Populations to Abrupt Increases in Amino Acid Concentration. Journal of the Marine Biological Association of the United Kingdom. 2009/05/11. Cambridge University Press; 1970;50:871–81.
Available from: https://www.cambridge.org/core/article/heterotrophic-utilization-of-dissolved-organic-compounds-in-the-sea-ii-observations-on-the-responses-of-heterotrophic-marine-populations-to-abrupt-increases-in-amino-acid-concentration/E7BB7FA8A41E6C63A21492C3E55CE00

69. Lauro FM, McDougald D, Thomas T, Williams TJ, Egan S, Rice S, et al. The genomic basis of trophic strategy in marine bacteria. Proceedings of the National Academy of Sciences. 2009;106:15527 LP – 15533. Available from: http://www.pnas.org/content/106/37/15527.abstract

70. Lennon JT, Jones SE. Microbial seed banks: the ecological and evolutionary implications of dormancy. Nature Reviews Microbiology. Nature Publishing Group, a division of Macmillan Publishers Limited. All Rights Reserved.; 2011;9:119. Available from: https://doi.org/10.1038/nrmicro2504

71. Poretsky R, Rodriguez-R LM, Luo C, Tsementzi D, Konstantinidis KT. Strengths and limitations of 16S rRNA gene amplicon sequencing in revealing temporal microbial community dynamics. PLOS ONE. United States; 2014;9:e93827.

72. John A, Harris R. r- and K-Selection and Microbial Ecology. Advances in Microbial Ecology. 1986;

73. Agusti S, Duarte CM, Kalff J. Algal cell size and the maximum density and biomass of phytoplankton1. Limnology and Oceanography. John Wiley & Sons, Ltd; 1987;32:983–6. Available from: https://doi.org/10.4319/lo.1987.32.4.0983

74. Ratkowsky DA, Olley J, McMeekin TA, Ball A. Relationship between temperature and growth rate of bacterial cultures. The Journal of Bacteriology. 1982;149:1.

75. Wittebolle L, Marzorati M, Clement L, Balloi A, Daffonchio D, Heylen K, et al. Initial community evenness favours functionality under selective stress. Nature. Macmillan Publishers Limited. All rights reserved; 2009;458:623. Available from: https://doi.org/10.1038/nature07840

76. Osman RW. Intermediate Disturbance Hypothesis. Encyclopedia of Ecology. Academic Press; 2008;1986–94. Available from: https://www.sciencedirect.com/science/article/pii/B9780080454054005103

77. Kirchman DL. Microbial ecology of the oceans, second edition. Wiley; 2008.

78. Valdivia-Anistro JA, Eguiarte-Fruns LE, Delgado-Sapién G, Márquez-Zacarías P, Gasca-Pineda J, Learned J, et al. Variability of rRNA Operon Copy Number and Growth Rate Dynamics of Bacillus Isolated from an Extremely Oligotrophic Aquatic Ecosystem. Frontiers in microbiology. 2015;6:1486.

79. Allgaier M, Uphoff H, Felske A, Wagner-Döbler I. Aerobic Anoxygenic Photosynthesis in Roseobacter Clade Bacteria from Diverse Marine Habitats. Applied and Environmental Microbiology. 2003;69:5051 LP – 5059.
Available from: http://aem.asm.org/content/69/9/5051.abstract

80. Buchan A, González JM, Moran MA. Overview of the Marine Roseobacter Lineage. Applied and Environmental Microbiology. American Society for Microbiology Journals; 2005;71:5665–77. Available from: https://aem.asm.org/content/71/10/5665

81. Luo H, Csűros M, Hughes AL, Moran MA. Evolution of Divergent Life History Strategies in Marine Alphaproteobacteria. Azam Jonathan FZ, editor. mBio. 2013;4:e00373-13. Available from: http://mbio.asm.org/content/4/4/e00373-13.abstract

82. Moran MA, Belas R, Schell MA, González JM, Sun F, Sun S, et al. Ecological Genomics of Marine Roseobacters. Applied and Environmental Microbiology. 2007;73:4559 LP – 4569. Available from: http://aem.asm.org/content/73/14/4559.abstract

83. Cottrell MT, Kirchman DL. Transcriptional Control in Marine Copiotrophic and Oligotrophic Bacteria with Streamlined Genomes. Applied and environmental microbiology. United States; 2016;82:6010–8.

84. Xing P, Hahnke RL, Unfried F, Markert S, Huang S, Barbeyron T, et al. Niches of two polysaccharide-degrading Polaribacter isolates from the North Sea during a spring diatom bloom. The ISME Journal. 2014/12/05. Nature Publishing Group; 2015;9:1410–22.
Available from: https://www.ncbi.nlm.nih.gov/pubmed/25478683

85. Stoddard SF, Smith BJ, Hein R, Roller BRK, Schmidt TM. rrnDB: improved tools for interpreting rRNA gene abundance in bacteria and archaea and a new foundation for future development. Nucleic acids research. England; 2015;43:D593-8.

86. Singer E, Webb EA, Nelson WC, Heidelberg JF, Ivanova N, Pati A, et al. Genomic potential of Marinobacter aquaeolei, a biogeochemical "opportunitroph". Applied and Environmental Microbiology. 2011;77:2763–71.

87. Bornø G, Lie Linaker M. The Health Situation in Norwegian Aquaculture 2014. Fiskehelserapporten (Fish Health Report) 2014. 2014;8–14. Available from: http://www.vetinst.no/eng/Publications/Fish-Health-Report/Fish-Health-Report-2014

88. D'Alvise PW, Lillebø S, Prol-Garcia MJ, Wergeland HI, Nielsen KF, Bergh Ø, et al. Phaeobacter gallaeciensis reduces Vibrio anguillarum in cultures of microalgae and rotifers, and prevents vibriosis in cod larvae. PLOS ONE. Public Library of Science; 2012;7:e43996–e43996.
Available from: https://www.ncbi.nlm.nih.gov/pubmed/22928051

89. D'Alvise PW, Lillebø S, Wergeland HI, Gram L, Bergh Ø. Protection of cod larvae from vibriosis by Phaeobacter spp.: A comparison of strains and introduction times. Aquaculture. Elsevier; 2013;384–387:82–

6. Available from: https://www.sciencedirect.com/science/article/pii/S0044848612007351
90. Travers M-A, Boettcher Miller K, Roque A, Friedman CS. Bacterial diseases in marine bivalves. Journal of Invertebrate Pathology. 2015;131:11–31.
Available from: http://www.sciencedirect.com/science/article/pii/S0022201115001354
91. Boettcher KJ, Geaghan KK, Maloy AP, Barber BJ. Roseovarius crassostreae sp. nov., a member of the Roseobacter clade and the apparent cause of juvenile oyster disease (JOD) in cultured Eastern oysters. International journal of systematic and evolutionary microbiology. England; 2005;55:1531–7.
92. Attramadal KJK, Minniti G, Øie G, Kjørsvik E, Østensen M-A, Bakke I, et al. Microbial maturation of intake water at different carrying capacities affects microbial control in rearing tanks for marine fish larvae. Aquaculture. 2016;457:68–72.
Available from: http://www.sciencedirect.com/science/article/pii/S0044848616300631
93. Phadtare S. Recent developments in bacterial cold-shock response. Current issues in molecular biology. England; 2004;6:125–36.
94. Bren A, Hart Y, Dekel E, Koster D, Alon U. The last generation of bacterial growth in limiting nutrient. BMC systems biology. England; 2013;7:27.
95. Kinnunen M, Dechesne A, Albrechtsen H-J, Smets BF. Stochastic processes govern invasion success in microbial communities when the invader is phylogenetically close to resident bacteria. The ISME Journal. 2018;12:2748–56. Available from: https://doi.org/10.1038/s41396-018-0202-1
96. Smith WL, Chanley MH. Culture of marine invertebrate animals : proceedings of the conference on culture of marine invertebrate animals. New York: Plenum Press; 1975.
97. Bragt J van, Wageningen. L, Tuinbouwplantenteelt. A. Effects of sterilization on components in nutrient media. Wageningen: H. Veenman & Zonen; 1971.

List of appendices

Appendix A Cultivation medium recipes

f/2 media is a general growth medium for marine bacteria and algae (96). f/2 media enriched with M65 was used as a the cultivation media. The components and the amounts added per litre, see Table S1, were added to 0.2 μm filtered seawater and autoclaved at 121 °C. A temperature sensor was placed in a similar container with equal volume during autoclavation when volume was over one litre. Note; for future experiments, media components should be added after seawater has been filtered and autoclaved, as sugar components can caramelize and vitamin B be inactivated (97). M65 stock solution was prepared by combining the component presented in Table S2 to distilled water prior to filtration at 0.2 μm. The stock solution was distributed to Eppendorf tubes and stored at -20°C to reduce the risk of contamination. The f/2 trace metal- and vitamin solution recipes are presented in Table S3 and Table S4, respectively. It is recommended to prepare media and keep it at room temperature at least three days prior to introducing it to the system in order to test for potential contamination.

Table S1: Components and their respective volumes added to one litre prefiltered seawater.

Component	**Concentration**	**Add per litre medium**	**Final concentration**
M65 stock solution	150 g/L	33.3 μL	5 mg/L
	150 g/L	6.66 μL	1 mg/L
f/2 medium			
$NaNO_3$	75 g/L	20 μL	1.5 mg/L
NaH_2PO_4 *H_20	5 g/L	20 μL	0.1 mg/L
Na_2SiO_3 *9 H_20	30 g/L	20 μL	0.6 mg/L
Trace metal solution	7.7 g/L	20 μL	0.2 mg/L
Vitamin solution	0.2 g/L	10 μL	2 ng/L
0.2 μm filtrated seawater		1 L	

Table S2: M65 concentrated stock solution recipe, yielding a final concentration of 150 g/l.

Component	**Amount**	**Final concentration**
Yeast extract	5 g	50 g/L
Bacteriological peptone	5 g	50 g/L
Tryptone	5 g	50 g/L
Distilled water (Milli-Q)	100 mL	

Table S3: f/2 trace metal solution recipe. The components below were dissolved in 950 mL of distilled water, before the volume is brought up to 1 L, giving a final concentration of 7.7 g/L.

Component	Stock solution (g/L)	Add per litre	Final concentration
$FeCl_3 * 6H_2O$	-	3.15 g	3150 mg/L
$Na_2EDTA * 2H_2O$	-	4.36 g	4360 mg/L
$MnCl_2 * 4H_2O$	180	1 mL	180 mg/L
$ZnSO_4 * 7H_2O$	22	1 mL	22 mg/L
$CoCl_2 * 6H_2O$	10	1 mL	10 mg/L
$CuSO_4 * 5H_2O$	9.8	1 mL	9.8 mg/L
$Na_2MoO_4 * 2H_2O$	6.3	1 mL	6.3 mg/L

Table S4: Vitamin solution recipe. The components were dissolved in 950 mL distilled water before the volume was brought up to 1 L, giving a final concentration of 0.2 g/L.

Component	Stock solution (g/L)	Quantity used	Final concentration
Thiamine * HCl (vit B)	-	200 mg	200 mg/L
Biotin (vit. H)	1.0	1 mL	1.0 mg/L
Cyanocobalium (vit B_{12})	1.0	1 mL	1.0 mg/L

Appendix B Tris-EDTA-buffer recipe

Tris-EDTA buffer (TE-buffer) was made by combining the components presented in Table S5. The TE-buffer was diluted 1:10 in 0.2 μm distilled water to obtain a 1/10 TE-buffer and used to dilute SYBR-green II.

Table S5: Components and amounts used to make 1x Tris-EDTA buffer.

Component	Amounts	Final concentration
2 M Tris-HCl pH 7.5	2.5 mL	10.0 mM
0.5 M EDTA pH 8.0	1.0 mL	1.0 mM
dH_2O	496.6 mL	-

Appendix C Nutrient pulse solution

A nutrient pulse solution was prepared as described in Table S6, filtered at 0.2 μm and aliquoted to Eppendorf tubes prior to storage at -20 °C.

Table S6: Recipe for the nutrient pulse solution. The components were dissolved in 100 mL of distilled water.

Component	**Concentration**	**Amount**	**Final concentration**
M65 stock solution	150 g/L	166.7 µl	53.6 g/L
f/2 trace metal solution	7.7 g/L	100 µl	1.65 g/L
$NaH_2PO_4 \cdot H_2O$	5 g/L	100 µl	1.1 g/L
NH_4Cl	0.9 M	100 µl	0.19 M

Appendix D M65 agar media

M65 agar was prepared by combining the components in Table S7, followed by autoclaving at 121 °C for 20 minutes. The agar was set to cool until the solution reached about 60 degrees, and then poured into standard agar plates.

Table S7: M65 agar recipe yielding 1 litre of cultivation agar.

Component	Amount
Yeast extract	0.5 g
Bacteriological peptone	0.5 g
Tryptone	0.5 g
Distilled water (Milli-Q)	200 mL
0.2 μm filtered seawater	800 mL
Agar	15 g

Appendix E Qiagen DNeasy PowerSoil Kit Protocol

Quick-Start Protocol June 2016

DNeasy® PowerSoil® Kit

The DNeasy PowerSoil Kit can be stored at room temperature (15–25°C) until the expiry date printed on the box label.

Further information

- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Perform all centrifugation steps at room temperature (15–25°C).
- If Solution C1 has precipitated, heat at 60°C until precipitate dissolves.
- 2 ml collection tubes are provided.

1. Add 0.25 g of soil sample to the PowerBead Tube provided. Gently vortex to mix.
2. Add 60 µl of Solution C1 and invert several times or vortex briefly.
   Note: Solution C1 may be added to the PowerBead tube before adding soil sample
3. Secure PowerBead Tubes horizontally using a Vortex Adapter tube holder (cat. no. 13000–V1–24).
4. Vortex at maximum speed for 10 min.
   Note: If using the 24-place Vortex Adapter for more than 12 preps, increase the vortex time by 5–10 min.
5. Centrifuge tubes at 10,000 x *g* for 30 s.
6. Transfer the supernatant to a clean 2 ml collection tube.
   Note: Expect between 400–500 µl of supernatant. Supernatant may still contain some soil particles.
7. Add 250 µl of Solution C2 and vortex for 5 s. Incubate at 2–8°C for 5 min.

Sample to Insight

Note: You can skip the 5 min incubation. However, if you have already validated the DNeasy PowerSoil extractions with this incubation we recommend you retain the step.

8. Centrifuge the tubes for 1 min at 10,000 x *g*.
9. Avoiding the pellet, transfer up to 600 µl of supernatant to a clean 2 ml collection tube.
10. Add 200 µl of Solution C3 and vortex briefly. Incubate at 2–8°C for 5 min.

    Note: You can skip the 5 min incubation. However, if you have already validated the PowerSoil extractions with this incubation we recommend you retain the step.
11. Centrifuge the tubes for 1 min at 10,000 x *g*.
12. Avoiding the pellet, transfer up to 750 µl of supernatant to a clean 2 ml collection tube.
13. Shake to mix Solution C4 and add 1200 µl to the supernatant. Vortex for 5 s.
14. Load 675 µl onto an MB Spin Column and centrifuge at 10,000 x *g* for 1 min. Discard flow through.
15. Repeat step 14 twice, until all of the sample has been processed.
16. Add 500 µl of Solution C5. Centrifuge for 30 s at 10,000 x *g*.
17. Discard the flow through. Centrifuge again for 1 min at 10,000 x *g*.
18. Carefully place the MB Spin Column into a clean 2 ml collection tube. Avoid splashing any Solution C5 onto the column.
19. Add 100 µl of Solution C6 to the center of the white filter membrane. Alternatively, you can use sterile DNA-Free PCR Grade Water for this step (cat. no. 17000–10).
20. Centrifuge at room temperature for 30 s at 10,000 x *g*. Discard the MB Spin Column. The DNA is now ready for downstream applications.

    Note: Solution C6 is 10 mM Tris-HCl, pH 8.5. We recommend storing DNA frozen (–20° to –80°C) as Solution C6 does not contain EDTA. To concentrate DNA see the Hints & Troubleshooting Guide.

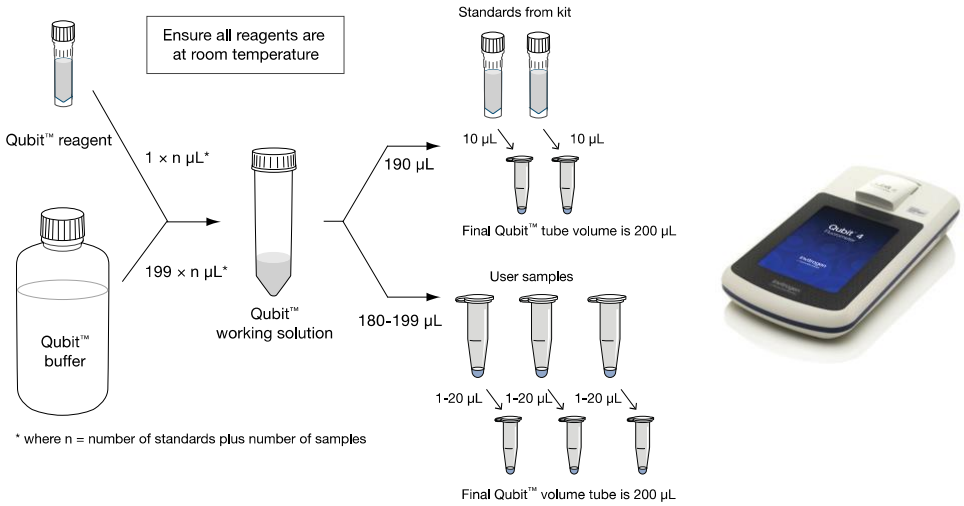
Appendix F Qubit 4 protocol

invitrogen

1. Set up two assay tubes for the standards (three for the protein or RNA IQ assay) and one assay tube for each sample.
2. Prepare the Qubit™ working solution by diluting the Qubit™ reagent 1:200 in Qubit™ buffer. Prepare 200 µL of working solution for each standard and sample.†
3. Prepare the assay tubes* according to the table below.
4. Vortex all tubes for 2–3 seconds.
5. Incubate the tubes for 2 minutes at room temperature (15 minutes for the Qubit™ protein assay).
6. Insert the tubes in the Qubit™ Fluorometer and take readings. For detailed instructions, refer to the Qubit™ Fluorometer manual.

	Standard assay tubes	User sample assay tubes
Volume of working solution† (from step 2) to add	190 µL	180–199 µL
Volume of Standard (from kit) to add	10 µL	—
Volume of user sample to add	—	1–20 µL
Total Volume in each assay tube	200 µL	200 µL

† Qubit 1X dsDNA Working Solution needs to be prepared only once, and subsequently can be stored as a ready-to-use working solution.
* Use only thin-wall, clear 0.5 mL PCR tubes. Acceptable tubes include Qubit™ assay tubes (set of 500, Cat. No. Q32856).



Find out more at thermofisher.com/qubit

Appendix G SequalPrep Normalization Plate (96) kit protocol

invitrogen™

SequalPrep™ Normalization Plate (96) Kit

Catalog no: A10510-01 **Store at room temperature (15–30ºC)**

Contents and Storage

The components included with the SequalPrep™ Normalization Plate (96) Kit are listed in the table below. Sufficient reagents are included to perform 10 × 96 purification/normalization reactions. Upon receipt, **store all components at room temperature (15–30ºC)**. Store plates for up to 6 months.

Components	Quantity
SequalPrep™ Normalization Plate (96)	2 bags of 5 plates each
SequalPrep™ Normalization Binding Buffer	40 ml
SequalPrep™ Normalization Wash Buffer	50 ml
SequalPrep™ Normalization Elution Buffer (10 mM Tris-HCl, pH 8.5)	40 ml

Description

The SequalPrep™ Normalization Plate Kit allows simple, one-step, high-throughput amplicon purification and normalization of PCR product concentration (2–3 fold range) via a limited binding capacity solid phase. Each well of the SequalPrep™ Normalization Plate can bind and elute ~25 ng of PCR amplicon. Eluted PCR amplicon can be subsequently pooled and subjected to a variety of massively parallel sequencing analyses. The SequalPrep™ Normalization Plate is compatible with any automated liquid handling workstations without the need for shakers, magnets, or vacuum. The SequalPrep™ Normalization Plate Kit when used with SequalPrep™ Long PCR Kit provides a complete PCR enrichment and amplicon normalization system that is designed to complement amplicon sequencing workflows such as next-generation sequencing.

The conventional next generation sequencing workflows require laborious sample prep methods consisting of amplicon purification, quantitation, and manual normalization to adjust amplicon concentration. The SequalPrep™ Normalization Plate Kit eliminates the tedious amplicon quantitation and manual normalization steps.

SequalPrep™ Normalization Plate Kits utilize ChargeSwitch® Technology that provides a switchable surface charge depending on the pH of the surrounding buffer to facilitate nucleic acid purification. Under low pH conditions, the positive surface charge of the ChargeSwitch® coating binds the negatively charged nucleic acid backbone. Proteins and other contaminants (such as short oligonucleotide primers) are not bound and are simply washed away.

System Overview

The SequalPrep™ Normalization Plate Kit is a solid phase, high-throughput amplicon purification and normalization system in a 96-well plate format. PCR products (5–25 µl) are added to a SequalPrep™ Normalization Plate well and mixed with the Binding Buffer. DNA binding to the plate is performed at room temperature for 1 hour. The wells are washed with Wash Buffer to efficiently remove contaminants. Purified PCR products are eluted using 20 µl Elution Buffer at normalized concentrations.

System Specifications

Starting Material:	At least 250 ng PCR product (amplicon) per well
DNA Fragment Size:	100 bp to 20 kb
Elution Volume:	20 µl
DNA Yield:	Up to 25 ng per well
Normalization Range:	2–3-fold
Plate Dimensions:	Standard SBS (Society for Biomolecular Screening) footprint, semi-skirted 96-well plate
Plate Capacity:	0.2 ml

Accessory Products

The following products may be used with the SequalPrep™ Normalization Plate Kit. For details, visit www.invitrogen.com.

Product	Quantity	Catalog no.
SequalPrep™ Normalization Wash Buffer	4 × 50 ml	A10510-03
SequalPrep™ Long PCR Kit with dNTPs	1,000 units	A10498
Platinum® PCR Supermix	100 reactions	11306-016
Platinum® PCR Supermix High Fidelity	100 reactions	12532-016
Quant-iT™ PicoGreen® dsDNA Assay Kit	1 kit	P7589
PureLink™ Foil Tape	50 tapes	12261-012
E-Gel® *96* gels 1% (or 2%)	8 gels	G7008-01 (G7008-02)

Part no: 100003531 Rev. date: 5 May 2008

General Guidelines

- Wear a laboratory coat, disposable gloves, and eye protection when handling reagents and plate.
- Always use proper aseptic techniques when working with DNA and use only sterile, DNase-free tips to prevent DNase contamination.
- If you are using only part of the plate for DNA purification, cover unused wells with the Plate Seal and leave them attached while purifying DNA in the other wells. The plates can be stored at room temperature for up to 6 months.
- The SequalPrep™ Normalization Plates are compatible for use with automated liquid handling workstation; the workstation must be capable of handling and manipulating 96-well plates.
- If you are using automated liquid handling workstations for purification, you may need additional Wash Buffer depending on your type of workstation. See previous page for Wash Buffer ordering information.

Generating PCR Amplicon

You can generate the PCR amplicon using a method of choice. General recommendations for generating PCR amplicons are listed below:

- To obtain the best results, we recommend using the SequalPrep™ Long PCR Kit with dNTPs (page 1) which provides a robust system for long-range, high-fidelity PCR for use in next-generation sequencing applications.
- Other commercially available PCR supermixes and enzymes such as Platinum® PCR Supermix (page 1), Platinum® PCR Supermix High Fidelity (page 1), or equivalent are suitable for use.
- Perform PCR in a separate plate. **Do not** use the SequalPrep™ Normalization Plate to perform PCR.
- You need at least 250 ng amplicon per well to use with the SequalPrep™ Normalization Plate (see below).

Sample Amount

To achieve robust normalization, we recommend adding at least 250 ng/well of amplicon. This input amount is easily achieved using only a fraction of most PCR amplification reactions. An average efficiency PCR (20 µl reaction volume) produces product in the range of 25–100 ng/µl, allowing you to purify 5–10 µl using the SequalPrep™ system.

Elution Options

Depending on the nature of the downstream application and target nucleic acid concentrations desired, the SequalPrep™ kit offers the flexibility to elute purified DNA in a variety of options.

The **standard elution** method described in the protocol below is designed to elute purified DNA from each well using 20 µl elution volume to obtain each amplicon at a concentration of 1–2 ng/µl.

The **optional sequential elution** method is designed to sequentially elute multiple rows or columns using the same 20 µl of elution buffer to obtain higher amplicon concentrations. The amplicon concentrations will be additive as sequential wells are eluted. For example, dispense 20 µl of elution buffer into the first column (A1–H1), mix well, and incubate for 5 minutes at room temperature. Then, simply move this column of elution buffer to the next column (A2–H2), and again incubate for 5 minutes. Continue this step to obtain your specific elution needs for the downstream application of choice.

Materials Needed

- PCR reactions containing amplicons of the desired length (see **Generating PCR Amplicon**, above)
- DNase-free, aerosol barrier pipette tips
- *Optional:* automated liquid handling workstation capable of handling and manipulating 96-well plates
- *Optional:* PureLink™ Foil Tape (see previous page)

Binding Step

1. Transfer the desired volume of PCR product (5–25 µl PCR reaction mix, at least 250 ng amplicon/well) from the PCR plate into the wells of the SequalPrep™ Normalization plate.
2. Add an equivalent volume of SequalPrep™ Normalization Binding Buffer.
   For example: To purify 10 µl of PCR product, add 10 µl SequalPrep™ Normalization Binding Buffer.
3. Mix completely by pipetting up and down, or seal the plate with PureLink™ Foil Tape (page 1), vortex to mix, and briefly centrifuge the plate.
4. Incubate the plate for 1 hour at room temperature to allow binding of DNA to the plate surface. Mixing is not necessary at this stage.
   Note: Incubations longer than 60 minutes do not improve results. However, depending on your workflow you may perform overnight incubation at room temperature for the binding step.
5. **Optional:** If >25 ng DNA/well yield is desired, transfer the amplicon/Binding Buffer mixture from Step 4 to another, fresh well/plate to sequentially bind more DNA. Perform DNA binding at room temperature for 1 hour.
   Note: After binding is complete, you can remove the amplicon/Binding Buffer mixture from the well and store at –20°C for up to 30 days to perform additional purifications at a later time.
6. Proceed to **Washing Step**, next page.

Washing Step

1. Aspirate the liquid from wells. Be sure not to scrape the well sides during aspiration.

   Note: If you wish to store the amplicon/Binding Buffer mixture for additional purifications at a later time, aspirate the liquid from wells into another plate and store at –20ºC for up to 30 days.

2. Add 50 µl SequalPrep™ Normalization Wash Buffer to the wells. Mix by pipetting up and down twice to improve removal of contaminants.

3. Completely aspirate the buffer from wells and discard.

   To ensure complete removal of wash buffer and maximize elution efficiency, you may need to invert and tap the plate on paper towels depending on the pipetting technique or instrument used. A small amount of residual Wash Buffer (1–3 µl) is typical and does not affect the subsequent elution or downstream applications.

4. Proceed to **Elution Step**, below.

Elution Step

Review **Elution Options** (previous page).

1. Add 20 µl SequalPrep™ Normalization Elution Buffer to each well of the plate.

   Note: Do not use water for elution. If you need to elute in any other buffer, be sure to use a buffer of pH 8.5–9.0. If the pH of the buffer is <8.5, the DNA will not elute efficiently.

2. Mix by pipetting up and down 5 times or seal the plate with PureLink™ Foil Tape (page 1), vortex to mix, and briefly centrifuge the plate. Ensure that the buffer contacts the entire plate coating (up to 20 µl level).

3. Incubate at room temperature for 5 minutes.

4. Transfer and pool the purified DNA as desired or store the eluted DNA at 4°C (short-term storage) or –20°C (long-term storage) until further use.

Expected Yield and Concentration

The expected DNA concentration is 1–2 ng/µl when using 20 µl elution volume. The expected DNA yield is ~25 ng/well normalized.

Optional: DNA Quantitation

The SequalPrep™ Normalization Plate Kit is designed to eliminate the quantitation and manual dilution steps typically performed for normalization in next-generation sequencing workflows. You can pool the eluted amplicon and use the pooled amplicons directly for your downstream applications without DNA quantitation.

However, if your downstream application requires DNA quantitation, you may determine the yield of the eluted amplicon using Quant-iT™ PicoGreen® dsDNA Assay Kit (page 1). We **do not** recommend using UV spectrophotometric measurements (A_{260}/A_{280} nm), as this method is inaccurate for low DNA concentrations.

Downstream Applications

The SequalPrep™ Normalization Plate Kit is designed to produce purified PCR products with normalized concentrations and substantially free of salts and contaminating primers. PCR amplicons purified from this system can be used individually or pooled in any downstream application for which normalization is an important sample preparation criterion such as next generation sequencing applications.

Pooled amplicons purified using the SequalPrep™ Normalization Plate Kit have produced successful data from massively parallel sequencing-by-synthesis on the Illumina/Solexa Genome Analyzer indicating that the amplicon purity is suitable for other next-generation sequencing platforms (Roche/454 FLX, Applied Biosystems SOLiD™ system). For detailed sample preparation guidelines, refer to the instrument manufacturer's recommendations.

Continued on next page

Appendix H Supplementary figures and tables

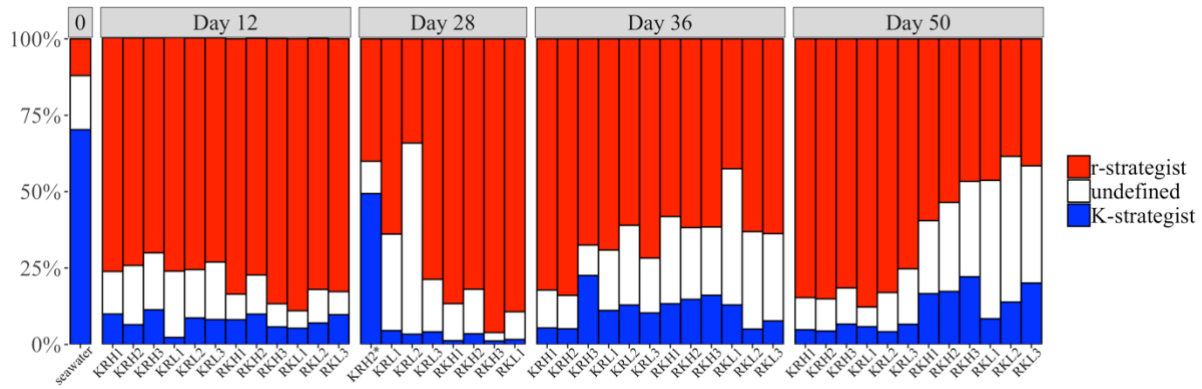


Figure S1: Fraction of r- and K-strategists in community samples determined by nutrient pulsing. KRH and RKL is only represented by one replica on day 28. RK: r-selection from day 0-28, K-selection from day 28-50. KR: K-selection from day 0-28, r-selection from day 28-50. H: high carrying capacity, L: low carrying capacity.

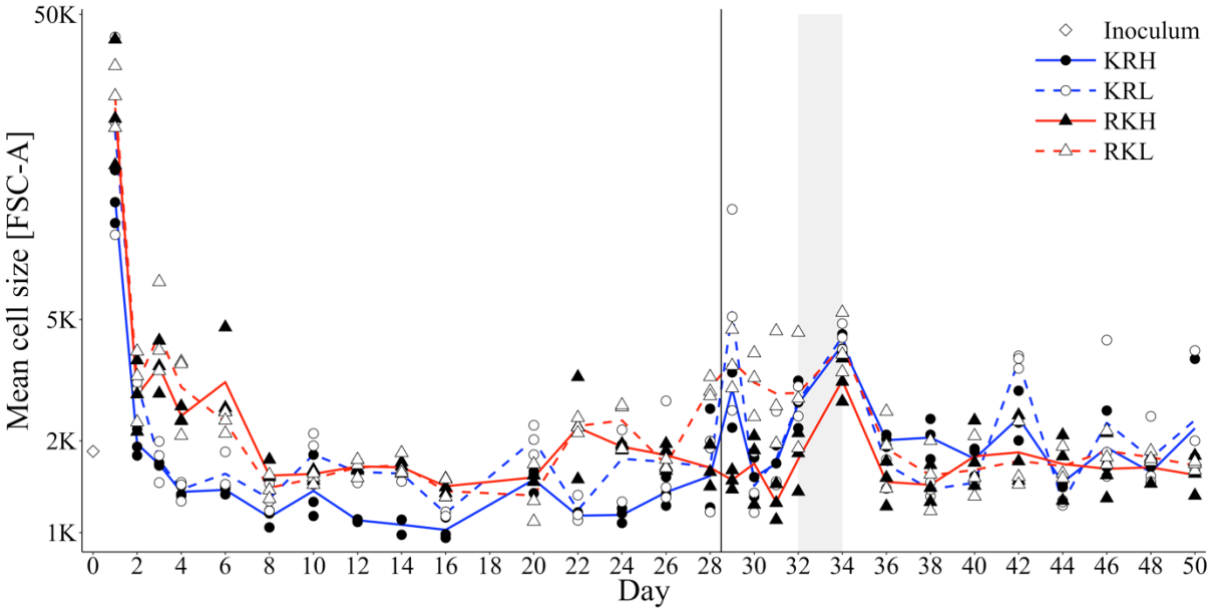


Figure S2: Mean cell size, measured as FSC-A, for all bioreactors and the inoculum natural seawater. Notice FSC-A axis is with log scale. Inoculum (◇): natural seawater. RK(△ and ▲): r-selection from day 0-28, K-selection from day 28-50. KR(○ and ●): K-selection from day 0-28, r-selection from day 28-50. H (filled): high carrying capacity, L (blank): low carrying capacity.

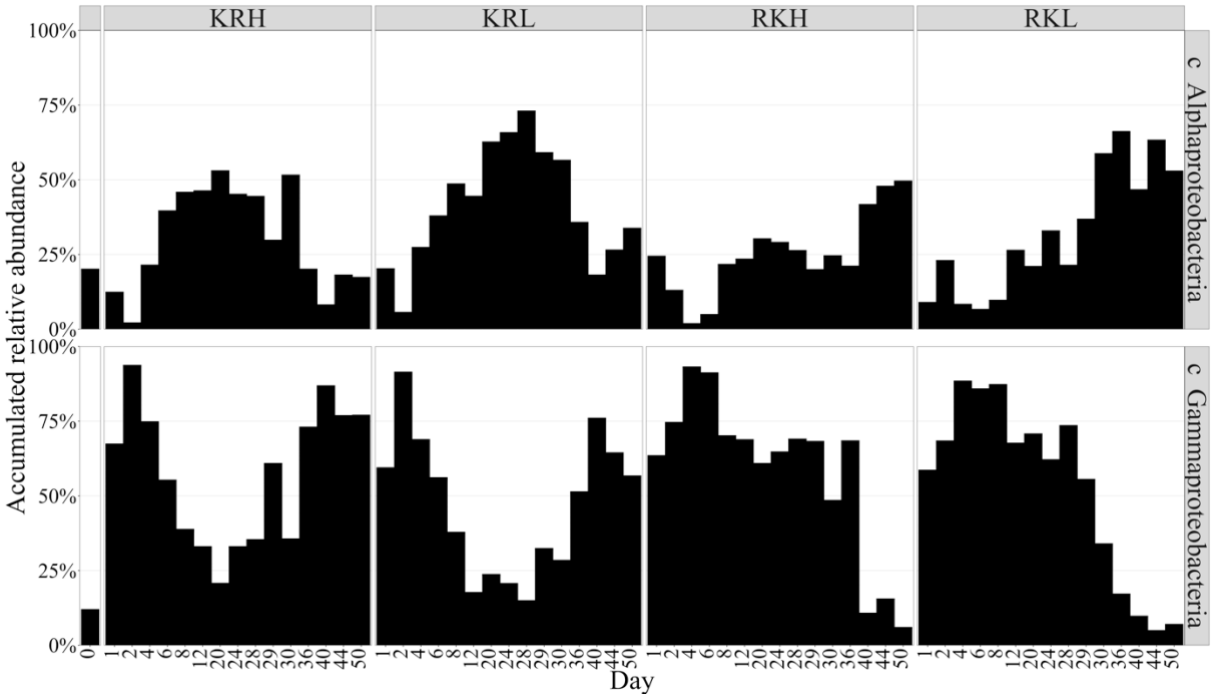


Figure S3: Accumulated relative abundance of *Gamma-proteobacteria* and *Alpha-proteobacteria* for the cultivation regimes (n=3) over time. RK: r-selection from day 0-28, K-selection from day 28-50. KR: K-selection from day 0-28, r-selection from day 28-50. H: high carrying capacity, L: low carrying capacity.

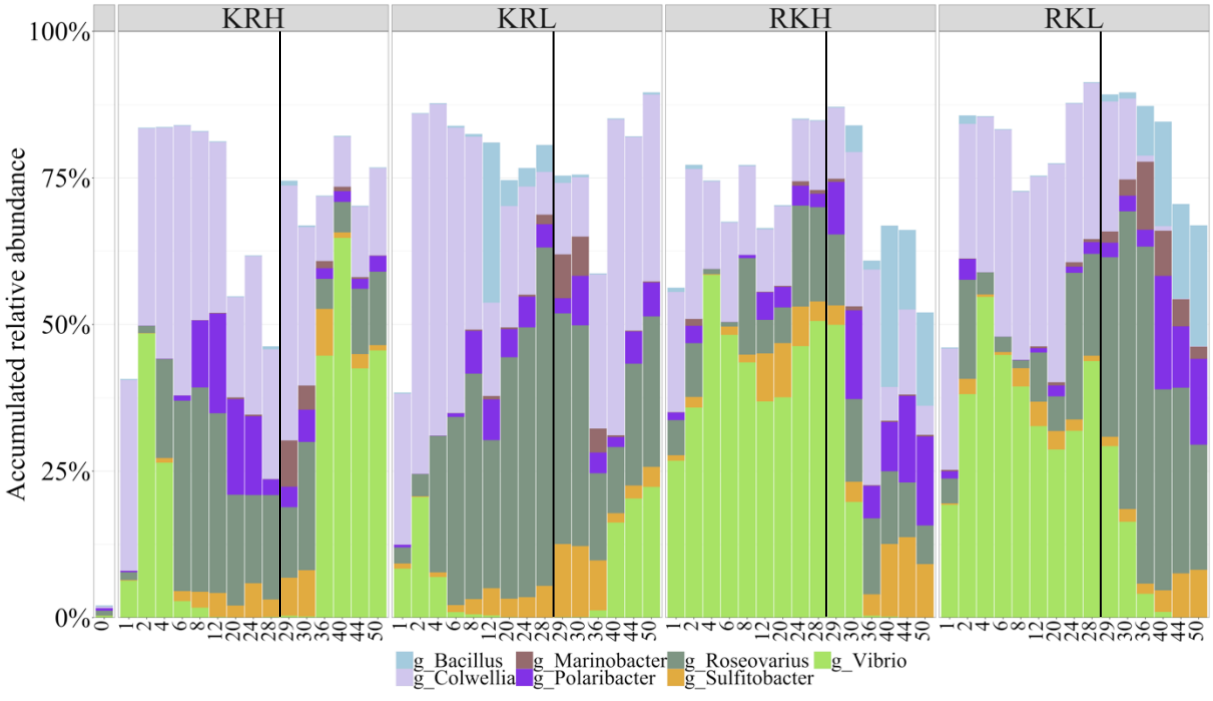


Figure S4: Accumulated relative abundances of the major six genera observed during the experimental period for the cultivation regimes (n=3). White space above bars indicate filtered out OTUs, and the solid vertical line indicates the r- and K-selection swtich. RK: r-selection from day 0-28, K-selection from day 28-50. KR: K-selection from day 0-28, r-selection from day 28-50. H: high carrying capacity, L: low carrying capacity.

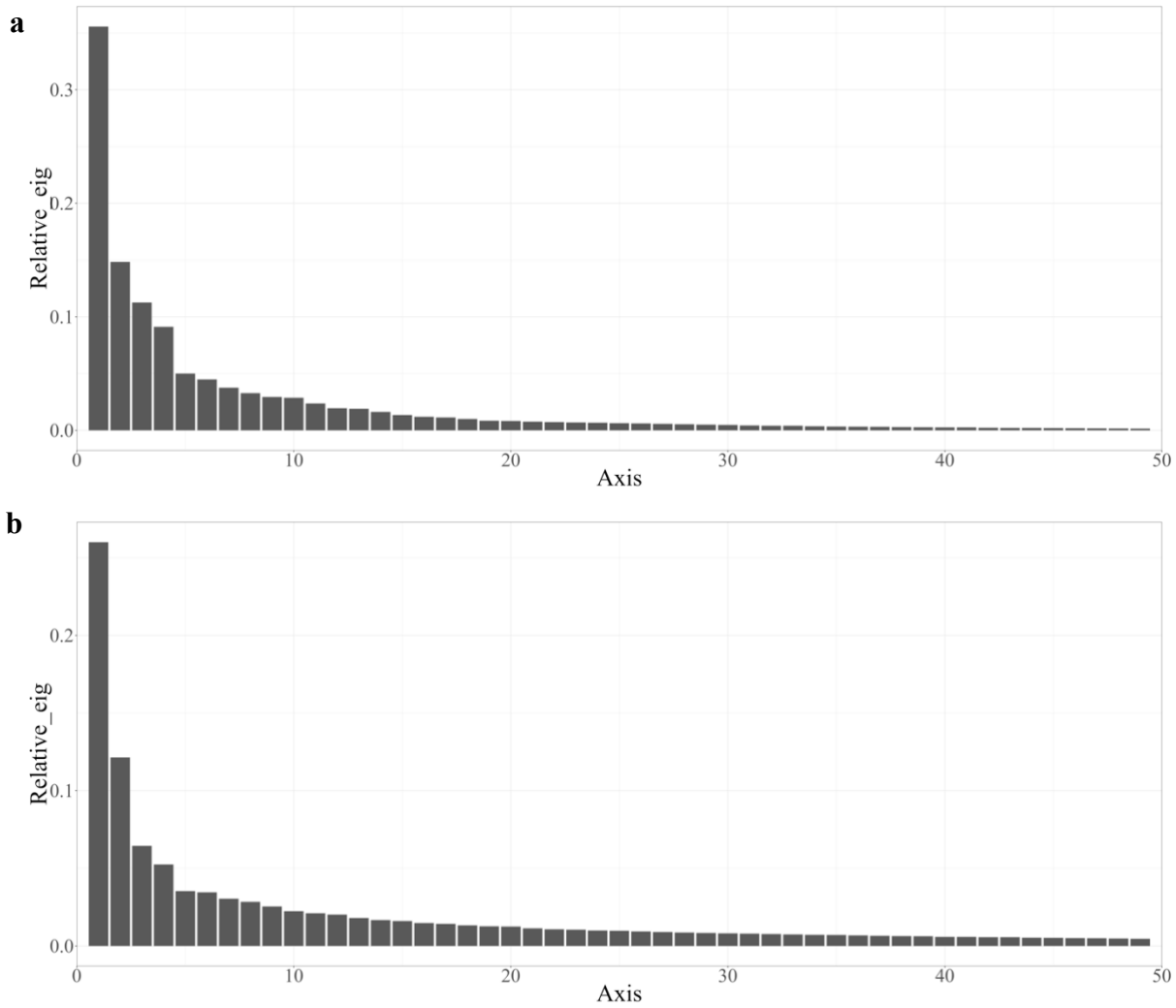


Figure S5: Scree plot of the first 50 axis of the PCoA ordination of a) the Bray-Curtis matrix and b) the Sørensen dissimilarity matrix.

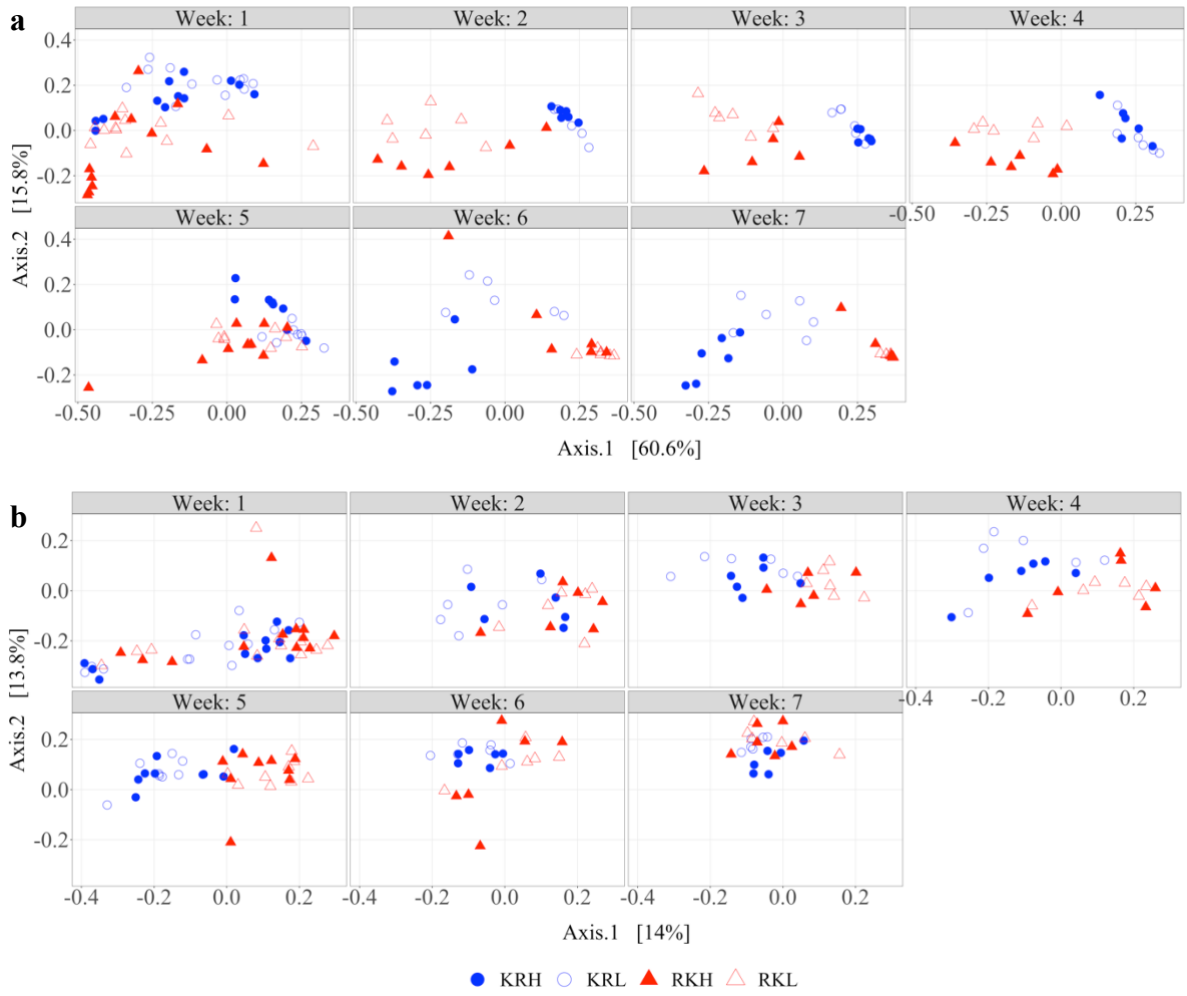


Figure S6: Dissimilarity of the samples over time calculated by a) weighted and b) unweighted UniFrac projected with PCoA. The switch in r and K-selection was between week 4 and 5.RK: r-selection from day 0-28, K-selection from day 28-50. KR: K-selection from day 0-28, r-selection from day 28-50. H: high carrying capacity, L: low carrying capacity.

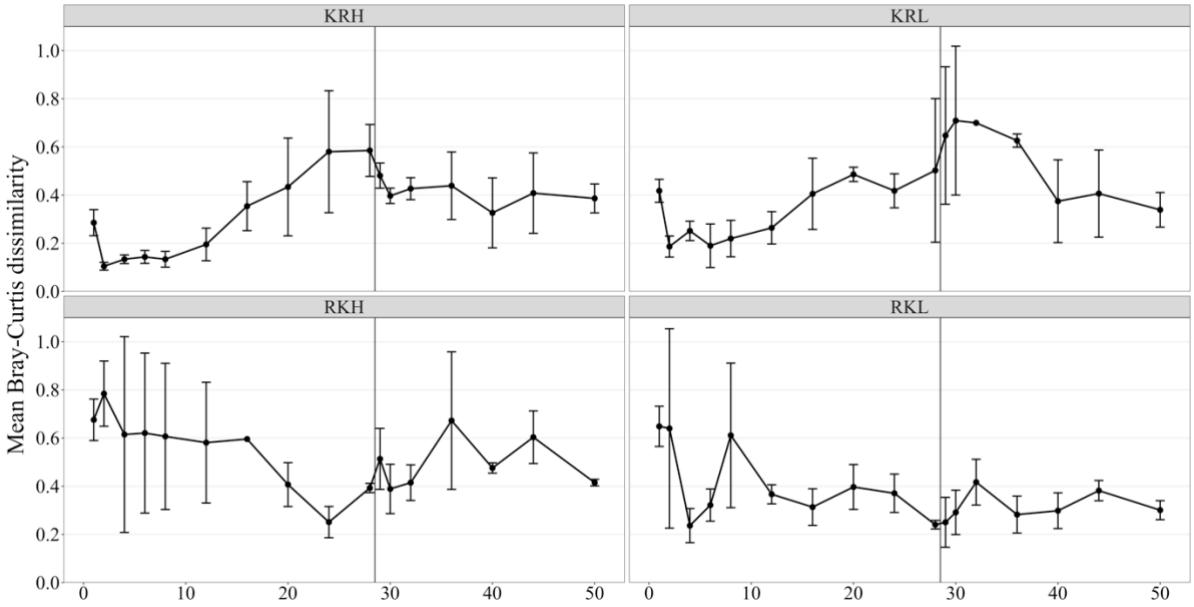


Figure S7: Mean Bray-Curtis dissimilarity per day for the four cultivation regimes over time. Error bars indicate standard deviation. The switch in r and K-selection was between day 28 and 29. No standard deviation could be calculated for KRL day 32 and RKH day 16 because there were only two replicas in the dataset. RK: r-selection from day 0-28, K-selection from day 28-50. KR: K-selection from day 0-28, r-selection from day 28-50. H: high carrying capacity, L: low carrying capacity.

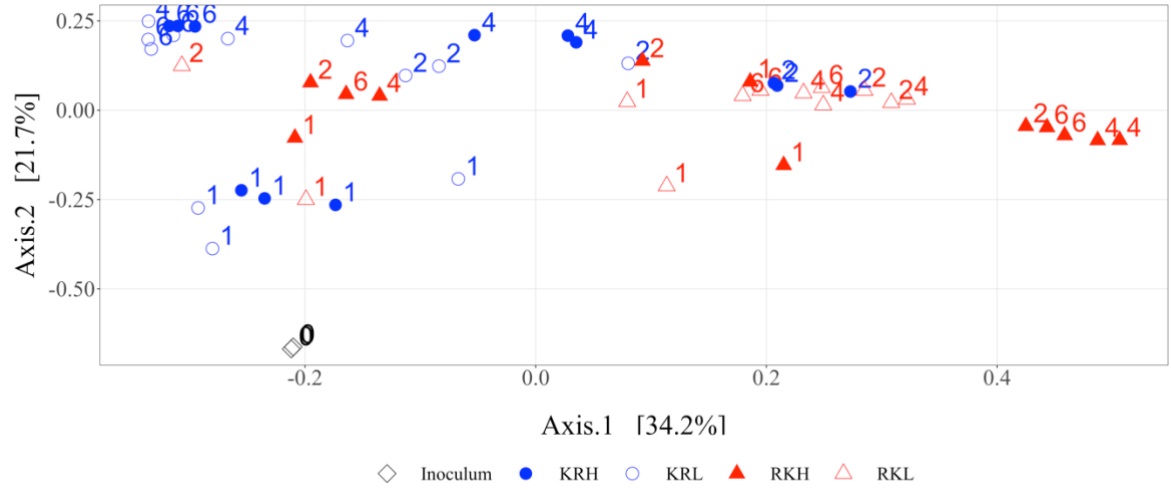


Figure S8: PCoA ordination based on Bray-Curtis dissimilarity of day 0-6. RK: r-selection, KR: K-selection H: high carrying capacity, L: low carrying capacity.

Appendix I Results from SIMPER analysis

Table S8: The OTUs that contributed more than 1% to the variation between r- and K-selection in the KRH, KRL, RKH and RKL group, and had a p-value <0.1, determined by SIMPER analysis. The mean abundances are based on day 6-28 in the first selection period and day 36-50 for the second period. RK: r-selection from day 0-28, K-selection from day 28-50. KR: K-selection from day 0-28, r-selection from day 28-50. H: high carrying capacity, L: low carrying capacity.

Group	OTU	Genus	Variation contribution	K-selection	r-selection	p-value
KRH	OTU_1	Vibrio	24 %	1 %	49 %	0.005
	OTU_10	f_Rhodobacteraceae	5 %	10 %	0,2 %	0.055
	OTU_11	Pseudoalteromonas	4 %	0,1 %	8 %	0.005
	OTU_2	Roseovarius	9 %	25 %	9 %	0.005
	OTU_307	o_Alteromonadales	1 %	0,0 %	2 %	0.005
	OTU_5	Polaribacter	5 %	11 %	2 %	0.095
	OTU_8	Colwellia	4 %	9 %	2 %	0.005
KRL	OTU_1	Vibrio	7 %	0,3 %	15 %	0.005
	OTU_2	Roseovarius	11 %	39 %	18 %	0.030
	OTU_6	Colwellia	9 %	3 %	20 %	0.005
	OTU_9	f_Alteromonadaceae	3 %	0,4 %	6 %	0.035
RKH	OTU_1	Vibrio	20 %	0,2 %	41 %	0.005
	OTU_10	f_Rhodobacteraceae	4 %	8 %	0,1 %	0.005
	OTU_16	f_Rhodobacteraceae	1 %	2 %	1 %	0.020
	OTU_5	Polaribacter	4 %	11 %	3 %	0.005
	OTU_6	Colwellia	7 %	7 %	10 %	0.020
	OTU_7	Bacillus	7 %	15 %	0,0 %	0.005
	OTU_8	Colwellia	4 %	7 %	2 %	0.010
	OTU_9	f_Alteromonadaceae	4 %	8 %	1 %	0.015
	OTU_98	f_Flavobacteriaceae	2 %	4 %	0,0 %	0.010
RKL	OTU_1	Vibrio	17 %	1 %	36 %	0.005
	OTU_14	f_Rhodobacteraceae	3 %	7 %	0 %	0.005
	OTU_2	Roseovarius	13 %	36 %	11 %	0.005
	OTU_3	Sulfitobacter	2 %	5 %	2 %	0.040
	OTU_4	Marinobacter	3 %	7 %	1 %	0.005
	OTU_5	Polaribacter	5 %	12 %	1 %	0.005
	OTU_6	Colwellia	8 %	0,2 %	17 %	0.005
	OTU_7	Bacillus	8 %	16 %	0,0 %	0.005