Aquatic bacterioplankton communities along salinity gradients: insights into composition, community assembly and functional traits

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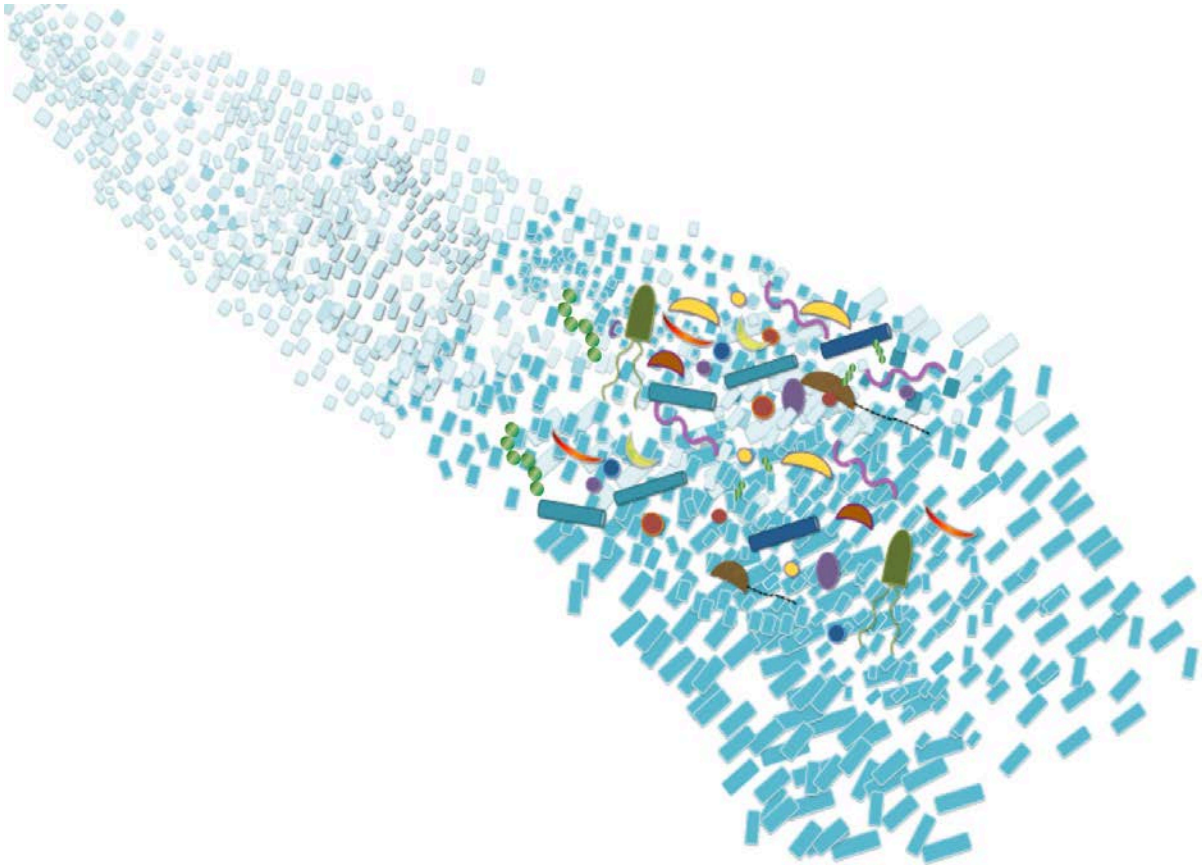
"…There are things we know we know. We also know there are known unknowns; that is to say we know there are some things we do not know. But there are also unknown unknowns – the ones we don't know we don't know…"

Donald Rumsfeld

(Former Secretary of Defense of the United States of America)



Designed by me 'Imagining some microbes in the water mixing zone'

Although he was not referring to microbes, I found this phrase was probably the best summary of microbial ecology:

"*things we know we know*" are relatively few, such as, rare bacteira as well as relative importance of different community assembly processes;

"*We also know there are known unknowns*" are abundant and culturable bacteria, such as, their ecological interactions;

"*Unknown unknowns*" remain to be discovered, such as novel bacteria and their metabolic pathways.

List of publications and submitted manuscripts

The present thesis has used the materials from the publications and manuscripts that are listed below in Roman numerals. The author contributions to the respective papers are clearly addressed as follows:

I. Sara Beier*[1], **Dandan Shen**[1], Thomas Schott[1], Klaus Jürgens[1]. (2017). **Metatranscriptomic data reveal the effect of different community properties on multifunctional redundancy**. *Molecular Ecology* **26**: 6813-6826.

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Author contributions: SB performed the experiment, analyzed the metagenomic / metatranscriptomic data, developed the metric for measuring FR and wrote the manuscript. DS performed sampling during the cruise and the experiment, analyzed 16S rRNA data and wrote the methods concerning the 16S rRNA gene analyses and the measurement of bacterial production. TS supported bioinformatics processing and translated the developed metric into perl programming language. KJ provided the funding and conceived the experimental design with SB and DS. All authors have commented and approved the manuscript.

II. **Dandan Shen***[1], Klaus Jürgens[1], Sara Beier[1]. (Accepted Article). **Experimental insights into the importance of ecologically dissimilar bacteria to community assembly along a salinity gradient**. *Environmental Microbiology*. doi: 10.1111/1462-2920.14059. This article is protected by copyright. All right reserved.

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Author contributions: DS conceived the study and wrote the manuscript. DS performed sampling during the cruise, the experiment, analyzed the samples and data, and wrote the manuscript. KJ designed the experiment together with DS and SB, provided the guidance for writing and commented on the manuscript. SB performed the experiment, provided the guidance for the writing, and commented on the manuscript. All authors discussed the results and approved the final version of the manuscript.

III. **Dandan Shen***[1], Silke Langenheder[2], Klaus Jürgens[1]. **Effects of dispersal on the diversity and composition of bacterioplankton following a salinity change**. (*Submitted, Frontiers in Microbiology*)

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Author contributions: DS conceived the study and wrote the manuscript. DS and KJ designed the experiment. DS performed sampling during the cruise and the experiment, analyzed samples and data, and wrote the manuscript. SL and KJ provided guidance in the writing, edited and commented on the manuscript. All authors discussed the results and approved the final version of the manuscript.

IV. **Dandan Shen***[1], Sara Beier[1], Klaus Jürgens[1]. **Is metabolic specificity or functional plasticity as a strategy for the marine – oligohaline transitions of ecologically dissimilar taxa?** (*Manuscript*)

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Author contributions: DS, SB and KJ conceived the study and designed the experiment. DS performed sampling during the cruise, the experiment, data processing, and wrote the manuscript. SB performed the experiment and the metagenome / metatranscriptome data pre-processing, shared her knowledge of meta-'omics' application and some of the R scripts for those data analyses to proceed; SB also wrote the method description on the metagenome / metatranscriptome data pre-processing. KJ discussed the results with DS and SB, also well as provided guidance for the writing.

All authors listed in the publications and manuscripts have agreed on the use of all materials from the respective manuscripts for the present thesis. I hereby declare that, I performed the experiments, data processing, and wrote Paper **II**, **III** and **IV**, accouting for > 90% of the contribution to the respective papers, and I contributed 30 % among the coauthors to Paper **I**.

The permission to usage of the materials from Paper **I** and Paper **II** has been granted by the Wiley publisher of Molecular Ecology and Environmental Microbiology, respectively. Specifically, license No. 4279381191036 is granted to Paper **I**. It should be noted that Paper **II** is under review and waiting to be published, at the time of the thesis submission.

Additional contribution to other manuscript not included in the thesis

Dandan Shen[1], Anna-Lena Höger[1,2], Daniel Herlemann[1,3], Klaus Jürgens[1]. **Stable abundance-occupancy ranks despite high taxonomic variability between particle-attached and free-living bacterial communities**. (*Manuscript in preparation)*

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Originality-Significance statement

Aquatic environments are typically not homogenous, but characterized by environmental gradients with regards to abiotic parameters, such as salinity and nutrient availability. Such heterogeneity of aquatic environments creates multiple niches allowing bacteria with varying niche breadth and functional traits to coexist. Quantifying individual taxon's response, unraveling the processes that underlie community assembly, and linking the emergent community structure to their microbially mediated functions after environmental perturbations remain a challenge and have been understudied, however. Here I identified ecological assembly processes driving community dynamics after exposure to changing salinity, as well as metabolic traits of bacteria exhibiting a varying degree of salinity preference. The obtained data can be informative for understanding when and where the relative conribtuions of community assembly process to community changes are ecologically relevant with regards to the corresponding functional attributes. Furthermore, I assessed functional performance of the disturbed communities by quantifying the degree of multi-functional redundancy at varying salinity disturbances, using metatranscriptomics to produce a more comphrenhensive understanding of community behavior under shifting environmental conditions.

Abstract

Bacterial communities are determined by the complex interplay between environmental factors and ecological interactions among the respective community members. Mechanisms influencing community assembly and functional behavior of bacterial populations remain unclear, particularly under shifting environmental conditions, however. I could show that bacteria employed two major ecological strategies with regards to niche breadth when responding to salinity disturbances. The deterministic processes, like environmental filtering and competitive exclusion differently impacted the assembly of habitat specialists and generalists. Specialist species significantly expressed multiple genes involved with respiratory chain, energy production and carbon metabolism when coping with un-favored salinity conditions. At the community level, disturbance intensity may influence the extent of functional redundancy depending on community sensitivity. Further, dispersal altered community diversity and composition, and the degree of such changes is likely dependent on environmental contexts. The present thesis also demonstrates the feasibility of using novel experiments and integration of 16S rRNA and meta-'omic' data to address ecological aspects.

Keywords: Bacteria; reciprocal transplants; salinity; seed bank; community assembly; redundany; gene expression

Zusammenfassung

Bakteriengemeinschaften werden durch das komplexe Zusammenspiel von Umweltfaktoren und ökologischen Interaktionen zwischen den jeweiligen Mitgliedern der Gemeinschaft bestimmt. Mechanismen, die die Zusammensetzung und das funktionelle Verhalten von Bakterienpopulationen beeinflussen, bleiben jedoch unklar, insbesondere unter sich verändernden Umweltbedingungen. Diese Arbeit zeigt, dass Bakterien zwei wichtige ökologische Strategien in Bezug auf die Nischenbreite anwendeten, wenn sie auf Salinitätsstörungen reagierten. Die deterministischen Prozesse wie Umweltfilterung und Konkurrenzausschluss wirkten sich unterschiedlich auf die Zusammensetzung von Habitatspezialisten und Generalisten aus. Spezialisierte Spezies exprimierten signifikant mehr Gene, die an der Atmungskette, der Energieproduktion und dem Kohlenstoffmetabolismus beteiligt sind, wenn sie unter ungünstigen Salzgehaltsbedingungen leben. Auf Gemeinschaft-Ebene kann die Störungsintensität, abhängig von der Sensitivität der Gemeinschaft, das Ausmaß der funktionellen Redundanz beeinflussen. Darüber hinaus hängt die, durch Verteilung veränderte, Vielfalt und Zusammensetzung der Gemeinschaft und der Grad dieser Veränderung wahrscheinlich von den herrschenden Umweltbedingungen ab. Die vorliegende Arbeit demonstriert auch die Machberkeit neuartige Experimente und die Integration von 16S rRNA und meta-'omischen' Daten zu verwenden, um ökologische Aspekte zu adressieren.

Abbreviations

ANOSIM	Analysis of similarities
ANOVA	Analysis of variance
BCC	Bacterial community composition
BEF	Biodiversity-Ecosystem functioning
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DNA	Deoxyribonucleic acid
DT	Dispersal treatment
FC	Functional change
FL	Free living
FR	Functional redundancy
mRNA	Messenger RNA
ND	Non dispersal
NMDS	Non-multidimensional scaling
NRI	Net relatedness index
NTI	Nearest taxon index
ORFs	Open reading frames
OTU	Operational taxonomic unit
PA	Particle attached
PCA	Principal component analysis
PD	Phylogenetic diversity
PERMANOVA	Permutational multivariate analysis of variance
RDP	Ribosomal Database Project
RNA	Ribonucleic acid
SLE	St. Lawrence estuary
TFC	Theoretical community functional change

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Summary

A central pursuit of microbial ecology is to accurately describe and explain changes in microbial community composition and functions that microbes perform in response to environmental changes. This goal requires a thorough understanding of individual taxon's response and processes guiding the assembly of microbial population that feature similar response traits, as well as functional traits that correspond to the response traits. However, most microbial ecology studies focus on assessing changes of average population abundances, rather than on quantifying response traits, phylogenetic relatedness and variability of bacterial taxa in an emergent community after exposed to environmental gradients or disturbances. To address these research aspects, four case studies are presented in the context of marine-oligohaline transitions that reflects a historical and evolutionary dimension of microbes. Here, mechanisms by which salinity influences community assembly, metabolic processes of bacteria, magnitude of functional redundancy and how dispersal modifies community outcome are explicitly explored in each of the 4 case studies:

Case study 1: Importance of ecologically dissimilar taxa to community assembly. The response of locally adapted bacteria to marine-oligohaline transitions and the processes that underlie community assembly remain unclear, particularly with regards to bacteria differing in ecological strategies. Here, a transplant experiment was implemented using water and bacterioplankton from three salinity regions of the Baltic Sea (~3, 7, and 28 psu), where they were exposed to each other's native environmental conditions for a 4-day incubation. Initial and final bacterial communities were then analyzed using 16S rRNA gene Illumina sequencing. By analyzing absolute abundance patterns two major ecological strategies of bacterial taxa were identified as a response to a large change in salinity: habitat specialists exhibited a high abundance in one habitat (i.e., salinity level) but lower in two others, whereas habitat generalists displayed approximately equal abundance in all three habitats. Regardless of the origin of the communities, habitat specialists were more abundant than generalists after the exposure. Most specialists found in their native habitat tended to originate from common taxon pool of the starting communities; whereas taxa specialized to non-native habitats were favorably selected from initially rare taxon pool. By contrast, the taxon's initial abundance was less important for the selection of generalist taxa compared to that of specialist taxa. These results suggest that rare taxa are viable outside their preferred salinity zones and can resume active growth when environmental conditions are suitable. In particularly, I found that seed banks of brackish bacteria exist within both oligohaline and marine species pools. Further, phylogenetic relatedness analyses showed that habitat filtering strongly influenced the assembly of habitat specialists, whereas competitive exclusion was more relevant for that of generalists. Altogether, this study suggests that

distinguishing assembly mechanisms of different community members helps understand and predict community dynamics in response to environmental change.

Case study 2: Metabolic specificity and functional plasticity of ecological dissimilar taxa to the marine-oligohaline transitions. I further examined whether the abundance of the identified specialist / generalist taxa pattern in their transcriptional activities. In other words, do response traits (ecological strategies) of bacteria correlate with functional traits in the face of new environments? The transcriptional activities of the specialist taxa were not positively correlated with their abundances, suggesting that high transcriptional activity may occur when taxa encounter non-favored environments. The metatranscriptomic data were further analyzed to infer the mechanisms used by the ecological groups to adjust their metabolisms: a preferential growth in one habitat or maintenance of equal growth in the three habitats. The overlapping of significantly differentially expressed genes was larger between the environments with smallest salinity distance, and the number of those shared genes decreased when increasing in salinity differences between the treatments. These findings imply that functional similarity appeared to be high at the individual taxon level when a taxon was grown in the similar environmental contexts. The central metabolic processes varied among the specialist strategy groups. An oligohaline specialist responded to marine environments by enhancing expression of genes encoding CO_2 fixation and one carbon pool by folate metabolism, as a strategy to cope with oxidative stress induced by high salt situation. In contrast, central metabolic processes were suppressed in a marine specialist when facing oligohaline conditions, including carbohydrate metabolism and membrane transporters for energy production. A brackish specialist was shown to regulate the expression of gene *accC* involved in carbohydrate and lipid metabolism to maintain the osmotic balance, as a response to saline conditions. Collectively, the variations in composition of transcripts that mediate energy conservation and cellular carbon storage were pronounced between the oligohaline and marine specialists. These results further suggest that, specialists to certain salinity are metabolically specific, and they also exhibit functional plasticity to some extent through basal maintenance energy requirement and high energetic costs to respiration and carbon metabolism when crossing marine-oligohaline boundaries.

Case study 3: Quantifying the magnitude of functional redundancy (FR) between the disturbed communities. FR acts as an ecosystem buffer of functional performance for a microbial community that have experienced environmental disturbance. Understanding the degree to which FR occurs in the disturbed communities is critical for predicting the degree to which shifts in community composition affect ecosystem processes. In this study, a metatranscriptome-based approach was developed in order to assess multiple functions performed by all taxa within a community. FR among prokaryotes was measured in water samples after exposure to salinity change in a transplant experiment. I hypothesized that disturbance intensity would influence the degree of FR being

expressed in the disturbed communities. The differences in salinity conditions significantly affected functional performance of the marine communities, and the communities experiencing a large degree of disturbance, i.e. 16 / 19 psu difference) showed a significant greater FR than those experiencing 3.4 psu difference (P <0.05). FR in the brackish communities, however, was not affected by the intensity of changing salinity (P >0.05). Instead, functional diversity and phylogenetic diversity appeared to correlate with FR, which was higher in the brackish compared to marine communities. Furthermore, the degree of FR varied at different taxonomic ranks for both marine and brackish assemblages. Higher FR occurred between phylogenetically closely related organisms of the disturbed communities than between distant relatives when exposed to similar environmental conditions. Overall, disturbance intensity can influence the magnitude of FR, particularly pronounced in the bacterial communities that were highly sensitive to changes in salinity. This study also highlights the importance of community property in examining FR after disturbances.

Case study 4: Dispersal alters the diversity and composition of bacterial communities following a salinity change. Dispersal is increasingly recognized as an important factor influencing the assembly of microbial communities, and can modify the responses of microbes to environmental changes. The present study focuses on how dispersal and its interactions with different environmental contexts alter the responses of bacterioplankton at the community- and population-levels. Using a reciprocal transplant experiment with brackish and marine waters from St. Lawrence estuary, I could show that dispersal compensated for the loss of local diversity in case of marine, but not for brackish communities. Dispersal also caused an increase in community similarity, which was mainly driven by changes in relative abundances rather than the replacement of abundant taxa. Independent of the origin of the communities, some variation in the relative abundances of different phylogenetic groups occurred in response to dispersal and was especially pronounced at low taxonomic levels and under brackish conditions. Taken together, both dispersal and the interactions with the incubation environment played important roles in explaining the observed patterns. To my best knowledge, this is the first experimental study to demonstrate that the effects of dispersal and its interactions with different environmental conditions vary among bacterial taxa in the active fraction of bacterial communities.

Collectively, the present thesis provides a better understanding of the outcome of the disturbed communities in terms of compositional and functional changes, therefore enhancing our ability to predict bacterial responses to changing environments.

1. Introduction

Microbes constitute fundamental bases of every ecosystem on earth (Whitman *et al*., 1998). Natural microbial communities are known to be highly biologically diverse - estimates exceed 10^5 cells in a few grams of soil or in one drop of water (Gans *et al*., 2005; Heip *et al*., 2009). They are often taxonomically diverse and determined by the complex interplay between environmental factors and ecological interactions among the respective community members (Little *et al*., 2008). Microbial compositions vary over space and time, such as down the ocean water column (Sunagawa *et al*., 2015), or across season (Fuhrman *et al*., 2006), and this variation can have pronounced effect on ecosystem functions (Allison and Martiny, 2008). The assembly mechanisms driving this variation remain unclear, because the entanglement of multiple mechanisms complicates the identification of causal relationships (Nemergut *et al*., 2013). Furthermore, characterizing the relationship of microbial composition and function is a major challenge for ecologists. Shift in microbial composition does matter to the ecosystem processes in some systems, but not in others. Many ecosystems are currently threatened by environmental change, such as disturbances due to climate change or anthropogenic activities. An understanding of the relative contribution of assembly mechanisms influencing the response of microbial communities to change, as well as when and where the communities are functionally relevant, is critical for guiding management efforts aimed at maintaining microbial diversity and ecosystem multifunctionality.

1.1 Ecological strategies

Ecological communities are naturally comprised of species with different degrees of ecological specialization (Futuyayma and Moreno, 1988; Devictor *et al*., 2010). For microorganisms, high densities, small body size and widespread dispersal enable them to travel across and colonize diverse environments (Tamames *et al*., 2010; Fierer *et al*., 2012; Sunagawa *et al*., 2015). In order to survive under constant movements and competitions with immigrant microbes, optimal ecological strategies exist that could lead to some species having a broader range of environmental optima (habitat generalists), while others exhibit a narrow environmental optimum (habitat specialists) (Futuyayma and Moreno, 1988; Van Tienderen, 1991).

1.1.1. Distribution and abundance of habitat specialists vs. generalists

Identifying ecological categories of microbial communities is considered as an important step towards quantifying indicator species that respond to environments as well as examning ecological interactions among those species. Previous studies have shown a positive relationship between abundance and occupancy at large spatial scales (van der Gast *et al.*, 2011). This positive

relationship suggests a great number of habitat specialists and few generalists, with generalists being more abundant. How well habitats are differentiated may affect the abundance and occupancy ranks between the two ecological categories (Mariadassou *et al*., 2015). If the targeted habitats are defined along abiotic gradients, habitat specialists are found in greater numbers and to be more abundant than generalists (Fortunato *et al*., 2013; Logares *et al*., 2013). Recent work with samples from wide environmental conditions has demonstrated that, habitat specialists consistently dominate microbial systems, and that the higher specificity taxa tend to have, the more abundant they are in that habitat (Mariadassou *et al*., 2015). This also implies that, habitat specialists in general are more sensitive to surrounding changes due to strong adaptation to their local environments, with generalists being more resilient to change (Clavel *et al*., 2011; Shade *et al*., 2012)

Environmental changes are implicated in community diversification, leading to the establishment of habitat specialists with high abundances (Logares *et al*., 2013). By contrast, shifting in envionrmental conditions may favor generalists because of their ecological versatility and broad environmental tolerance (Székely *et al*., 2013). The relationship between habitat specialists and generalists may change when disturbances or small changes to their surrounding environments are introduced. These relationships are likely dependent on how many generlaists and specialists are present in a community, as well as their perception of habitat differences (Langenheder and Székely, 2011). Accordingly, the extent to which habitat specialists and generalists exposed to new environments are selected in terms of their distribution and cell abundances remains unclear. Previous studies have shown that these generalist and specialist differently impact the dynamics of microbial community structures (Pandit *et al*, 2009; Székely and Langenheder, 2014; Liao *et al*., 2016). In general, habitat specialists seem to be mostly influenced by deterministic factors (Pandit *et al*, 2009), while habitat generalists appear to respond mainly to stochastic factors (but see, Székely and Langenheder, 2014).

1.1.2 Functional differences between habitat specialists vs. generalists

Niche breadth may not only be linked to abundance, but also the role of species in ecological functioning. It is suggested that life-history tradeoffs between specialist vs. generalist species, whereby specialists invest more energy in exploring their specific niches, but high functional performance once established (Lennon *et al*., 2012). On the other hand, high competition among generalists makes them less capable of exploiting the potential niche space (Büchi and Vuilleumier, 2014). A simulation model has recently revealed the difference in producing biogeochemical rates between these ecological groups, and that specialists can generate higher biogeochemical rates and adapt better to their local habitat than generalists (Graham and Stegen, 2017). The above studies indicate that functional separation may be a key for the balance of the two ecological groups within a community. Hence, it is becoming clear that the level of functional performance differs between

habitat specialist and generalist taxa, what remains unclear is that how well their respective functional traits correlate with their abundances along environmental gradients.

1.2 Community assembly

Community assembly involves four fundamental processes: drift, selection, speciation (diversification) and dispersal (migration) (Vellend, 2010). As summarized by Vellend, (2010) and Nemergut *et al*., (2013): drift encompasses stochastic processes leading to random changes in species abundance (e.g. events like birth and death), selection refers to deterministic fitness differences among taxa driven by abiotic conditions and biotic interactions, speciation is the process resulting in the generation of new genetic variation, dispersal is the movement of individuals between habitat patches. Dispersal, for microorganisms, is often regarded as stochastic process with respect to taxon identity and taxon abundance when considering taxon's abundance in a local community relative to its abundance in the regional species pool, as well as the rate of dispersal (Hubbell, 2001; Sloan *et al*., 2006). All these processes have been shown to play a role in microbial community dynamics (Costello *et al*., 2012). A great body of literatures has highlighted the importance of understanding the relative contributions of community assembly processes to community changes (Stegen *et al*., 2012; Nemergut *et al*., 2013; Vellend *et al*., 2014; Evans *et al*., 2016; Lee *et al*., 2017). These processes can also be informative for understanding community changes after a disturbance (e.g., Lee *et al*., 2017). Hence, investigating community assembly mechanisms helps us to predict the dynamics of bacterial communities in a changing environment. The role of speciation is an important topic within evolutionary biology (Rundle and Nosil, 2005), as well as the role of ecological drift that involves with spatial difference in taxon abundance; hence, the processes of drift and speciation are beyond the scope of the present thesis. The following sections focus on the processes of environmental filtering, biotic interactions (Koeppel and Wu, 2014), and dispersal.

1.2.1 Environmental filtering and competitive exclusion inferred from phylogenetic patterns

Recent work in microbial ecology has revealed substantial variation in bacterial communities among habitat types (Lozupone and Knight, 2007; Tamames *et al*., 2010). These observations suggest the dominant role of habitat specialization in the assembly of microbial communities (Andersson *et al*., 2010; Logares *et al*., 2013). This so-called habitat filtering is a classic deterministic process driven by contemporaneous environmental heterogeneity. Although deterministically environmental filtering is a dominate process on average in several field studies (Jones and McMahon, 2009; Stegen *et al*., 2012; Powell *et al*., 2015), other processes, including species interactions and stochastic processes (e.g. dispersal limitation) are also important and influence community structure and dynamics (Martiny *et al*., 2006; Stegen *et al*., 2012; Dini-Andreote *et al*., 2015). These processes could act simultaneously in the assembly of naturally occurring communities (Barberán and

Casamayor, 2010; Langenheder and Székely, 2011; Stegen *et al*., 2012; Valverde *et al*., 2014). Additionally, different underlying processes may be important in the assembly of different parts of the total bacterial community (Lindström and Langenheder, 2012). As such, insights into the processes that influence different functional guilds of bacteria (Fierer *et al*., 2007) or habitat specialists vs. generalists will provide a better understanding of microbial community assembly. At the same time, studies on the relative importance of different deterministic processes to different microbial community members have recently emerged (Monier *et al*., 2015; Powell *et al*., 2015).

Previous studies attempted to quantify the relative importance of ecological processes in microbial community assembly by partitioning the variation in the entire community or sub-communities into fractions explained by environmental variables. Studies based on this analysis reveal that the assembly of habitat specialists appears to be largely driven by environmental filtering, whereas the assembly of habitat generalists may also include dispersal-related processes (Székely and Langenheder, 2014; Liao *et al*., 2016). However, these studies ignore the biotic interactions that influence community assembly. In another approach, the phylogenetic relatedness of taxa within a community is used to infer the relative roles of species interactions (i.e., competition or facilitation) and of habitat filtering on community assembly. Although originally developed for macro-organisms (Webb *et al*., 2002), this approach also holds great promise for microorganisms. The ecological concept suggests that coexisting taxa are ecologically similar (Blomberg *et al*., 2003). If environmental filtering is the most influential assembly mechanism, coexisting taxa in a community should be more closely related than expected based on random community assembly. This leads to a high phylogenetic relatedness of taxa within the community. If competitive exclusion or facilitative interactions are strong, coexisting taxa in a community should be ecologically differentiated. Accordingly, those taxa should be more distantly related than expected by chance, which results in a lower phylogenetic relatedness. If stochastic processes primarily drive community structure and dynamics, community phylogenetic composition and dynamics should not differ significantly from expectations based on random community assembly. Several studies have evaluated the relative contributions of the different assembly processes to community changes by assessing the degree of the phylogenetic relatedness in entire microbial communities (Horner-Devine and Bohannan, 2006; Bryant *et al*., 2008; Stegen *et al*., 2012; Hamilton *et al*., 2013; Monier *et al*., 2015). Comparatively much less is known about the phylogenetic patterning of specific ecological subgroups within microbial communities. For example, bacterial taxa that responded with varying degrees of delay to changes in soil moisture had different degrees of phylogenetic relatedness (Placella *et al*., 2012; Evans and Wallenstein, 2014). However, phylogenetic relatedness has yet to be applied to examine the relevance of species interactions versus habitat filtering for habitat generalists and specialists.

1.2.2 The role of dispersal in shaping community dynamics

Dispersal limitation is one process that can increase stochasticity and obscure the relationship between environmental variable and microbial community composition. The concept of dispersal limitation (Martiny *et al*., 2006 and references therein) has disputed the idea "everything is everywhere – the environment selects." offered by Bass Becking (1934). That is, dispersal can be limited, and therefore, influences the evolution and coexistence of microbial diversity (Martiny *et al*., 2006; Hanson *et al*., 2012). Several studies have investigated the impact of dispersal limitation by quantifying community dissimilarity along geographic distance across globe (e.g. Martiny *et al*., 2011; Robeson *et al*., 2011). However, the question of when, where and to what extent the effect of dispersal on community dynamics remain unclear.

Dispersal acts as a link between local and regional community dynamics in the framework of metacommunities (Mouquet and Loreau, 2003; Leibold *et al*., 2004). Over the last decade, theoretical predictions (Loreau and Mouquet, 1999; Mouquet and Loreau, 2003) and experimental studies using natural communities (Matthiessen and Hillebrand, 2006; Lindström and Östman, 2011; Declerck *et al*., 2013; Albright and Martiny, 2018) have focused on how dispersal alters community diversity and composition. For microorganisms, the degree to which dispersal contributes to changes in community properties depends on (i) the magnitude of dispersal rates (Lindström and Östman, 2011; Declerck *et al*., 2013; Berga *et al*., 2015; Souffreau *et al*., 2014), (ii) initial diversity of communities experiencing dispersal (Zha *et al*., 2016), and (iii) the source of immigrants (Comte *et al*., 2017). In natural systems, passive migration of cells is often accompanied by a change in environmental conditions, particularly in aquatic system (Lindström and Langenheder, 2012; Rillig *et al*., 2015). Hence, it is difficult to tease apart the effect of dispersal from that of contemporary environmental conditions on the assembly of emergent bacterial communities. Only few experimental studies have assessed a direct effect of dispersal via exchange of microorganism on activity and overall structure of bacterial communities (Lindström and Östman, 2011; Severin *et al*., 2013). These studies, however, did not examine how different environments affect the importance of dispersal on bacterial community composition and whether dispersal varies among bacterial taxa.

Chesson, 2000 suggested that species interactions play a large role in regulating the colonization ability of immigrants, which leads to coexistence among local communities. Thus, different bacterial taxa vary in their ability to colonize new environments, and in their competitive capability with other community members (Mouquet and Loreau, 2002; Lowe and McPeek, 2014). This is rather important if dispersal is random with respect to taxon identity, given that most microorganisms are likely to disperse passively (i.e., wind-blow or water flows) (Martiny *et al*., 2006; Nemergut *et al*., 2013). The variability at both the community and the population level after dispersal is therefore assumed to reflect the cumulative effects of competition and colonization on species coexistence.

Dispersal can also influence the response of bacterial communities to disturbances or environmental perturbations (Shade *et al*., 2012). Without immigration, bacterial communities respond to a disturbance mainly through physiological acclimation during a short period (Martiny, 2015). This may lead to a decrease in total cell abundances (Baho *et al*., 2012) or proliferation of some bacteria from the seed banks (Jones and Lennon, 2010). However, in the presence of dispersal, immigrating bacteria can colonize niches opened by disturbances (Baho *et al*., 2012; Vuono *et al*., 2016; Comte *et al*., 2017), and fulfill the essential functions that were performed by taxa lost due to the disturbances (Székely and Langenheder, 2017). Thus, it is becoming clear that dispersal can modify the response of a bacterial community to environmental changes, but what remains unclear are how dispersal and its interactions with varying environmental conditions contribute to the compositional response of bacterial communities.

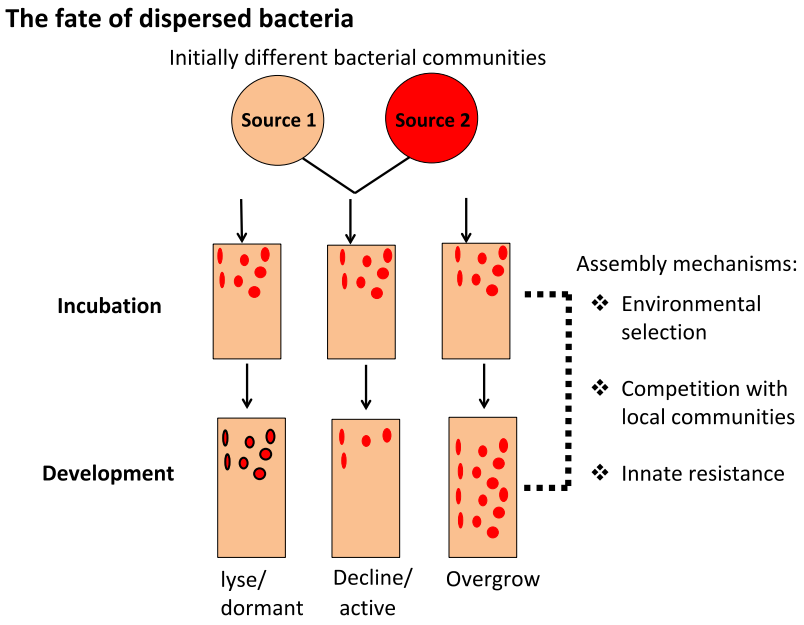

Figure 1 A schematic of the fate of bacteria from source 2 (red) after being dispersed to bacterial source 1 (orange). Three possible assembly mechanisms influence the development of the inoculated bacteria from source 2: environmental selection, competition with local communities for resources and innate resistance.

Figure 1 illustrates hypothecial results of the fate of dispersed bacteria with regards to their tolerance to the new environment and activity. If some bacteria from source 2 are dispersed to the environment of source 1 where bacterial composition differs from that of source 2, one could expect three possible developments of the mixed communities: i) scenario 1, dispersed bacteria from source 2 may be lysed or become dormant as a result of intolerance to the conditions encountered; ii) scenario 2, these bacteria may have declined their abundances but stay metabolically active due to a certain level of intrinsic resistance to environmental change; iii) scenario 3, they are able to outcompete the locally bacteria in source 1, possibly by employing opportunistic strategies with

regards to competitive ability and nutrient availability. The dispersed communities may be assembled through complex interplay between environmental filtering, competition with local communities for resources (Székely *et al*., 2013), as well as the extent of innate resistance under new abiotic and biotic conditions. Indeed, the relationships between the dispersed communities and these assembly mechanisms are likely complex. Thus, experiments will be required to separate one process from the others, enabling elucidate the role of dispersal in shaping bacterial communities.

1.3 Relationships between microbial composition and functional redundancy (FR)

Biodiversity-Ecosystem functioning is a pivotal research question in the ecology of macro and microorganisms and has been intensively addressed, including the influence of diversity patterns on system stability (Hooper *et al.*, 2005; Shade *et al.*, 2012; Srivastava *et al.*, 2012). Given the primary role of microorganisms in globally biogeochemical transformations (Falkowski *et al*., 2008), the relationship between bacterial composition and ecosystem functioning is a key to understanding where and when microbial communities are functionally relevant. During the past decade, a large body of studies attempted to investigate the potential impacts of disturbances on microbial composition and ecosystem processes (Allison and Martiny, 2008; Shade *et al*., 2012 and reference therein). The sensitivity of community composition to such change has been established in a framework considering the differences in composition before and after disturbances (Allison and Martiny, 2008). There are three main outcomes of these relationships: i) resistance, bacterial communities may be unaffected by the disturbances; ii) resilience, communities show certain level of sensitivity but recover quickly to its original composition; iii) functional redundancy (FR), communities remain altered but perform functions at similar rates as the original community (Allison and Martiny, 2008). Among the three possible outcomes of how changes in microbial composition could influence ecosystem functions, FR might be the most intriguing phenomena to be accurately predicted. For example, metabolic plasticity of individual community members (Comte *et al*., 2013), the appearance of phenotype plasticity (Beier *et al*., 2015), and the activity of multifunctionality (Mori *et al*., 2016) may blur the link between microbial community composition and the assessment of FR. High abundance (Meyer, 1994) and genetic diversity of microbes (Finlay *et al*., 1997) are usually considered as an argument for FR. Such argument can be interpreted in two ways: one is that, it is difficult to envision that biogeochemical cycling is limited by these features of microbes; another in the context of the survival strategies of species co-existences, it is difficult to imagine how thousands, or many more species may occupy finely differentiated niches, thus species are functionally redundant (Bell *et al*., 2009).

1.3.1 The challenges in the assessment of FR

Our knowledge on the distribution of functional traits across microbial taxa is generally limited (Schimel, 2001), particularly for bacteria from which phenotypic information is limited. It is not feasible to model all taxa and their responses individually; however, the assessment of characteristics of the functional groups may help predict the responses of ecosystem processes to disturbances (Allison and Martiny, 2008). Given that microbial taxa are generally sensitive to disturbances (Allison and Martiny, 2008 and references therein), they vary in their responses to change and the responsive taxa may not necessarily be the components of functional groups. Recent work shows that strong taxonomic variability within functional groups despite stable functional structure across aquatic microbial communities (Louca *et al*., 2016a). This indicates that a high FR in the regional microbial species pool makes it possible that the high taxonomic variation occurred within the functional groups (Louca *et al*., 2016a). Still, the probability of observing high levels of FR between communities decreases if multiple functions are simultaneously considered (Gamfeldt *et al*., 2008). The simultaneous assessment of multiple functions is challenging and time-consuming, and conclusions on FR expressed in a given community have been often drawn based on a limited number of bulk measurements or enzyme assays (Langenheder *et al*., 2005; Peter *et al*., 2011; Reed and Martiny, 2013). Furthermore, methodological difficulties in measuring functional performance of individual taxa in a complex microbial community have hindered the direct assessment of the extent to which specific members in one community compensate for the activity of specific members in another community. FR is inferred from the lack of congruent pattern between bacterial community structure and function, by relating differences in community composition to differences in bulk community functioning (Langenheder *et al*., 2005; Frossard *et al*., 2012; Comte *et al*., 2013). Assessing how well the patterns in composition (determined by 16S rRNA gene) and function (rate measurements) are connected, however, may lead to overestimation of FR of the bacterial communities; because bacterial taxa contributing to the change in community composition may not necessarily be involved in the measured functions.

1.3.2 Some factors influencing the degree of FR

For bacteria, the degree of FR expressed in the community may depend on (i) bacterial diversity (Langenheder *et al*., 2010; Peter *et al*., 2011), (ii) species identity and their interactions (Fetzer *et al*., 2015), (iii) the impact of environmental context (Comet *et al*., 2013). This also implies that FR can occur at both the species and community levels (Loreau, 2004), and the extent of FR at the species level may differ when scaling up to community level. It will be interesting to see that to what extent a community and its constituted species express FR when responding to disturbances, and how the degree of which could vary across different phylogenetic levels.

In this thesis, coauthors and I applied metatranscriptomics to assess the magnitude of FR being expressed between communities after salinity disturbances, where the model considers transcriptional behavior of individual taxa, aggregation of that at the community level as well as multi-functionality. The detailed information about the advantage of using metatranscriptome over metagenome data to quantify FR is provided in the section 1.6 Meta-'omic' data approaches in ecological frameworks.

1.4 Microbes to marine-freshwater transitions

1.4.1 Impact of salinity on bacterial biogeography

Salinity is a strong environmental force structuring not only for the community of macroorganisms (animals and plants, Remane and Schlieper, 1971) but also for microbes (Lozupone and Knight, 2007). Although microbes have larger population sizes, rapid growth rates, the potential for long-distance dispersal, and the ability to exchange genetic material via lateral gene transfer (Boucher *et al*., 2003), microbial crossing marine-freshwater boundaries occurred infrequently in evolutionary times (Logares *et al*., 2009). Therefore, marine and freshwater ecosystems are mainly inhabited by only distantly related bacterial lineages (Giovannoni and Stingl, 2005; Newton *et al*., 2011). While lakes are typically dominated by *Actinobacteria* and *Betaproteobacteria* (Newton *et al*., 2011), oceanic water are mainly populated by *Alpha-* and *Gammaproteobacteria* (Giovannoni and Stingl, 2005). *Flavobacteria*, *Sphingobacteria*, *Planctomycetes*, and *Verrucomicrobia* thrive in both habitats, but with representatives from distinct lineages (Giovannoni and Stingl, 2005; Glöckner *et al*., 1999). Numerous studies in aquatic systems catalogued taxa-specific changes in abundances along a salinity gradient (e.g. Bouvier and Giorgio, 2002; Herlemann *et al*., 2011). Drastic shifts in bacterial assemblage composition with a sequential replacement of freshwater taxa by brackish and marine ones are common along salinity gradients of estuaries (Cottrell and Kirchman, 2003; Campbell and Kirchman, 2013; Fortunato *et al*., 2013). This also implicated that some bacteria are vulnerable to altered salinity.

Marine-freshwater transitions are suitable for studying microbial assembly mechanisms because little overlap occurs in the abundant bacterial taxa inhabiting these two ecosystems (Lozupone and Knight, 2007; Logares *et al*., 2009; Tamames *et al*., 2010; Newton *et al*., 2011). Nevertheless, bacteria typically found in marine environments may be recruited from the less abundant members of freshwater or sediment communities following their exposure to marine conditions (Comte *et al*., 2014; Langenheder *et al*., 2016). This observation implies that rare or dormant taxa are viable outside their preferred salinity zone and can resume active growth when environmental conditions are favorable (Sjöstedt *et al*., 2012; Lindh *et al*., 2015). Several studies examine whether and how bacterial communities respond to changes in salinity (Berga *et al*., 2012; Sjöstedt *et al*., 2012; Székely *et al*., 2013; Comte *et al*., 2014); however, none examines the

phylogenetic patterns underlying the bacterial responses to the short-term environmental changes induced by shifts in salinity. An increased understanding of mechanisms by which salinity influences bacterial communities is important, given ongoing salinization of coastal habitats or basins due to climate changes or anthropogenic activity (Herbert *et al*., 2014; Mohrholz *et al*., 2015). Little is known about the fate of immigrant taxa transported from different salinity conditions, particularly when competition with local communities is present. The fate of dispersed communities may depend on the niche breath of immigrants, because changes in salinity may favor habitat generalists that have ecological versatility and broad salinity tolerance (Székely *et al*., 2013). Thus, characterizing diversity and composition of dispersed communities following a salinity change will enhance our ability to predict ecological consequences of a given microbial community under saltwater intrusion scenarios. In addition, salinity is easy to manipulate in aquatic systems. I can test, for example, how the locally adapted taxa respond to salinity shifts and what mechanisms drive the response.

1.4.2 The Baltic Sea – a large and stable environment with a natural salinity gradient

The Baltic Sea – a landlocked shelf-sea system that covers an area of 415, 266 km2 – is more stable with long water residence time, approx. 5 years (Reissmann *et al*., 2009). The saline inflows through the North Sea produce a lateral surface salinity gradient throughout the whole Baltic Sea, from high salinities (> 25 psu) in the transition surface zone of the Kattegate to low salinities (< 3 psu) in the Gulf of Bothnian. With the water mixing throughout the Baltic Sea, the middle water body presents a large brackish water zones spanning 62 % of the surface water (Figure. 2) (Reissmann *et al*., 2009). Thus, The Baltic Sea is characterized by a relatively stable salinity gradient that ranges from marine to oligohaline conditions, providing an ideal environment to investigate the impact of salinity as an environmental factor for the distribution and function of microbes.

Previous studies already investigated the bacterial composition in different salinity zones of the Baltic Sea (Holmfeldt et *al*., 2009; Herlemann *et al*., 2011). Typical freshwater bacteria were discovered in brackish water zones, while characteristic marine taxa were rarely observed (Riemann *et al*., 2008; Holmfeldt *et al*., 2009). Freshwater bacterial populations, i.e., ac1 *Actinobacteria*, LD12 *Alphaproteobacteria*, *Limnohabitans* spp. were abundant and active members of the bacterioplankton throughout one year in the coastal Baltic Sea and coexisted with the marine SAR11 lineage (Piwosz *et al*., 2013). A 16S rRNA gene survey with the high-resolution sampling campaign along a transect covering the whole Baltic Sea (2000 km) unveiled a sequential replacement of taxa along the salinity gradient (Herlemann *et al*., 2011). The major shift between freshwater-marine biomes occurred at around a salinity of 8 psu, comparatively to what is known about higher organisms (Remane and Schlieper, 1971). However, in contrast to a decline of diversity in higher organisms, bacterial diversity in the brackish waters was maintained at a high level, which was attributed to a community comprised of adapted bacteria immigrating from both marine and

freshwaters (Herlemann *et al*., 2011). Further, it is found that freshwater-marine siblings tribes, i.e., SAR 11-IV and LD 29 Verrucomicrobia, predominated the brackish-dwelling communities (Herlemann *et al*., 2011). However, the underlying reasons for the observed distribution of freshwater, brackish, and marine taxa along the salinity gradient of the Baltic Sea are still unclear. Specifically, it remains to be tested if salinity is the primary factor shaping microbial communities through e.g., changes in osmoregulation.

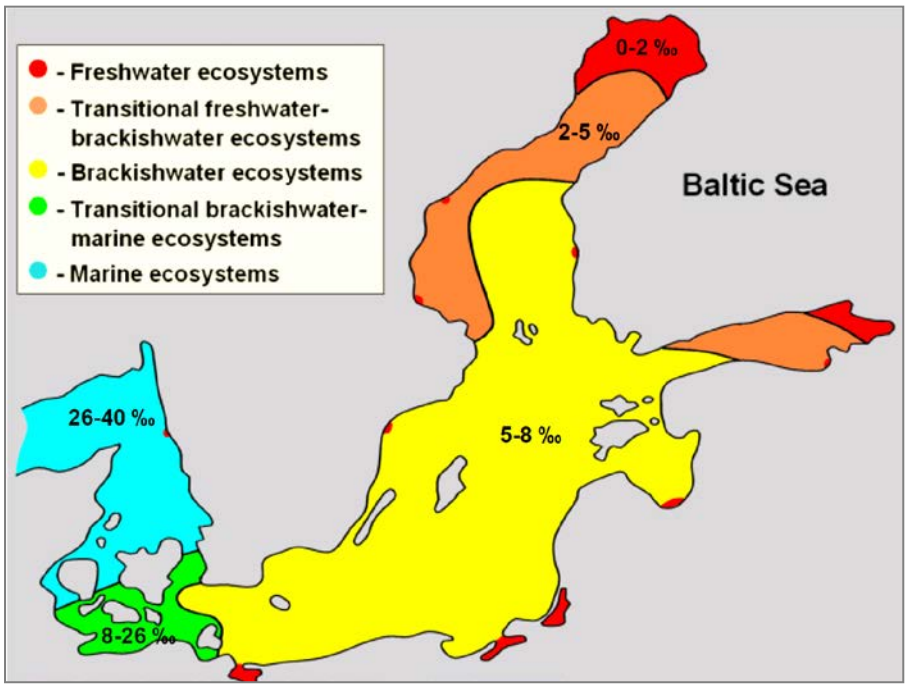

Figure 2 Salinity zones of the Baltic Sea (Modified based on Aladin and Plotnikov, 2009).

1.4.3 St. Lawrence estuary – a productive system with deep salinity gradients

SLE belongs to the deepest and largest estuaries in the world (Figure 3) (Vicent and Dodson, 1999; Dufour and Ouellet, 2007). It is located on the Canadian west coast and connects the St. Lawrence river over a length of 350 km with the Gulf of St. Lawrence, which again separates it from the North Atlantic by nearly 1000 km (Dufour and Ouellet, 2007). The St. Lawrence river begins as the outflow of the Laurentian Great Lakes and widens after 560 km into the large estuary near Île d'Orléans. The freshwater first encounters oceanic salt water and typical two layer estuarine circulation begins (Vicent and Dodson, 1999). The upper estuary ranges between salinities of 0.5 to 25 psu, as a highly turbid section of a complex bathymetry that is strongly influenced by tidal events. This area is dominated by marshes, which are responsible for a large amount of sediment-influx. The plume region is the most productive area of the whole SLE due to the mixing of surface waters with nutrients-rich water upwelled and advented from upstream regions (Therriault and Levasseur, 1985). Continuing downstream, the surface water of the Gulf of St. Lawrence becomes saltier. Ultimately a

true oceanic character is established at the head of the Laurentian Channel in the Gulf where strong upwelling bring deep water to the surface (Dufour and Ouellet, 2007). The semi-enclosed gulf, containing about 35, 000 km^3 of water, opens to the Atlantic Ocean through the Strait of Belle lsle, between Labrador and Newfoundland, and the Cabot Strait between Newfoundland and Cape Breton Island. Hence, compared to the hydrological dynamic of the Baltic Sea, SLE is relatively more dynamic aquatic ecosystem as a mixture of freshwater and saline water results in a salinity gradient of 1 – 31 psu. Due to the unpredictable tidal events and wind-driven event, bacterial assemblages inhabiting in the SLE could have exposed to high dispersion rate and short hydrodynamic residence time. Bacterial cell density decreased sharply along the salinity gradient in the SLE (Painchaud *et al*., 1996). When examining the factors influencing the distribution and activity of bacterial communities in SLE, Painchaud *et al*., (1996) suggested although the appearance of free bacteria appears to be controlled by hydrological parameters (dispersion and water residence time), the bacterial community composition is characterized by biological rates (growth activity, grazing rate). Bacteria in SLE encounter gradients of temperature and salinity, and changes in the bacterial community composition are expected. However, there is a large lack of studies with regards to the growth activity, distribution and composition of the bacterial communities inhabiting the SLE (but see Painchaud *et al*., 1996; Lovejoy *et al*., 1996; Mohit *et al*., 2014), let alone the information regarding the response of these estuarine bacteria assemblages to shifting in salinity conditions. In the present thesis, the upper and Gulf of SLE served as the second sampling campaign for my research. It allows me to extend the salinity-related researches from the Baltic Sea to a truly oceanic condition, while taking into accout the differences in hydrological dynamics for the experimental design.

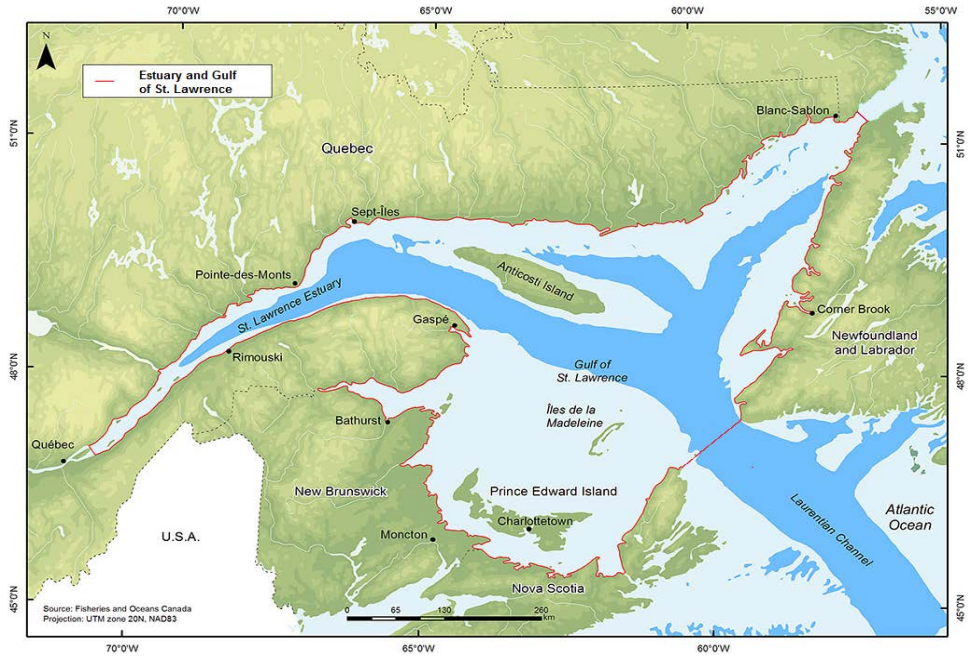

Figure 3 Map of the St. Lawrence river ecosystem (http://www.dfo-mpo.gc.ca).

1.5 Experimental systems

1.5.1 The debate on the use of experimental systems

Microbial communities in the field are often subjected to unknown, or uncontrolled variations across environmental conditions. The complexities in the both biological and environmental parameters make it difficult to identify the mechanisms that govern community dynamics or community behavior. Hence, various forms of model systems, likes microcosms, stable enrichment cultures, mono/co-cultivation of isolated organisms, have been proposed and used to mechanically understand the outcome of microbial interactions, the possible external drivers or the behavior of individuals (e.g., Bell *et al*., 2005; Langenheder *et al*., 2005; Reed and Martiny, 2013; Hausmann *et al*., 2016). However, general concerns are raised that experimental systems lack generality, rely on simplified communities, or are conducted at an inappropriate scale (Bell *et al*., 2009). Specifically, critics have been voiced that, the so-called "bottle effect" usually change expression activities and finally the growth behavior of microbial communities when microbes are not longer in their habitats (e.g., Stewart *et al*., 2012). Still, hypothesis testing requires a controlled setup or manipulation of the communities, which is rather difficult or even impossible to perform in the field (but see Albright and Martiny, 2018). If the research purpose is to understand more generally the processes that are important for community assembly, or this affects the community diversity and functions, there is no intrinsic reason to choose one system over another (Bell *et al*., 2009). Clearly, if the question under investigation is to understand how a community responds to changing environmental conditions, the experiments might be the straightforward system to predict the outcome of responses. With the line of reasoning, microbial communities appear to be the idea model system for biodiversity-functioning studies, as well as the studies unraveling principles of community assembly.

1.5.2 Reciprocal transplants

Reciprocal transplants are conducted by exposing organisms from multiple environments to each other's native environments. In marcroorganisms, this approach has been extensively used to study whether the extent of local adaption and the factors contributing to the distribution limit of an organism compared to its genotypes from other environments (e.g. Kawecki and Ebert, 2004; Hereford, 2009). However, microbial taxa in a community cannot be manipulated one at a time. In microbial ecology, the approach has been adopted for whole-community manipulation (Rawls *et al*., 2006). Reciprocal transplant experiments have been widely used to investigate microbial composition-functioning relationship under laboratory conditions (Langenheder *et al*., 2005; Lindh *et al*., 2015) or in the field (Balser and Firestone, 2005). These experiments are able to disentangle the effect of community composition on ecosystem functions from that of environment, while allowing individual effects of the two parameters and their interactions to be tested simultaneously (Reed and Martiny, 2007). For example, if different microbial communities produce different

processes rates in a common environment, then it can be inferred that the compositional differences lead to the functional differences.

The results obtained from reciprocal transplant studies may not always be straightforward to interpret for the purpose of distinguishing composition effects from environments on function; for example, at the time of sampling, the composition within inocula is variable as across treatments (i.e., the environments to which communities are exposed). Reed and Martiny, (2007) proposed that a short-term and long-term period of reciprocal transplants together could better tease apart community vs. environment effects. With a longer time of incubation, the effect of environment on functions should become stronger although the effect of community origin is still pronounced (Reed and Martiny, 2013). Apart from the caution of interpretation from transplant studies, there are other challenges in performing field experiments with microbial communities in terms of physiochemical nature as well as biotic factors. Using the habitat matrix, such soils seem to be impossible to archive an ideal transplant experiment. This is because geophysical properties of soil may not change after transplantation and the permeation of nutrients in new environment may not equilibrate with that in new environments within a short period (Balser and Firestone, 2005; Reed and Martiny, 2013). Another concern in the transplants is to prevent the immigration of microorganisms into the transplants, while allowing the abiotic conditions to permeate. Gasol *et al*., (2002) constructed a microbial 'cage' that can be applied in aquatic environments using dialysis bags with the desired molecular cutoff. Such dialysis bags-microcosms have been applied for testing a variety of ecological hypotheses in aquatic microbial ecology (Lindström and Östman, 2011; Shade *et al*., 2011; Berga *et al*., 2012; Comte *et al*., 2013; Székely *et al*., 2013). As mentioned in the last section, salinity is easy to manipulate in aquatic systems. In this regard, the osmotic equilibrium is relatively quick between dialysis bags and surrounding environments (which was observed in the pre-tests of my experiments). Given that salinity is a major driver for structuring community composition, I expect that a quick shift in salinity condition could result in at least some changes in the composition of the community inoculated in the dialysis bags. Despite the mentioned difficulties in implementation, transplant experiments are very informative for predictive models of ecological niche and factors driving species distributions in response to global change (Lee-Yaw *et al*., 2016).

1.6 Meta-'omic' approaches in ecological frameworks

Next generation sequencing techniques undoubtedly have revolutionized in the way microbial ecologists address diversity and behavior of microbial communities. The recent development of meta-'omic' approaches permit simultaneously mapping taxonomic and functional assignment to complex microbial communities, by using next generation high-throughput techniques to sequence genomic or transcriptomic data from a whole community (Jansson *et al*., 2012). Metagenomics offers the opportunity to look beyond the presence/absence of taxonomically defined communities,

and instead to understand the relationships between microorganisms as well as their activities and functionalities in a defined environment. As such, most metagenome studies attempted to understand the genetic potential of uncultured bacteria or archaea; the information collection generated by metagenomics is uncoupled with its ecological interpretation. Raes *et al*., 2011 proposed that functional assignment based on metagenome could be informative for quantitatively functional trait-based ecology. For example, recent metagenomics work has demonstrated how a trait-based framework can be used to understand community assembly (Burke *et al*., 2011; Barberán *et al*., 2012). Hence, in the light of functional trait-based ecology, meta-'omic' approaches therefore have the potential to advance BEF researches. Metatranscriptomics shed light on the actual activities and functional prolife of a microbial community, by looking at what genes are expressed by the entire microbial community at a specific time and place (Moran, 2009). The integration of meta-omics offers a comprehensive assessment of present (metagenomes) or active (metatranscriptomes) traits of individual species in a complex microbial community. Metatranscriptome data have recently been used to assess the extent of phenotypic plasticity of individual species within- or between-community after exposure to different sources of dissolved organic matter (DOM) (Beier *et al*., 2015). They developed a metric that quantified functional shifts within species as a measure of phenotypic plasticity, and have shown that the degree of phenotypic plasticity varied broadly among the inspected taxa within a community.

The above studies also demonstrate how meta-'omic' data can be used to explore microbial composition-function relationships. However, assigning shotgun environmental gene sequences to specific taxa is a notorious problem (Prosser *et al*., 2015) due to lack of reference genome in prokaryotes. Such noise is introduced to downstream analyses and would affect interpretations of gene expression patterns in natural communities. Furthermore, functional annotation and gene expression at the species level is challenging, owing to unknown variability in genomic microdiversity between very closely related organisms, as noted else (Bunse *et al*., 2016). However, the increasing coverage in reference databases and newly developed bioinformatics tools for taxonomic and functional binning of metagenome and metatranscriptome data (Alneberg *et al*., 2014), has greatly enhanced the quality of sequence annotations.

In the following sub-chapters of this section, I will provide the information on the use of meta-'omic' data in two case studies later presented in my thesis.

1.6.1 Quantifying multi-functional redundancy between communities

Quantifying and comparing the degree of FR require detailed knowledge about microbial populations that perform a specific function (Allison and Martiny, 2008). This is rather challenging in the context of multi-functionality. The advent of meta-'omic' makes it possible to address the

multi-functional attributes of individual taxa in microbial communities. Miki *et al*., (2014) developed a novel theoretical approach to evaluate multifunctional redundancy in a microbial community using the database of microbial metagenomics. Although metagenomic data may well describe the potential functional capacities of a given community, they fail to delineate functional regulation in response to specific environmental circumstances. The metric used in my study (reference to Beier *et al*., 2017) instead applies the memetatranscriptomic data to quantify FR between microbial communities, thereby reducing the drawbacks of previous approaches. In this case, the transcriptional pattern is a proxy of functional performance. Changes in the transcription level of each gene ortholog were presumably linked via the subsequent translation of mRNA into active proteins that alter rates of a theoretically measureable function. In accordance to this principal of the developed metric, here, FR is considered as the degree to which members in one community compensate for the taxon-level qualitative and quantitative changes in the transcription of gene orthologs in another community. Metatranscriptomic inventories the comprehensive detection of all functional assigned transcripts; thus, this metric provides a measure of multifunctionality that can be used to decipher the FR between two communities.

1.6.2 Investigation of bacterial response to changing salinity using meta-'omics'

Prior studies experimentally testing for the effect of bacterial community composition on the resulting functioning following salinity change yielded mixed results (Langenheder *et al*., 2005; Berga *et al*., 2012; Reed and Martiny, 2013; Lindh *et al*., 2015; Morrissey and Franklin, 2015; Berga *et al*., 2017). Crucially, however, these studies were limited to a number of enzyme assays or bulk measurements; they do not reflect genomic capacities of the responsive bacteria to such exposure in a broader context, for example, metabolic processes for osmoregulation, cell maintenance or energetic purpose.

Meta-'omics' data (especially metatranscriptomics) can be interpreted to better understand how a microorganism interacts with the surrounding environment (including other microorganisms) (Moran, 2009). So far, only few existing comparative metagenomics studies have shown the quantitative differences in the gene pool of freshwater and marine communities, including respiration, glycolysis and osmolyte transporters (Oh *et al*., 2011; Eiler *et al*., 2014; Dupont *et al*., 2014). While these observations on the functional potentials indicate that salinity influences both bacterial composition and their central metabolism, a metatranscriptomic survey has revealed high variation in gene expressions across a salinity gradient (Fortunato and Crump, 2015). Given rapid growth and fast adaptation of bacteria to new environmental conditions (Fukami *et al*., 2007; Shade *et al*., 2012), shortgun metagenomes alone is most likely to miss capturing actual metabolic activities of the emerging communities. Hence, key questions remain unclear how bacteria that are well-adapted to a certain salinity level respond to its change in terms of compositional and physiological

characterizations, and which metabolic pathways the bacteria invoked to respond to it. Salt stress is a highly complex situation characterized by ionic and osmotic stresses accompanying by many secondary stresses, which have been documented for model cyanobacteria at the physiological and molecular levels (Klähn and Hagemann, 2011; Pade and Hagemann, 2015). However, the integration of metagenome and metatranscriptome has not yet been applied in order to gain mechanistic understanding of physiological responses of bacterioplankton to salinity-induced shift in environmental conditions. Linking omics data with specific microorganisms (Gifford *et al*., 2013) and a group of microorganisms with similar ecological traits (Fierer *et al*., 2012) remain a challenge for the microbial ecologists. Controlled experiments are required to investigate what metabolic interactions or physiological traits of microbes impact their fitness and dynamics using 'omics' approaches (Brussaard *et al*., 2016). Experimental metatranscriptomics hold a great promise for understanding the regulation of activities and gene expression patterns in microbial communities when responding environmental clues or defined manipulations (Moran, 2009).

1.7 Aims of the thesis and outline

The present thesis explores the assembly mechanisms that underlie the bacterioplankton community, population variability, and their functional consequences after environmental perturbations, elucidating the link between bacterial community composition and functional traits that they express. These research aspects were addressed in the context of crossing salinity boundaries, as the marine-freshwater transition reflects a historical and evolutionary dimension of microbes. Two significant topics of the thesis that were broached in 4 specific case studies are:

1.7.1 Identification of ecological processes and their contribution to disturbed communities

First, it is essential to disentangle the interplay between community assembly processes; the investigation of their partitioning on the different community members helps understand if and how compositional differences and variability of populations occur within the disturbed communities. The degree of environmental mixing will influence how a taxon responds to environmental changes, including the change in resources, abiotic factors, and biotic interactions. The framework considering the addition of communities to a new environment (Figure 4, upper panel) provides a model system to study the emergent properties of the disturbed communities and if differently composed communities perceive the disturbance differently? As their survival strategies, one could expect that some microbes adapt to broad ranges of environments, while others specialize to certain habitats.

In this regard, Case study 1 investigates whether and how the response of locally adapted bacterial communities to changing salinity relies on the selection of habitat specialists or generalists, using bacterial assemblages from the contrasting habitats of the Baltic Sea. I tested the following specific hypotheses:

(i) Habitat specialists in their favored habitat are dominant over habitat generalists. This is because large changes in salinity create communities where specialist taxa are selectively favored and can fill in newly opened niches generated by salinity disturbance (Logares *et al.,* 2013).

(ii) The selection for specialists after salinity changes primarily relies on the recruitment of rare members from the starting community, while the selection for generalists should be independent of their initial abundance.

(iii) Different assembly processes are important for the dynamics of habitat specialists and generalists.

It should be noted that the setup was designed to exclude the potential effect of dispersal, i.e., exchange of individuals among local communities and to reduce complex interactions between assembly processes. Hence, deterministic processes should mainly drive the studied communities.

Second, dispersal, or the movement of organisms, has an important role in evolution and maintenance of biodiversity (Martiny *et al*., 2006). However, quantifying bacterial dispersal and its role in shaping communities remain a challenge, especially its interaction with contemporatry environmental conditions considered. The framework that compares the communities experiencing regular exchange of cells over time to that without any exchange provides a model system to investigate the effect of dispersal on the diversity or composition (Figure 4, lower panel). In particular, such model can be easily adopted in a different environment, thereby testing how contribution of dispersal to community outcome is dependent on environmental context.

In this regard, Case study 4 investigates the effect of dispersal on the diversity and composition of bacterial communities following a salinity change. Specifically, I want to test the following hypotheses:

(i) Since immigrating taxa can colonize newly open niches after a disturbance, we expect that dispersal can compensate for a potential loss in local diversity following the salinity change.

(ii) Dispersal will influence the compositional response of the communities to a change in salinity, by introduction of taxa that are viable and can thrive under the new environmental conditions.

(iii) The importance of dispersal events differs among bacterial phylogenetic groups.

To test these hypotheses, I implemented a full-factorial experiment using dialysis bags in which brackish and marine bacterioplankton originating from the SLE were incubated under their own waters and the other's environment, with and without dispersal of cells between the two inoculum sources. Hence, this experiment was designed to disentangle the effect of dispersal from that of

contemporary environmental conditions; meanwhile, it enabled us to examine at which salinities dispersal or their interactions are relatively important for explaining community outcome.

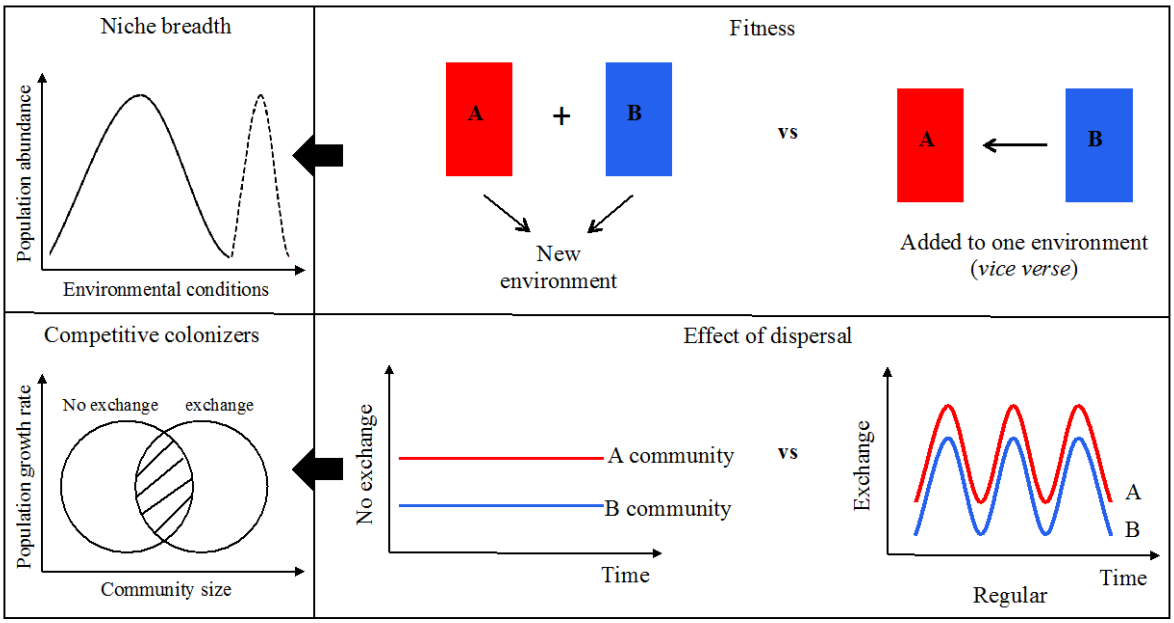

Figure 4. The framework designed in this thesis to examine outcome of community assembly processes. Upper panel: specificially, nitche breadth can be estimated by experimentally testing fitness of bacterial community A and B in a new environment and one another's environmental condition. Population density is plotted against environmental conditions that they are present in order to obtain the niche breadth of a particular population. Lower panel: specifically, competitive colonizers can be identified by expreimetnally manipulating exchange ratio of the community A and B, that is, no exchange vs. exchange regularly over time. The overlap in the communities with and without exchange is a collection of potential colonizers; and the population whith high growth rate in the overlapping is likely the competitive colonizers among all identified ones. This conceptual framekwork was inspired by the work of Rillig *et al*., (2015).

1.7.2 Metabolic processes of ecological groups and the assessment of functional redundancy

Environmental conditions strongly shape the metabolic functions of microbial communities (Louca *et al*., 2016b). The question of when and where microbial communities are functionally relevant, in the face of environmental changes/gradients, are poorly understoond. To address this uncertainty, it is essential to know first if how well response traits from a disturbed community can be correlated with their functional traits. Second, the extent of FR measured after disturbances may imply how relevant a specific function is to change in community composition. Importantly, functional potential described in these earlier studies may not translate directly to gene or enzyme expression (e.g, Fierer *et al*., 2012; Amend *et al*., 2016). Here, I investigated functional attributes of the disturbed communities using metagenomic and metatranscriptomic libraries.

In this regard, Case study 2 identifies the physiological traits that link to the ecological strategies of bacterial taxa following a salinity change. I want to address two main questions:

(i) Do ecological strategies (habitat specialists / generalists) with respects to growth preference pattern in their transcriptional activities at three salinity levels? This is to test an earlier suggestion that similarity in niche (habitat) uses correlated more with the functional genes than with bacterial species, as suggested earlier (Burke *et al*., 2011). I could expect that transcriptional activity of specialists should be higher in their favored than non-favored habitats due to their high functional performance once established (Lennon *et al*., 2012; Graham and Stegen, 2017).

(ii) Do changing salinity induce specific gene expressions of these ecological categories, if so, how difference in transcriptional responses among the specialists with their fitness optima at each of the salinity levels?

Case study 3 investigates whether FR expressed in the communities alter as the disturbance intensity (environmental mixing) changes? Here, a metatranscriptome-based metric that allows multiple functions to be assessed simultaneously was developed and was used to tested the following hypotheses:

(i) Disturbance intensity would influence the degree of FR expressed in the communities. This is based on an earlier finding that FR may vary in different environments as species roles and interactions differ under those conditions (Fetzer *et al*., 2015).

(ii) Environmental conditions are more relevant for the magnitude of FR than the characteristics of the compared communities, as noted elsewhere (Comte *et al*., 2013).

(iii) A greater FR is expressed among closely related organisms when the compared communities are grown under similar than distinct environmental conditions. This is because closely related taxa share more similar traits, such as similar salinity preference (Martiny *et al*., 2015). A change to similar environmental conditions other than a change to distinct environmental conditions would accordingly allow phylogenetically closely related taxa to replace the functional attributes by the originally active taxa at high frequencies (Blomberg *et al*., 2003).

2. Materials and methods

Water samples analyzed in this thesis were sourced from the Baltic Sea and the upper part of the St. Lawrence estuary (SLE), spanning a salinity gradient of 3-28 psu and 24-31 psu, respectively. Two transplant experiments were performed using the water from the two aquatic environments. A number of ecological aspects were assessed using the Illumina Miseq sequencing: richness (number of distinct operational taxonomic units, OTUs defined at the 97% sequence similarity level), evenness (equitability of OTUs), structure (representation of dominant and rare OTUs), phylogenetic diversity (the number and relationship of phylogenetic lineages), and composition (relative abundance of specific taxa defined as phylotypes). Additionally, metatranscriptomic data were analyzed to assess the performance of multi-funtionality in bacterial communities, as well as investigated the central metabolism (functional annotation of individual gene orthologs of a taxon) after exposure to changing salinity.

2.1 Sampling of the Baltic Sea and transplant experiment 1

2.1.1 Study sites and experimental setup

The Baltic Sea was characterized by a relatively stable salinity gradient that ranges from marine to oligohaline conditions (Reissmann *et al*., 2009; Herlemann *et al*., 2011), thereby providing an ideal environment to investigate how locally adapted bacteria responded to changing salinity. Surface water (3m) from three regions of the Baltic Sea area was collected during the Alkor 439 German research cruise: Skagerrak (salinity 28 psu; 58°7’ N, 9°59’E; June 9, 2014), Bothnian Bay (salinity 3 psu; 514 65°26’ N, 23°17’ E; June 12, 2014), and Baltic Proper (salinity 7 psu; 58°35’ N, 18°14’ E; June 14, 2014). For simplicity, we refer to the three locations as ‘marine’, ‘oligohaline’ and ‘brackish’ sites, respectively. Approximately 110 L of the sampled water from each site was filtered through 0.2 μm, 142 mm cellulose acetate filters (Sartorius, Göttingen, Germany) on-board. The filtered water from the three sites filled three tanks, correspondingly, which were kept in the dark at a constant temperature of 18 °C. The filters collected during the filtration were stored at - 20 °C and were later used to prepare the nutrient supplements as described in the following section. Moreover, 40 L of sampled water were pre-filtered through a 25 μm mesh and were collected in canisters, which were stored at 4 °C in darkness until the laboratory setup of the experiment.

Before the start of the transplant experiment 1 (June 30, 2014), the 110 L water in each tank was re-filtered as described above to ensure the water free from bacteria as possible. After the re-filtration, 100 L of these filtrates from each tank later served as oligohaline (O), brackish (B), or marine (M) sterile media, respectively. In order to obtain protist-free inoculum, the pre-filtered 40 L water from each canister was further filtered onto 0.8 μm, 142 mm cellulose acetate filters; the

filtered water were used as oligohaline (o), brackish (b), or marine (m) inoculum. The filters collected from the on-board pre-filtration were further flash-frozen using liquid nitrogen, and then were broken down into pieces. These pieces from all filters, regardless of the medium of origin, were then autoclaved together in 300 mL of deionized water in a glass bottle. The autoclaved water was subsequently filtered through 0.22 µm, 47 mm membrane filters (Millipore, Billerica, USA) to remove the suspended particles and filter pieces and then used as a nutrient supplement. Each medium and inoculum was amended with 50 mL of the nutrient supplement in the evening before the experiment was initiated, in order to sustain bacterial growth.

Dialysis tubing with molecular weight cutoff of 12 – 14 kD (Spectrum Laboratories, CA, USA) was chosen to ensure free exchange of dissolved organic matter and nutrients without allowing the movement of microorganisms (protists, bacteria, viruses). Dialysis tubing pieces were soaked in sterile water for 18 h before the setup and were rinsed again. Triplicate dialysis bags were filled with 3 L of o, b or m source inoculum and were placed in three incubation tanks for a total of 30 microcosms (Figure 5A). The microcosms were incubated in darkness at 23 °C for 4 days; this short incubation time assured that the permeability of the dialysis bags were maintained without biofilm formation on the bag surfaces.

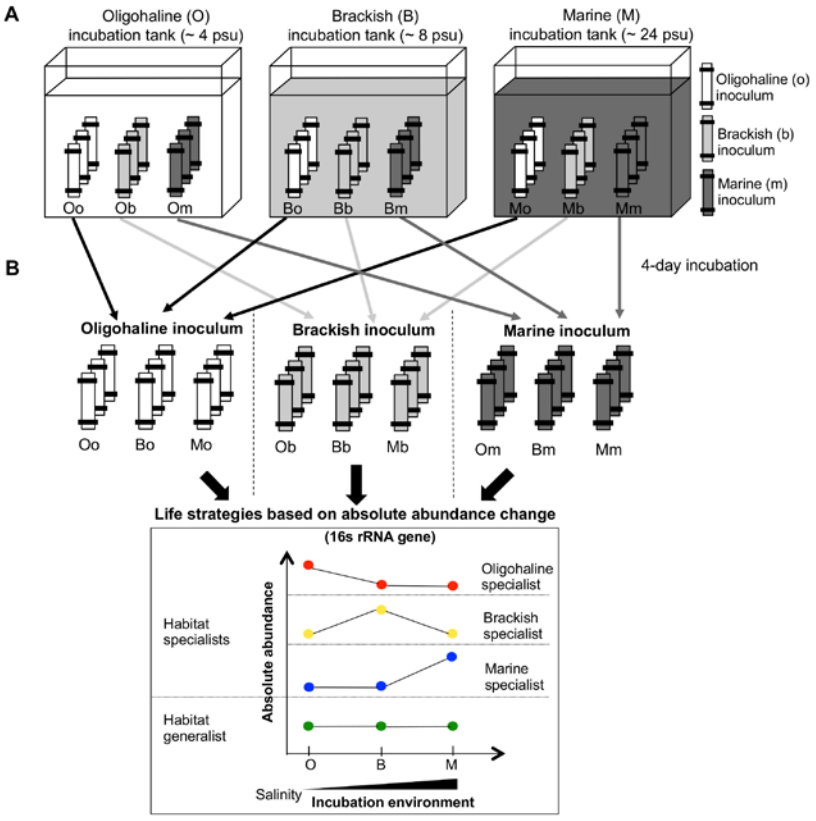

Figure 5 A) experimental design. Triplicate dialysis bags containing oligohaline (o), brackish (b), and marine (m) bacterial assemblages reciprocally incubated in oligohaline (O), brackish (B), and marine (M) tanks. Capital letters O, B and M represent incubation environments, while lowercase letter o, b and m represent inoculum source. **B**) schematic overview illustrating the responses of different life strategies. The upper part of

the figure shows that the microcosms that were established from the same inoculum source were compared for assessing the responses of individual OTUs across media with distinct salinities. The lower part of the figure illustrates the life strategies that were determined based on changes in taxon's absolute abundance across three incubation environments. (Shen *et al*., accepted; doi: 10.1111/1462-2920.14059; the article is protected by copyright. All rights reserved)

2.1.2 Bacterial abundance and heterotrophic production

Samples for bacterial abundance and heterotrophic production were collected from the initial inocula, as well as daily from each microcosm. Bacterial abundance was determined by flow cytometry, as described in (Gasol and del Giorgio, 2000). Briefly, bacterial cells (4 mL aliquots) were preserved with 1% paraformaldehyde and 0.05% glutaraldehyde, and flash frozen in liquid nitrogen. The fixed samples were stained using 10 x SYBR Green I before being enumerated in a FASCalibur flow cytometer (BectonDickinson, Fremont, USA). Heterotrophic bacterial production was estimated based on [^{3}H] thymidine incorporation assay. For each sample, triplicate aliquots (5 mL) and a formaldehyde-killed control were incubated with 25 µl of [^{3}H] thymidine (at a final concentration of 20 µM) for 1 h at a room temperature. A theoretical conversion factor of 20 fg C cell^{-1} was used to convert thymidine incorporation rates to bacterial carbon production (Lee and Fuhrman, 1987).

2.1.3 Environmental parameters analyses

Salinity, temperature, and pH were monitored daily during the experiment. For the quantification of inorganic nutrients and dissolved organic carbon (DOC), 18 mL of samples was collected from the initial inocula (day 0) and all microcosms at the end of the experiment (day 4) as well as the nutrient supplement, and was filtered through GF/F 25-mm glass-fiber filters (Whatman, Dassel, Germany). Concentration of inorganic nutrients (NO_3^-, NO_2^-, PO_4^{3-}, NH_4^+, and SiO_2^-) was determined colorometrically according to Grasshoff *et al*., (1999) by means of a Seal Analytical QuAAtro Automated nutrient analyzer (SEAL Analytical GmbH, Norderstedt, Germany). DOC was analyzed on TOC-VCPH TOC Analysator (Shimadzu Europe GmbH, Duisburg, Germany). Supplementary Table S1 summarizes the water chemical and biological measurements.

2.1.4 Nucleic acid extraction and sequencing

For nucleic acid extractions, 3 L of the initial inocula were collected before filling the dialysis bags, and then fixed by the addition of 10 volume % of an ethanol:phenol mix (19:1) for mRNA preservation (Feike et al., 2012). The fixed samples were then filtered through 0.22 µm pore-size, 47 mm diameter membrane filters (Millipore, Billerica, USA). All filters were immediately submerged in RNAlater (Qiagen, Valencia, USA) and stored at - 80°C until nucleic acid extraction. The same volume of water was also harvested from all dialysis bags at the end of the experiment (day 4), and processed in the same manner.

DNA and RNA from a total of 30 samples (initial inocula of three sources on day 0 and all microcosms on day 4) were simultaneously extracted from the same filter using the AllPrep DNA/RNA mini kit (Qiagen, Valencia, USA) following the manufacturer's protocol. A total of 30 DNA extract was sent for paired-end Illumina Miseq amplicon sequencing (2 x 300 bp, LGC Genomics GmbH, Berlin, and Germany) for bacterial community analysis. The hypervariable V3-V4 region of the 16S rRNA gene was sequenced using the forward primer 341F 5'-CCTACGGGNGGCWGCAG and the reverse primer 805R 5'-GACTACHVGGGTATCTAATCC (Herlemann *et al*., 2011).

For metagenome sequencing, the total DNA derived from the biological triplicate of each treatment was pooled and sent for Illumina Hiseq paired-end sequencing after library preparation with the Rubicon Thruplex kit (2 x 150 bp, SciLifeLab, Stockholm, Sweden). The metagenomic data were used to create assembled contigs for functional and taxonomic annotations to which metagenomic reads as well as reads from the below described metatranscriptomic data were mapped. In addition, the metagenomic data was used to estimate community functional diversity and average genome size for each treatment.

Genomic DNA in the RNA extracts from the 27 replicate samples (initial samples were not included) was removed by DNase treatment using the TURBO DNA-free kit™ (Invitrogen, Darmstadt, Germany). The DNase-treated RNAs were tested for traces of DNA by PCR amplification with the bacterial 16S rRNA gene primers of two sets: comf1/r2ph (Stolle *et al*., 2011) and 341f/805r (Herlemann *et al*., 2011). The products were visualized on 1.2 % agarose gels before being cleaned using the RNA Clean & Concentrator™-5 kit (Zymo Research, Irvine, USA). The above-described DNase treatment and the tested PCR step were repeated until no positive DNA amplification was detected. Quality and concentration of the purified RNA extracts were checked on an Agilent 2100 Bio-analyzers. The purified RNA extracts were sent for Illumina Hiseq paired-end metatranscriptome sequencing (2 x 150 bp), after library preparation with the TruSeq chemistry (TruSeq Stranded RNA HT kit) combined with RiboZero ribosomal depletion (bacteria), to the SciLife-Lab (Stockholm, Sweden) or Fasteris (Plan-les-Ouates, Switzerland). All replicates of treatment 'Mo' and one replicate of 'Bm1'were not included in the metagenome and metatranscriptomic analyses due to the unsuccessful library preparation for metatrancriptome sequencing.

2.2 Transplant experiment 1: bioinformatics

2.2.1 16S rRNA gene sequence processing

The paired-end reads were merged in FLASH (Magoč and Salzberg, 2011), which were later processed using MOTHUR v.1.34.0 (https://www.mothur.org/wiki/MiSeq_SOP, assessed: 20 January 2014) (Kozich *et al*., 2013). A total of 895,209 reads were quality controlled in the

following settings: any reads with a length < 450 base pairs, ambiguous bases > 0, and homopolymer length > 6nt were removed for further analysis. The resulting unique sequences were aligned using the SILVA v119 bacterial reference database. The sequences were further screened for putative chimeras with UCHIME (Edgar *et al*., 2011). The remaining non-chimeric 110,504 sequences were taxonomically classified using the naïve Bayesian classifier (Wang *et al*., 2007) and the RDP database. OTUs were assigned at a 97% sequence similarity (average neighbor method). The representative sequence for an OTU was the sequences with the smallest maximum distance to the others. All sequences of 586 Archaea, Eukaryota, chloroplasts, and mitochondria, in addition to unknown sequences and singletons (OTUs with only one sequence), were discarded from the data set. In order to standardize the uneven sequencing effort, all samples were normalized to 5,748 reads (the smallest library size; Table S1).

2.2.2 Meta-'omic' sequence processing

Metagenomic protein-coding reads from all samples were assembled using the ray metagenome assembler (Boisvert *et al*., 2012) and a kmer size of 51. Coding regions within the assembled contigs (open reading frames = ORFs) were identified using the prodigal software (Hyatt *et al*., 2010). Individual protein-coding reads from metagenomic and metatranscriptomic data were mapped on the contigs with predicted ORF sequences from the assembly using the Bowtie2-aligner (Langmead & Salzberg, 2012). Subsequently, the predicted ORFs were annotated using BLASTP (Altschul *et al*., 1997) against the KEGG database and the RefSeq database. The resulting ORFs were manually extended with 30 assembled genomes from the Baltic Sea (Hugerth *et al*., 2015). The first BLAST hit was used as the annotation reference, with 10^{-4} as the e-value cut-off taxonomic annotation. In addition to the e-value cut-off, the ratio of the score against KEGG database to the score derived from a reciprocal BLAST search exceeded 0.4 (Rasko *et al*., 2005); only the hit that met such criteria were considered for functional annotation. All contigs (or reads that were mapped on this contig) were sorted into taxon bins according to the genome of the first BLASTP hit against the RefSeq Database, as described previously (Beier *et al*., 2015). Archaea representing < 0.008% of all reads across samples, only contigs that had been assigned to bacterial genomes and to KEGG gene orthologs were selected for downstream analyses. Table S2 summerzies metagenome and metatranscriptome sequences reads. Additional details on bioinformatics for metagenome and metatranscriptome data are reported in Appendix Text 1.

2.3 Transplant experiment 1: life strategy groups and their community assembly

2.3.1 Community analyses and experimental conditions

To assess the growth of bacterial inocula in the three environments after 4-day incubation, the significant difference in bacterial abundance was analyzed using Kruskal-Wallis test. The effect of

inoculum source, incubation environment and their interaction on bacterial community composition (BCC) was tested using a permutational multivariate analysis of variance (PERMANOVA). Differences in community composition of the inocula and the microcosms (β-diversity) were visualized using a non-multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity matrix. The variability in experimental conditions, including nutrients, DOC, salinity) among the samples was analyzed using a principal component analysis (PCA).

2.3.2 Grouping of OTUs into life strategies

Previous studies have used either the presence/absence or the abundance data to define ecological groups (Székely *et al*., 2013; Morrissey and Franklin, 2015). However, the use of presence/absence data seems to be inappropriate in this study: the residual DNA of non-viable cells that were intolerant to salinity changes may still be detected in the 16S rRNA gene libraries after a short incubation period (Jones and Lennon, 2010). Thus, OTUs were sorted into life strategies by comparing their abundance data across treatments (Figure 5B).

The OTU-table that contained a total of 2927 OTUs (after subsampling) was separated into three sub-datasets according to the inoculum source. Before detecting the OTUs' life strategies, low abundance OTUs were filtered out from each dataset to reduce noise and identify the OTUs that responded to altered salinity; that is, OTUs either exhibited < 3 sequences in all microcosms or that were not detected in at least two of the triplicate microcosms (Evans and Wallenstein, 2014). To account for variability in cell abundances across treatments, absolute abundance of each OTU was obtained by multiplying relative abundance (proportion of total sequence reads) with the total prokaryotic cell counts in each of the samples, as applied elsewhere (Andersson *et al*., 2010; Props *et al*., 2017; Ward *et al*., 2017). Owing to numerous inherent biases of DNA extraction, amplification as well as differences in gene copy number, absolute abundances were not interpreted as direct cell counts of the OTUs, rather as an estimate for the dynamics of their cell abundance across samples. By contrast, 16S rRNA gene relative abundance is not suited to track OTUs' dynamics in communities that differ in total cell numbers (Widder *et al*., 2016), because simply enrichment of taxa does not necessarily relate to the outgrowth of taxa, i.e. increase in absolute abundance (Props *et al*., 2017). For instance, OTUs whose relative abundance increases may have actually decreased in bacterial cell numbers.

Within each of the three OTU datasets after filtering, difference in absolute abundance of individual OTUs among incubation environments (O, B, and M) were analyzed using ANOVA. Significance values were corrected for *post-hoc* pairwise comparisons using Bonferroni correction procedure with an adjusted alpha of 0.05 (alpha-error < 0.05). OTUs whose absolute abundance did not differ significantly among treatments (ANOVA, alpha-error $\geqslant$0.05) were subjected to false-negative detection (ANOVA, beta-error < 0.05); OTUs below this cutoff were defined as 'habitat

generalists' (namely, OTUs exhibited highly similar absolute abundances in all three habitats; Figure 5B). The OTUs that did not pass statistical filtering at either the alpha- or beta-error cutoff were defined as 'undetermined', as no specific life strategy assigned.

For the OTUs that exhibited a significant difference in absolute abundances following salinity change in each data set, hierarchical cluster analysis (Pearson's correlation, average clustering criterion) was used to group those OTUs according to the in absolute abundance across the three habitats. Dendrogram was used to cluster the OTUs that had similar abundance pattern, in dependence of the maximal abundance (growth) in the O, B or M habitat, respectively, without any information on phylogeny. Accordingly, those OTUs were defined as 'habitat specialists' (namely, OTUs that showed a high absolute abundance in one habitat but lower in the two other habitats; Figure 5B). ANOSIM was used to verify the clusters in order to determine whether the groupings of the specialist strategy were significantly different from one another.

After assigning life strategies to individual OTUs, mean relative abundances in the favored habitat of specialists were compared with that of generalists found in that habitat. Difference in mean relative abundances was tested for all 9 microcosms together using a repeated-measures t-test on log-transformed data, followed by independent two-sample t-tests for each microcosm. Significance *P*-values of the latter tests were manually corrected using the Bonferroni correction procedure.

2.3.3 Recruitment of initially rare and abundant members

One aim of the study was to examine if the selection for habitat specialists after exposed to changing salinity relied on the recruitment of rare members from the starting communities. For this purpose, the proportions of both initially rare and initially abundant OTUs among the OTUs belonging to each life strategy were inspected. Following the abundance threshold in Hausmann *et al*., (2016), initially abundant members were defined as OTUs with a relative abundance of $\geqslant$ 0.1% in the initial inoculum, while initially rare with a relative abundance of < 0.1%.

2.3.4 Phylogenetic relatedness of the life strategy groups

A phylogenetic tree of the OTUs that were assigned to life strategies was constructed using FastTree (Price *et al*., 2009) in QIIME (Caporaso *et al*., 2010). Phylogenies of these OTUs were visualized using iTOL v3 (Letunic and Bork, 2016).

Following the method of Webb (2000, 2002), the net relatedness index (NRI) and nearest taxon index (NTI) were used to measure the degree of phylogenetic relatedness among OTUs displaying the same life strategy within each data set. In other words, whether OTUs in a particular strategy were more closely related to one another than to the OTUs in other strategies and originating from the same source inoculum? The degree of phylogenetic clustering of co-occurring species within a community indicates the ecological processes that influence their assembly. Both indices are

standardized measures of the phylogenetic distance of target species, relative to the phylogeny of a species pool (Webb 2002). The key difference is that calculation of the NRI is based on average phylogenetic distance between all co-occurring taxa, while the NTI considers only the average distance between co-occurring closest phylogenetic relatives (Koeppel and Wu, 2014). Due to the slight differences in algorithms for determining phylogenetic distance, the NTI focuses on clustering at lower taxonomic levels and is less sensitive to the higher-level phylogenetic structure than the NRI (Webb, 2000). The statistical significance of observed NRI and NTI values was tested using a two-tailed test against the expected values calculated from artificially constructed phylogenies. The artificial phylogenies were obtained by randomly shuffling taxa labels (1000 random permutations) across all considered OTUs. Positive NTI/NRI values (and low *P*-values: $P < 0.05$) indicate that the considered OTUs are (significantly) more closely related (phylogenetically clustered) than expected by chance, whereas negative values (and high P-values: $P > 0.95$) indicate that the considered OTUs are (significantly) less closely related (phylogenetically over-dispersed) than expected by chance. The NRI and NTI were computed using the Picante package (V.1.6.2; Kembel *et al*., 2010) in R.

2.4 Gene expression analyses of bacteria after exposure to changing salinity

2.4.1 Matching OTUs generated from 16S rRNA gene data with meta-omic data

The utility of the metatranscriptomes was to assess transcriptional activities and expression of ecological groups (habitat specialists / generalists) that were previously identified. To screen their transcriptional activity and gene expression at different salinities, I chose habitat specialist or generalist OTUs that met the following criteria: if they showed the same environment-preference in the treatments inoculated with at least two inoculum sources; and no contradicting preference was detected in the treatments inoculated with the third source, including absence or low abundance of the OTUs. These criteria allowed us to assume that, each of the selected OTUs belong to a 'truly' single organism, despite a general bias to the taxonomy defined by a 97 % sequence similarity of 16S rRNA gene sequences. The first 10 hits for each of the selected OTUs were generated through a BLASTN search of its representative sequence against the RefSeq RNA database, and then we extracted its taxonomy at the genus level. Additionally, the mean relative abundance obtained from the triplicates of each treatment of each OTU for all 9 treatments, was correlated with the relative abundance of protein-coding metagenomic reads that were mapped to contigs of each genome bin using Pearson's correlation. Subsequently, all contigs being annotated to genome bins that (i) correlated to the abundance of an OTU at Pearson's correlation coefficient $r > 0.9$, and (ii) were annotated to the same genus as this OTU, were pooled into a taxon bin. The metagenomic data were used to create assembled contigs for functional and taxonomic annotations to which metagenomic reads and metatranscriptomic reads were mapped. Subsequently, all metagenome or metatranscriptome reads that were mapped to the contigs of such taxon bin were considered as

genome or transcriptome reads originating from the corresponding OTUs.

The number of transcripts for each OTU per liter of water was calculated from metatranscriptome data from the corresponding taxon bin as published elsewhere (Satinsky *et al.*, 2013), while assuming the average length of an transcript with 1000 nt. The average number of transcripts for each cell of a certain OTU was estimated by dividing the number of transcripts per liter by the number of cells l^{-1} for an OTU. The latter was calculated by multiplying the overall cell counts measured by flow cytometry with the relative abundance of each OTU (i.e., the number of the metagenome transcripts that were assigned to that OTU). The OTU (absolute) abundance estimated here was retrieved from the metagenome reads, but not from amplicon reads as previously described for the assignment of OTUs strategies.

To explore the overall patterns in the transcript abundance for the selected OTUs belonging to each of the four strategy groups, one-way ANOVA was performed and the significant level was detected using Tukey's *post hoc* test.

2.4.2 Differential gene expression

To test if the selected OTUs within each ecological group share a unique set of significantly expressed genes in response to different growth media, transcriptomic data of each taxon bin were subjected to differential expression analyses using the DeSeq2 package implemented in R (Love *et al.*, 2014). Pairwise comparisons of differential expression for each gene by individual taxa were performed between any of the two incubation environments. Differentially expressed genes at a significance level of $P < 0.01$ were then extracted. Transcriptomic data retrieved from the treatments inoculated with three sources but in the same medium were used as replicates. Only taxon bin exceeding 1,000 reads in each replicate were included. Subsequently, Transcriptomic content of all gene orthologs were mapped to KEGG ontology (Kanehisa *et al*., 2007) in order to gain the functional information and metabolic pathways of the studied ecological groups. To account for uneven sequencing depths, the data were normalized using the default setting in the DeSeq2 and the degree of changes in relative abundance of gene orthologs was presented as log2 fold changes.

2.5 The assessment of functional redundancy using metatranscriptomics

2.5.1 Estimation of functional redundancy (FR)

FR was obtained by subtracting functional change in the combined community (FC) from theoretical community functional change (TFC), based on metatranscriptome data (Figure 6). Specifically, difference in metatranscriptome gene ortholog count tables for either between-community or in individual taxon bins were calculated using the Bray-Curtis dissimilarity metric for FC in the combined community or in taxon bins. TFC was defined as the sum of the functional changes for each taxon bin weighted for average transcript abundance in the two compared communities. There

are two outcomes derived from the calculations of the two parameters: 1) TFC will be higher than FC if there is compensation by gene ortholog transcription among taxa: i.e., when the transcription of a certain gene is associated with one genome bin in one community and with another genome bin in the second community; 2) TFC and FC will be identical if the transcription of specific gene orthologs is never compensated among different taxon bins. Hence, the difference between the TFC and FC is a quantitative indicator of the degree to which gene transcription was compensated, which is used here to quantify FR between two communities. Additional details on the calculation of TFC and FC are reported in Appendix Text 2.

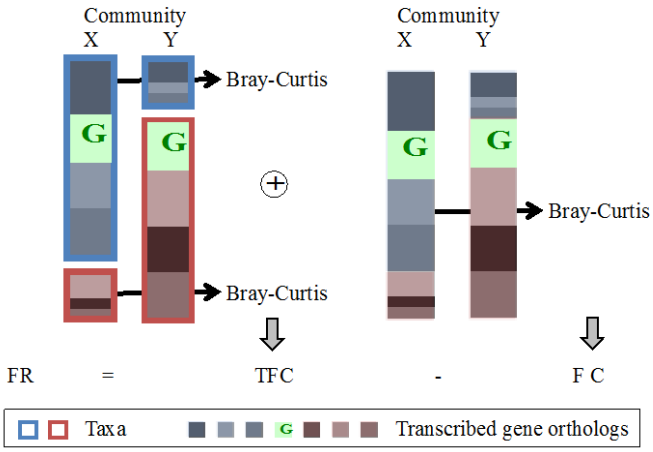

Figure 6 Schema illustrating the metric used to quantify functional redundancy (FR) based on metatranscriptome data. The outer lines or fillings of the same color indicate identical taxa or gene orthologs, respectively. The theoretical community functional change (TFC) was calculated by summing the transcriptional changes in each individual taxon bin (estimated by Bray-Curtis distances and considering transcript composition and relative abundance), which were weighted for the taxon's mean relative transcript abundance in the compared communities. FC is the transcriptional change in the bulk community (estimated by Bray-Curtis distances). If transcriptional changes in one taxon are compensated by those in another, as illustrated for gene ortholog G, TFC will be larger than FC, because taxon-specific transcriptional changes in gene G affect TFC, but not FC. The difference between TFC and FC was interpreted as FR, which estimates the degree to which taxon-specific translation changes within individual taxa in one community are compensated by the activity of other taxa in the other community. (Beier *et al*., 2017; the permission is granted for the passage)

All community pairs of either brackish or marine inoculum, i.e., two communities originating from the same source inoculum but grown under different salinity conditions, were constructed for the calculation of FC, TFC, and FR; that is, OB: oligohaline vs. brackish medium, ~ 3.4 psu salinity difference; BM: brackish vs. marine medium, ~ 16 psu salinity difference; OM: oligohaline vs. marine medium, ~ 19 psu salinity difference). In addition to quantification of FR between-community, the degree of FR at different taxonomic ranks was assessed. The genome level was considered as the species level in this case, while all other phylogenetic levels were defined by the RefSeq database (genus, family, order, class, phylum, superkingdom). FR at the kingdom level

was, by default, equal to 0, provided that only prokaryotes belonging to the same kingdom (Bacteria) were included in the analyses. The differences in TFC, FC and FR at different salinity disturbances were analyzed separately for the marine and brackish inoculum using ANOVA. In addition, another ANOVA test was used to test the effect of incubation environment and inoculum source on TFC, FC and FR.

2.5.2 Analyses of diversity indexes and effective genome size

On the basis of 16S rRNA gene, species diversity (Shannon index) in each sample was estimated from the number of OTUs, while a phylogenetic tree and a rarefied OTU-table were used to calculate the abundance-weighted phylogenetic diversity (PD) as described elsewhere (Vellend *et al*., 2011). For the metagenome-based data, the effect genome size was calculated for each metagenome with the parameters for a, b, and c set to estimate marker gene density as described in Raes *et al*., (2007), and indicated the average genome size of cells in a sample. Functional diversity was estimated via the Shannon diversity of KEGG gene orthologs detected in the metagenome. All diversity indexes and the effective genome size were calculated with taxon or gene abundance-weight measures included, as comparably FR was also weighted for both the transcript abundance of taxa and gene orthologs. To assess the influence of the different diversity measures and the effective genome size on FR, a PCA was performed after scaling and centering the values for all parameters.

2.6 Sampling in the St. Lawrence estuary (SLE) and transplant experiment 2

2.6.1 Study site and experimental setup

Microorganisms inhabiting SLE are not only experiencing fluctuations in salinity but are also transported via currents or tidal events (Mucci *et al*., 2011; Dinanuer and Mucci, 2017), thereby providing a suitable aquatic system for our purpose. Surface water (3 m) from two regions of the Saint Lawrence estuary was collected using a Rosette sampler: the Gulf of St Lawrence (47° 11.1547′N, 59° 32.2932′W; Salinity ~30.35 psu) on August 26, 2015 and Lower SLE (48° 38.3388′N, 68° 37.9090′W; Salinity ~24.29 psu) on August 30, 2015. For simplicity, we refer to sampling locations as ‘marine’ and ‘brackish’ sites, respectively; water from these sites served as a source for both medium and inoculum. Approximately 200 L of the sampled water from each site was then filtered through a 200 µm mesh into 30 L carboys to remove large zooplanktons. To prepare the medium, the pre-filtered water was subsequently filtered through 142 mm GF/F, filters (Whatman, Dassel, Germany) in order to remove protists. For the microbial inoculum, the < 200 µm water was further filtered through a 25 µm mesh to remove large phytoplankton. All inocula and media were stored at + 4°C in the dark until initiation of the experiment.

The same type of dialysis bags as used in the transplant experiment 1 was chosen. Dialysis bags with the length of 45 cm were rinsed thoroughly 24 hours before use and soaked in Milli-Q water overnight, and were rinsed again. All inocula and media were acclimated to a constant temperature of 19 °C for 12 hours before the setup of the experiment on August 30. Each dialysis bag was filled with 1.5 L of either brackish or marine inoculum; and two incubation tanks were filled with ~ 95 L of the brackish and marine media, respectively. Dialysis bags were then placed in the tanks and the incubations were carried out in triplicates for 5 days in the dark at 19 °C, resulting in a total of 30 microcosms (Figure 7). Salinities were measured and equalized between the dialysis bags and incubation tank in < 12 h.

The dispersal manipulation was initiated right after the equalization of salinity (12 h after the start of incubation) to assure that cells between two inoculum sources were dispersed in the similar incubation environment. For the dispersal treatment, 50 mL of water was exchanged by pipetting between dialysis bags inoculated with brackish and marine inoculum twice per day (every 12 h), representing a daily exchange of 6 % of each microcosm volume (dispersal treatments, DT, Figure 7). To keep the same level of physical disturbance in all microcosms, the other half of the microcosms were subjected to the same action, e.g., pipetting water out-and-in from the same dialysis bag without exchanging microorganisms among them (non-dispersal treatments, ND, Figure 7). All dialysis bag-microcosms were destructively sampled on the 5-day of the incubation (that is, 12 h after the last dispersal manipulation on day 4). One replicate of treatment Bb_ND was not included in all analyses due to water loss during sampling. Nutrients and dissolved organic carbon (DOC) were determined as described elsewhere (Grasshoff *et al*., 1999). See Appendix text 3 for details on the analyses of environmental parameters.

2.6.2 Cell abundance of bacteria and protists

Samples for microbial abundance analysis were collected from the initial microbial incoula (day 0) and all microcosms (day 5). For bacterial abundance, 4 mL aliquots of each sample were preserved with formaldehyde at a final concentration of 2% and immediately flash frozen in liquid nitrogen until determination of flow cytometry. Samples were stained using 10 x SYBR Green I (Life Technologies, Darmstadt, Germany) before being counted in a FASCalibur flow cytometer (BectonDickinson, Fremont, USA), as described elsewhere (Gasol and del Giorgio, 2000). Cell abundance of protists was determined using epifluorescence microscopy, according to (Weber *et al*., 2012) with minor modifications. Briefly, 10 mL from each microcosm were fixed with formaldehyde at a final concentration of 2% and stored at 4 °C. After about 4 h fixation, samples were filtered onto 0.8 μm, 25 mm black filters (Whatman, Dassel, Germany), and filters were stored at – 20 °C until further processing. For enumeration, cells on the filters were stained using 4′,6-diamidin-2-phenylindol and randomly selected microscopic fields were inspected using a Zeiss Axioskop 2 mot

plus microscope (Zeiss, Oberkochen, Germany) with 63x magnification. Technical triplicates were carried out for each sample.

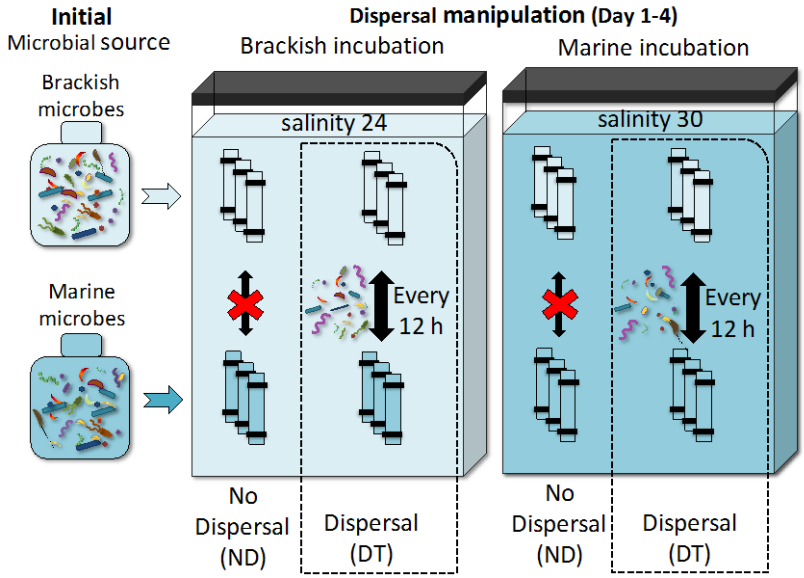

Figure 7 The design of transplant experiment 2 using dialysis bags. Triplicate microcosms containing either a brackish or marine inoculum were reciprocally incubated in the brackish and marine environments. Capital letters B and M represent the brackish and marine incubation environments into which the dialysis bags were placed, respectively. Lowercase letters b and m represent the source (origin) of the initial brackish and marine inocula, respectively. ND indicates the treatments with no dispersal, while DT indicates the treatments with dispersal. The combination of these letters represents the particular combination of the incubation environment for the microcosms, the inoculum source and dispersal. For example, microcosms 'Bb_DT' indicates the microcosms in which the brackish inoculum was incubated in the brackish environment, and was subjected to dispersal.

2.6.4 Active bacterial community composition

Bacterial communities were analyzed from two of the initial microbial inocula and the microcosms on day 5. Water samples (1.5 L) from the initial inocula and ~ 1.4 L of water from each dialysis bag were filtered through 0.22 µm pore size filters (Millipore, Darmstadt, Germany) and immediately flash-frozen in liquid nitrogen. All filters were later stored at – 80 °C until nucleic acid extraction. RNA in a total of 29 samples was extracted using the RNeasy mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The removal of gemonic DNA from the RNA extracts was carried out in the same manner as described in the experiment 1. The purified RNA extracts were reverse transcribed using the iScript cDNA synthesis kit (Bio-Rad, München, Germany). The hypervariable V3-V4 region of the bacterial 16S rRNA gene was targeted using primers 341f/805r. This allowed us to determine mainly the metabolically active fraction of the bacterial communities (Blazewicz *et al*., 2013) that responded to experimental conditions and dispersal treatments. The amplicons were sequenced using the Illumina Miseq system (2 x 300 base pairs) at the LGC

sequencing center (Berlin, Germany).

2.6.5 Sequence processing

Sequences were processed using MOTHUR v.1.36.1 following the Miseq SOP with minor customized modifications (http://www.mothur.org/wiki/MiSeq_SOP, assessed: 1 May 2016) (Kozich *et al*., 2013). After paired-end sequences were merged, sequences were quality filtered. The remaining sequences were aligned to the SILVA v123 reference database. Bayesian classifier was used to classify the aligned sequences against the RDP (Wang *et al*., 2007), and only classifications above an 80% bootstrap cutoff value were included in the analyses. Sequences were taxonomically assigned to OTUs at a 97% sequence similarity. Additional details on sequence processing are reported in Appendix Text 4.

2.6.6 Statistics

Three-way ANOVAs were used to test the effects of dispersal, incubation environment, inoculum source and their interactions on microbial cell abundances, alpha diversity, species richness, and evenness of the bacterial communities at the end of the experiment (day 5). All three factors were used as fixed effects in all linear models that were tested by ANOVA. To assure the fulfillments of the assumptions of ANOVA and where data needed to be transformed, the normal distribution of the residuals of linear models were tested using Shapiro-Wilk normality test and the homogeneity of variance were tested using Levene's test using the package 'car' (v2.1-4). In case of significant effects, Welch's t-test was carried out owing to unequal variances, in order to further explore differences between non-dispersal and dispersal treatments for the brackish and marine communities, as well as differences between the two initial microbial inocula. Species richness (S.Obs) and alpha diversity (Shannon index: H) were computed from the average of each of 100 iterations using the 'vegan' R packages (v2.4-1; Oksanen et al., 2016). Evenness was calculated from the product of H / ln S.Obs.

Differences in the BCC were visualized using non-metric multidimensional scaling based on Bray-Curtis dissimilarity metric, and with fitting environmental variables (i.e., salinity, nutrient contents and protist abundance) to the ordination. Significant differences in between-community variation among non-dispersal and dispersal treatments of each environment were analyzed based on the test of homogeneity of multivariate dispersions (beta-dispersion) (Anderson, 2006). The effects of dispersal, incubation environment, inoculum source and their interactions on BCC in all microcosms were analyzed using three-way PERMANOVA (Anderson, 2001). A large fraction (52.23 %) of the variance in the differences in communities could be attributed to the inoculum source. Therefore, we excluded this variance to improve the estimates of dispersal and incubation environment, by performing two-way PERMANOVA tests separately for the brackish and marine

communities.

The three-way ANOVAs was further used to test the effects of dispersal, incubation environment, inoculum source and their interactions on abundances of bacterial phyla/classes, or order /families. The normality and homogeneity of variance were checked in a similar manner as described above, and data were arcsine square-root-transformed if necessary in order to fulfill ANOVA requirements.

To explore the occurrence patterns of abundant OTUs (mean relative abundance > 1% in any microcosm), hierarchical analysis with Pearson's correlation was used to cluster OTUs that exhibited similar relative abundances. The dendrograms grouped taxa according to their occurrence patterns, without any information on phylogeny. The heatmaps with color gradients were used to present the trend in relative abundance of each OTU. Among the abundant OTUs, we further identified the OTUs with potentially high dispersal ability (termed 'abundant dispersers') in each environment. For this, we determined OTUs that were detected in the pool of abundant OTUs in the dispersal treatments, but were absent from either brackish or marine communities in the non-dispersal treatment. Defining OTUs with good dispersal capabilities only from the abundant OTU pool is somehow arbitrarily; however, it offers additional information from that defined by taxonomy or functional capacity (Lindström and Langenheder, 2012). All statistical analyses and data visualization were performed in R (v3.4-0).

2.7 Data accessibility

Raw sequence data and metadata from the transplant experiment 1 are publically available at the European Nucleotide Archive (http://www/ebi.ac.uk/ena) under the Accession no. PRJEB14197 and the source code for calculating FC, TFC and FR from the annotated metatranscriptome data is available at https://github.com/tsciow/FuncRed.

The FASTQ files and associated with metadata obtained from the transplant experiment 2 have been deposited and are now publically available at the European Nucleotide Archive under the Accession no. PRJEB23259.

3. Results and discussion

3.1 Experimental conditions and community analysis of the transplant experiment 1

The three sites were characterized by distinct salinities and nutrient concentrations as illustrated by PCA, including the three initial inocula, which reflect the conditions of the sampling sites (Figure S1). The salinities between the dialysis bags (inocula) and the tanks (media) equalized within <12h after the experimental setup, and initial/final salinities in each tank were oligohaline: ~3/4 psu, brackish: salinity ~7/8 psu, marine: salinity ~28/24 psu. At the end of the experiment (day 4), salinity, inorganic nutrients and DOC were similar among the communities that were exposed to the same environment, irrespective of the inoculum source (Figure S1; Table S1).

Bacterial cells in the inoculum increased from 0.6–1.3 × 10^6 cells ml^{-1}, reaching a plateau in all incubation environments on day 3 or 4 of the experiment with 1.1–6.3 × 10^6 cells ml^{-1} (Table S1). The growth of oligohaline bacteria was significantly higher (Kruskal–Wallis test, P <0.05) in the oligohaline or brackish habitat than that in the marine habitat (Figure 8A). Neither brackish nor marine bacteria differed significantly in their growth in the three habitats on day 4 (Figures 8B, C).

The composition of the bacterial communities in all three inocula changed after 4 days under different environmental conditions, and the communities originating from the same inoculum source clustered closely (Figure S2). The result of PERMANOVA showed that the inoculum (F = 102.98, R^2 = 0.62, P < 0.001), the incubation environment (F = 22.48, R^2 = 0.13, P < 0.001) and their interaction (F = 15.87, R^2 = 0.19, P < 0.0001) had significant effects on compositional differences among all microcosms. The obtained F-values indicate that the inoculum source had a stronger effect on the community composition than the incubation environment. The stronger effect of inoculum source on the BCC was also visualized in the NMDS plot, as the communities that had the same origin clustered closer than those from different origin. This was not unexpected after a relatively short incubation period; with longer incubation time, the effect of the incubation environment should become stronger than that of the origin of the starting community (Reed and Martiny, 2013). By contrast, a pronounced effect of the incubation environment was observed at the family level (Figure 9): for the oligohaline incolua, the relative abundance of Comamonadaceae dropped from 21.25% - 0.27% when increasing salinity between the incubation conditions, and *vice verse* for the abundance of Rhodobacteraceae originating from the marine inocula (19.95% to 40.60%). Members of Microbacteriaceae were previously reported to exhibit a high abundance in the brackish water of the Baltic Sea (Riemann *et al*., 2008). In the microcosms, abundance of Microbacteriaceae decreased in response to the opposing conditions, which was more pronounced when they originated from the brackish inocula (Figure 9).

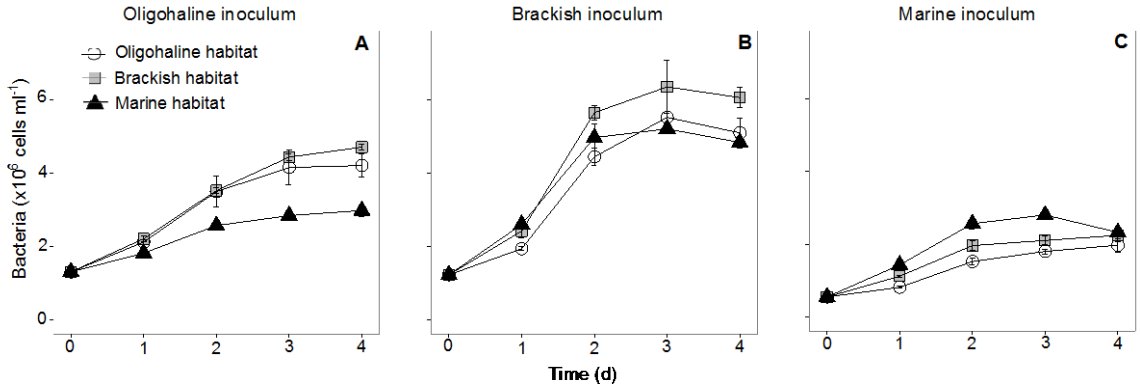

Figure 8 Bacterial cell counts of communities originating from oligohaline (**A**), brackish (**B**), and marine (**C**) inocula that were incubated in oligohaline, brackish, and marine habitats. Error bars display the standard deviation among triplicates. (Shen *et al*., accepted; doi: 10.1111/1462-2920.14059; the article is protected by copyright. All rights reserved).

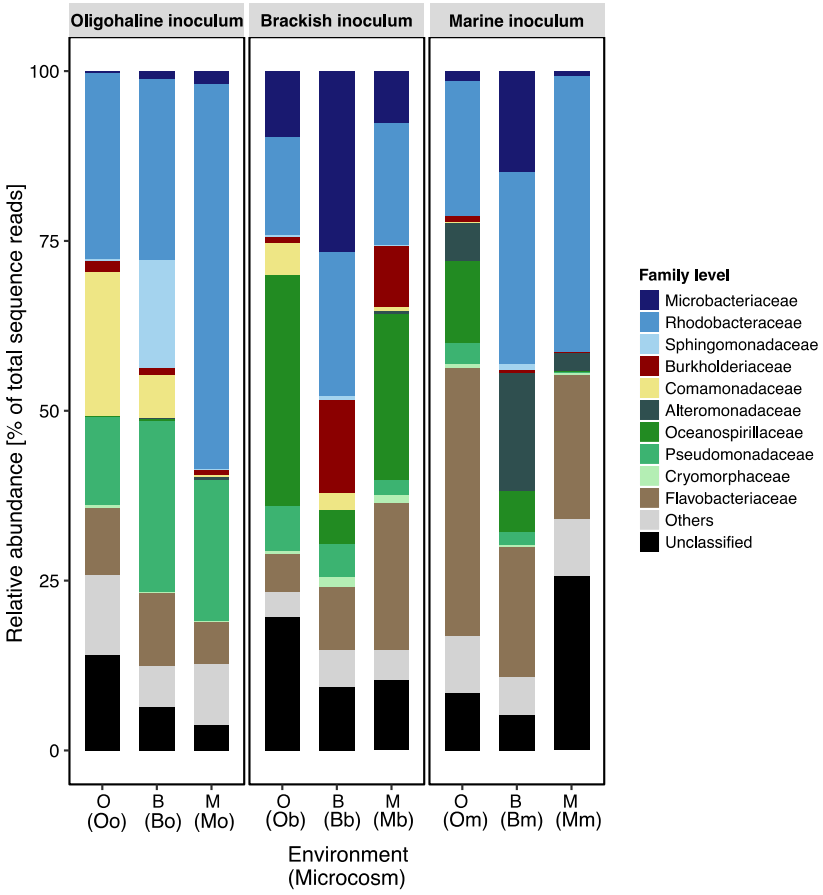

Figure 9 Taxonomic composition of bacterial communities in microcosms. Relative abundance was calculated from the percentage of total sequence reads, and is presented as the average of triplicate samples. Abbreviation: 'O', 'B' and 'M' represent the oligohaline, brackish and marine incubation environment, respectively. The combination of each capital and lower-case letter (i.e., origin of the environment and the inoculum) in the brackets indicate the corresponding microcosm ID.

3.2 The importance of ecological dissimilar bacteria to community assembly during marine-oligohaline transitions (Case study 1)

Noted: Section 3.2 uses the materials from Paper **II** (Shen *et al*., accepted; doi: 10.1111/1462-2920.14059; the article is protected by copyright. All rights reserved). The permission is granted for the passage.

Marine-oligohaline transitions are suitable for studying microbial assembly mechanisms, as there is little overlap in the abundant bacterial taxa inhabiting these two ecosystems. The present study focuses on how changes in salinity affect the community assembly of ecologically dissimilar taxa originating from oligohaline, brackish, and marine sources in terms of the phylogenetic patterning of bacterial responses. Overall, this transplant experiment showed that large changes in salinity lead to a greater abundance of habitat specialists than generalists. Phylogenetic clustering analyses showed that habitat filtering strongly influenced the assembly of closely related habitat specialists, whereas facilitative interactions were more relevant for that of distantly related generalists. To our knowledge this is the first study to demonstrate that, in the absence of dispersal processes, deterministic events vary during the community assembly of bacterial taxa differing in their life strategies.

3.2.1 Grouping individual OTU responses into life strategies

The filtering of low-abundance OTUs from each dataset (according to source inoculum) resulted in 257 OTUs originating from oligohaline, 216 from brackish, and 223 from marine inocula (Table 1). By analyzing the abundance patterns across treatments, 44–49% of these OTUs within each data set were identified as habitat specialists, ~20% were habitat generalists, and 32–39% could not be assigned to a specific life strategy (Table 1). According to the incubation environments in which the growth optima were detected, the grouping of 'habitat specialists' exhibited three distinct clusters: oligohaline, brackish, and marine specialists, correspondingly (Figure 10). The results of ANOSIM showed that the three specialist clusters differed significantly from one another within the communities originating from the same inoculum source (oligohaline: R = 0.987, P = 0.001; brackish: R = 0.906, P = 0.001; marine: R = 0.994, P = 0.001). Overall, ~50% of all specialist OTUs specialized to their native habitat (i.e., the incubation habitat and the inoculum originated from the same site). For the other half, the abundances of these OTUs were higher in non-native habitats than in native ones (Figure 10).

To examine whether specialist OTUs for each habitat rather than generalist OTUs dominated the microcosms, the mean relative abundances between the two life strategy groups were compared. OTUs with specialization to a particular habitat were significantly more abundant than the generalists found in that habitat, in all cases (repeated measures t-test, $P = 1.7 \times 10^{-6}$; independent

two-sample t-tests: $P < 0.05$; Figure 11). Similar patterns were also observed when analyzing absolute abundance data (Figure S3).

Table 1 Number of OTUs originating from the inocula of three sources and with assigned life strategies.

Life strategy	Oligohaline inoculum	Brackish inoculum	Marine inoculum
Oligohaline specialists	68	32	31
Brackish specialists	22	45	23
Marine specialists	35	21	44
Generalists	49	45	37
Undetermined	83	73	88
Overall OTU pool*	257	216	223

indicates all OTUs considered for life strategy assigment after filtering out low abundance OTUs.

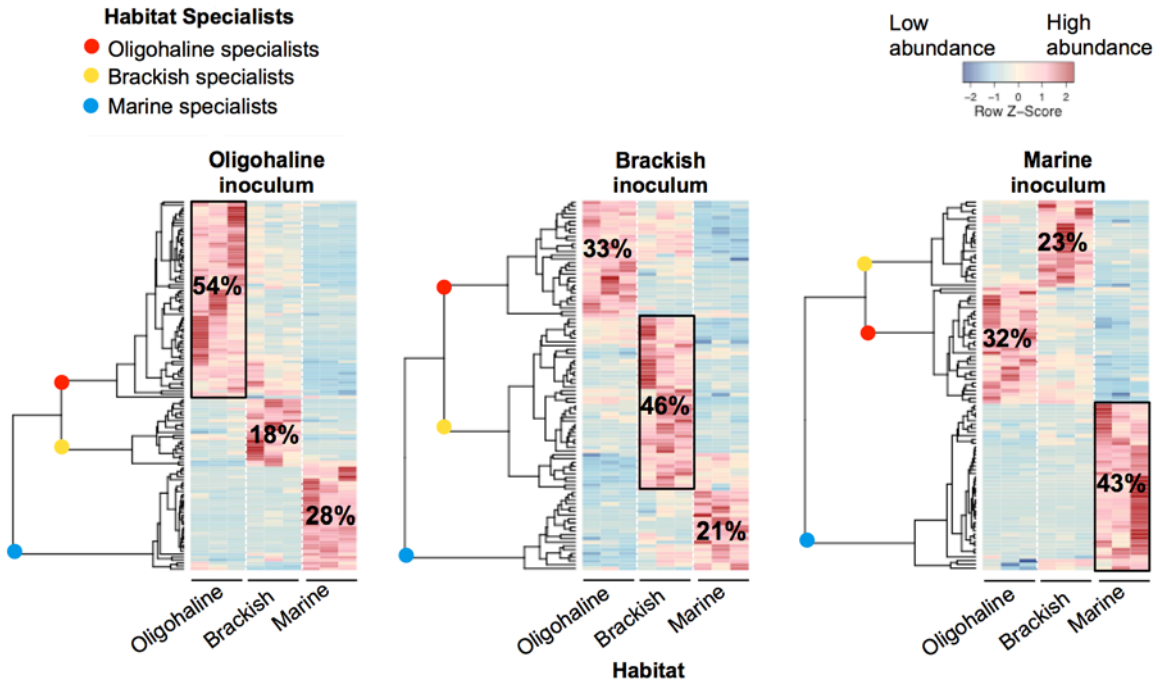

Figure 10 Dynamics of specialist OTU's absolute abundances using hierarchical cluster analysis (Pearson's correlation, average clustering criterion). Heat maps illustrate the changes in the absolute abundance of each OTU (rows) originating from the same inoculum source when incubated in the three habitats (O, B or M, x-axis, grouped in triplicate). Side dendrograms cluster OTUs that have similar abundance patterns. Percentages in black represent the proportion of OTUs that were assigned to different specialist strategies. Squares indicate the specialists specialized to their native habitat.

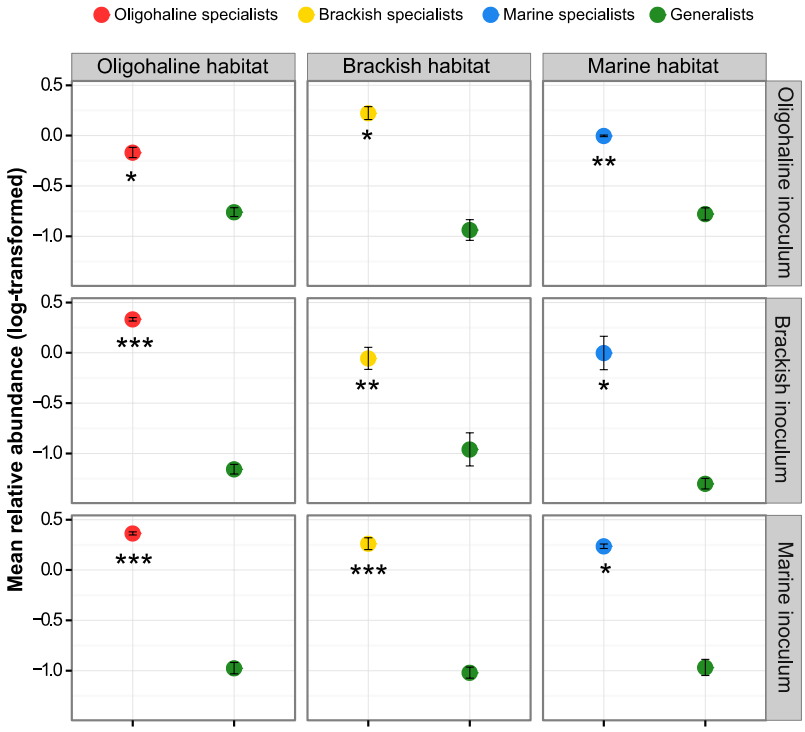

Figure 11 Mean relative abundances of OTUs assigned to a specific life strategy in all microcosms at the end of the experiment. The abundance data were plotted after log-transformation. In each panel, only the specialists that were specialized to the respective habitat are shown and compared with generalists found in the same microcosms. Asterisks indicate a significance level of difference in mean relative abundance between specialist taxa and generalist taxa in each microcosm ($P < 0.05$ *, $P < 0.01$ **, $P < 0.005$ ***), and error bars represent the standard deviation among the triplicate microcosms.

These results supported the first hypothesis that specialist OTUs in the favored habitat would dominate over the generalists found in that habitat. The findings further supports an earlier finding that a positive correlation between abundance and habitat specificity (Mariadassou *et al*., 2015). The dominance of specialists may have been particularly pronounced in this experiment. This is because salinity is a major barrier for microbial transitions (Lozupone and Knight, 2007; Logares *et al*., 2009) and within a wide range may strongly impede the appearance of abundant generalists. Moreover, unlike other habitats with salinity gradients (e.g., estuaries), surface salinity fluctuation in the Baltic Sea is comparatively small due to long water residence time (Reissmann *et al*., 2009). This stable salinity gradient might hinder the establishment of taxa with a broad salinity tolerance (Herlemann *et al*., 2011). Apart from the few number and low abundance of generalists, the data revealed a small variability in the total cell abundances among the microcosms inoculuated with the same source across three habitats (Figure 8). This could be the result of high growth in the favored habitat of specific taxa (Mariadassou *et al*., 2015). As such, many well-growing specialists were selected even in the incubations that were exposed to changing salinity.

3.2.2 Recruitment of initially rare and abundant members for the groupings

To evaluate the contribution of rare and abundant members from the starting communities to the selection of habitat specialists/generalists, the proportions of initially rare and abundant OTUs in each of four strategy groups were analyzed, and then compared to that of the overall OTU pool from the same source inoculum (Figure 12). The proportion of initially abundant members among specialists specialized to their native habitats (Figures 12B, H and N) was higher than that in the corresponding overall OTU pools (Figures 12A, F and K). Conversely, initially rare members were overrepresented in 4 of 6 cases among the specialists specialized to non-native habitats (Figures 12C, D, G and M). In two other cases (marine specialists originating from the brackish inocula: Figure 12I; oligohaline specialists originating from the marine inocula: Figure 12L), initially abundant members were overrepresented compared with the corresponding overall taxon pools (Figures 12F and K, respectively). The proportion of either rare or abundant members among generalist taxa was similar to that in the corresponding overall OTU pools (Figures 12E, J, O vs. Figures 12A, F, K, correspondingly), despite a slightly higher proportion of initially rare members in the case of generalists from the brackish inocula (Figure 12J vs. Figure 12F).

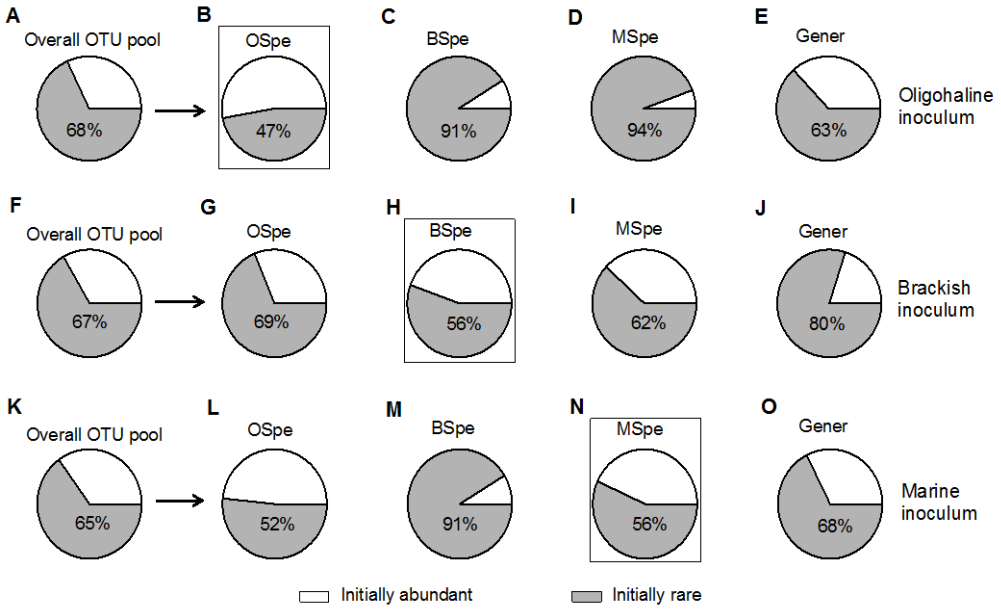

Figure 12 Proportion of the initially rare (relative abundance of <0.1%) and initially abundant OTUs (relative abundance of ≥0.1%) among the overall OTU pools originating from each inoculum source (after filtering out low-abundance OTUS; **A**, **F**, **K**), and among the OTUs sorted into each of four strategies (**B**-**E**: OTUs originating from the oligohaline inoculum; **G**–**J**: OTUs originating from the brackish inoculum; **L**–**O**: OTUs originating from the marine inoculum). The framed pie charts indicate specialist OTUs that were specialized to their native habitats. Abbreviations 'OSpe', 'BSpe', 'MSpe' and 'Gener' represent oligohaline specialists, brackish specialists, marine specialists and generalists, respectively.

I hypothesized that selection for habitat specialists after environmental change primarily depends on the recruitment of rare members. Generally the results obtained from this experiment support an earlier observation that members of the rare biosphere can eventually achieve large population sizes when the environment changes to suitable conditions (Jones and Lennon, 2010; Crump *et al*., 2012; Sjöstedt *et al*., 2012; Shade *et al*., 2014). Specifically, the brackish specialists were largely recruited from initially rare members of the oligohaline and marine inocula when exposed to brackish environments (Figure 12C and Figure 12M, respectively). This suggests that seed banks of brackish bacteria exist within both oligohaline and marine species pool. However, initially abundant members of the brackish and marine inocula emerged to an unexpected extent as habitat specialists in non-native habitats (Figure 12I and Figure 12L). These OTUs were abundant in the initial inoculum but exhibited preferential growth in another environment with a different salinity, suggesting their plasticity to changing salinity. I assume that the preferential growth of the initially abundant OTUs in their non-native habitat could have been due to species interactions. NMDS plot showed that the composition of the communities incubated in their native habitat differed from their initial composition in the inoculum (Figure S2). Possibly, newly arising competitors that limited the growth of some OTUs in their native habitats during the incubation were not present in the habitats where preferential growth of those OTUs was detected. On the other hand, I also found that majority of the specialists with preferential growth in their native habitats (no environmental change induced) were initially abundant (Figures 12B, H and N). The study of Herlemann *et al*., (2011) indicated that the abundant bacteria found at different salinity zones of the Baltic Sea are often those best adapted to the local environmental conditions. Likewise, specialists with preferential growth in their native environment were mainly selected from the abundant pool of the inoculum. I further expected that the selection for generalists should, however, be independent of their initial abundance in the starting community. This was supported by my finding that the proportions of rare/ abundant in the generalist grouping were similar to that in the corresponding OTU pool, suggesting that the initial taxon's abundance is of minor relevance as a determinant of the growth behavior of generalists.

3.2.3 Phylogenetic relatedness of strategy groupings

Overall, habitat specialists were phylogenetically clustered as evidenced by the significant and positive NRI and/or NTI indexes regardless of different specialist strategies or the inoculum source. Such phylogenetic clustering was evident at deep branches according to 5 positive NRI values of 9 cases. Particularly, specialists clustered more at finer taxonomic scales, as indicated by 8 positive NTI values, which were statistically significant in 4 cases ($P < 0.05$; Table 2). One exception to this trend was marine specialists originating from brackish inocula that exhibited significant and negative NTI values. By contrast, habitat generalists originating from all source inocula tended to be over-

dispersed as evidenced by negative NRI and/or NTI values, with one significant signal ($P > 0.95$; Table 2). One exception to this trend was generalists originating from the marine inocula, which were phylogenetically clustered near the tips of the tree (NTI > 0; Table 2).

Table 2 Phylogenetic relatedness indices of life strategies.

	Life strategies	NRI		NTI	
		Value	*P*-value	Value	*P*-value
Oligohaline inoculum	Oligohaline specialists	-1.273	0.902	1.407	0.071
	Brackish specialists	2.577	**0.014**	1.855	**0.03**
	Marine specialists	1.851	**0.044**	1.288	0.102
	Generalists	-0.945	0.826	-0.949	0.835
Brackish inoculum	Oligohaline specialists	-0.031	0.465	4.135	**0.001**
	Brackish specialists	1.673	0.063	1.894	**0.028**
	Marine specialists	-0.528	0.666	-1.904	**0.974**
	Generalists	-1.053	0.866	-0.298	0.623
Marine inoculum	Oligohaline specialists	1.365	0.11	1.771	**0.034**
	Brackish specialists	3.005	**0.006**	0.6	0.274
	Marine specialists	-0.068	0.496	0.085	0.476
	Generalists	-1.589	**0.953**	1.414	0.082

P-values in bold indicate $P < 0.05$ or $P > 0.95$ obtained from a two-tailed test, which determined whether the observed phylogenetic distances between OTUs were greater or less than expectations based on random community assembly. Low *P*-values and positive indices indicate OTUs in a particular life strategy group were significantly more closely related than expected by chance (phylogenetic clustering) for the inocula of each source; high *P*-values and negative indices indicate OTUs were less closely related than expected by chance (phylogenetic over-dispersion).

The third hypothesis that community assembly processes would differ between the two life strategies was also supported by the phylogenetic relatedness analyses. Habitat specialization may be a phylogenetically conserved trait, as proposed by (Székely and Langenheder, 2014), and in this study, the primary tendency for the habitat specialists was phylogenetic clustering (NRI > 0 and/or NTI > 0). Unlike simple traits, complex traits involving many genes are conserved at a deep phylogenetic level (Martiny *et al*., 2013). Phylogenetic clustering can be interpreted as evidence of habitat filtering in which closely related taxa share physiological traits that allow them to persist in a particular environment (Webb *et al*., 2002). The phylogenetic clustering of specialists was specifically evident at finer taxonomic scales (NTI > 0), with the exception of a few clusters at deep branches, such as at the class-level (NRI > 0). This clustering suggests that the different traits that gave rise to a specific salinity tolerance might have emerged in parallel among closely related taxa. A phylogenetic tree was constructed to visualize each OTU's strategy following a salinity change (Figure 13). Independent of the inoculum, most β-Proteobacteria were specialized to oligohaline and most Actinobacteria to either oligohaline or brackish water, while other lineages did not (Figure 13). This implies a less-pronounced ecological coherence among the bacterial classes in response to

salinity than expected from an earlier study (Morrissey and Franklin, 2015). Different incubation environments with specific abiotic factors likely selected for a set of growth-promoting traits in bacteria. Incubation environments established in this study differed besides salinities also in their nutrient levels and probably organic matter composition that might have an additional impact on bacterial community composition (Herlemann *et al*., 2017). However, Dupont *et al*., 2014 reported that salinity is a main determinant of microbial compositional changes and also impacts the presence of core metabolic functions. Traits associated with adaption to a certain salinity level could be related to microbial energy costs and osmoregulation (Logares *et al*., 2009; Dupont *et al*., 2014), or to the metabolic changes triggered by salt (e.g., the ability to uptake dissolved organic carbon) (Stepanauskas *et al*., 2000).

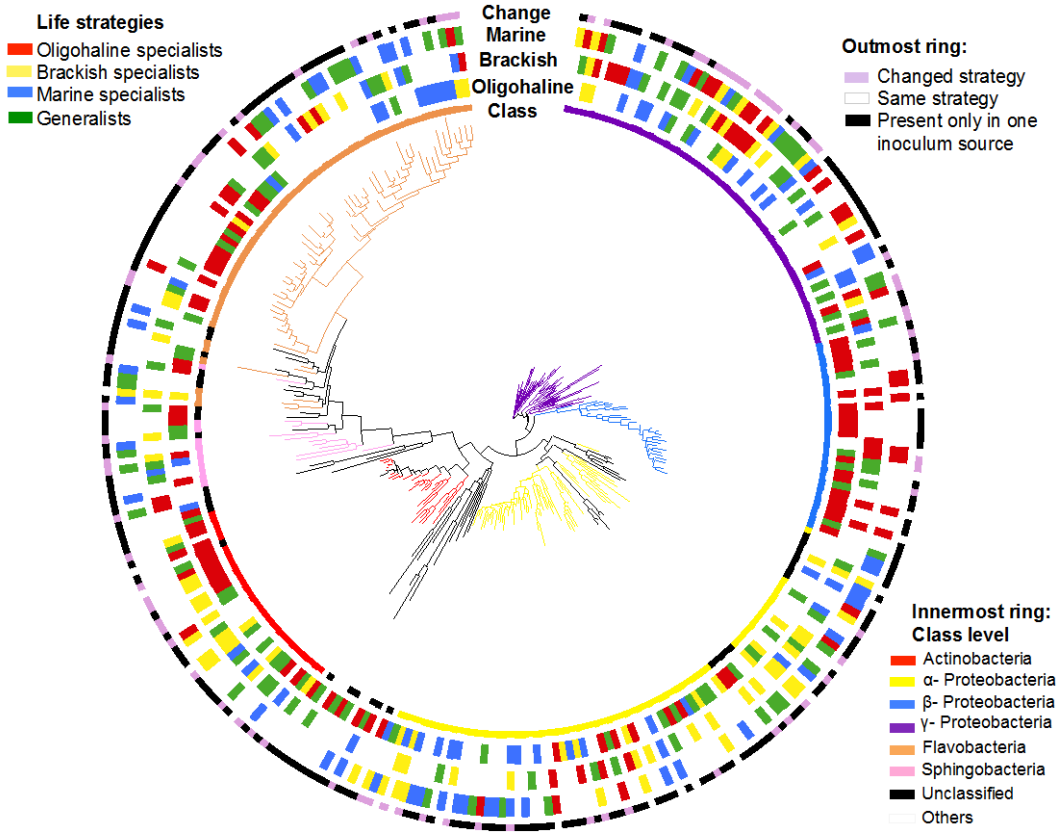

Figure 13 Phylogeny of bacterial OTUs and the life strategy of each OTU separated for their origin from either the oligohaline (inner-wide ring), brackish (middle-wide ring) or marine inoculum (outer-wide ring). In total, 304 OTUs were identified across samples. Branch colors and the innermost ring indicate the dominant bacterial classes. The outermost ring (purple-labeled) indicates whether the OTUs differed in their life strategies in dependence of the inoculum source.

By contrast, in most cases, habitat generalists were characterized by phylogenetic overdispersion (NTI and/or NRI < 0). To date, little is known about the specific traits inherent to

bacteria with a wide salinity tolerance. Overdispersion can be further interpreted as the result of competitive interactions among closely related species or facilitative interactions among distantly related species (Webb *et al*., 2002; Cavender-Bares *et al*., 2004). Thus, the assembly of habitat generalists in our study most likely resulted from the net outcome of both competitive exclusion from co-existing specialists and facilitative interactions among those generalists. However, two other factors should be considered: (i) phylogenetic matrices are sensitive to factors such as the taxonomic scale and 16S rRNA sequence identity, and (ii) communities can be subjected simultaneously to habitat filtering and competition (Horner-Devine and Bohannan, 2006; Koeppel and Wu, 2014). The results of this study, nevertheless, indicated that one process should be of greater importance despite simultaneous operation of both processes to some extent.

3.2.4 Distribution of OTUs' life strategies and difference in strategies within an OTU

Across all samples, a total of 304 OTUs were assigned to life strategies: 112 were shared in at least two inoculum sources, while the other 192 OTUs were exclusively present in the microcosms inoculated either with oligohaline, brackish or marine inocula (Figure 13; Table S4). Only 34 of the 112 shared OTUs were consistently assigned to a unique life strategy, whereas 78 shared OTUs were assigned to different life strategies depending on the origin of the inoculum (Figure S4B). Different strategies of a single OTU were apparent in the shift between: generalists-any specialist type (52 OTUs) > brackish-oligohaline (or marine) specialists (17 or 6 OTUs, respectively) > oligohaline-marine specialists (3 OTUs). At the class level, some phylogenies preferentially displayed the same strategy while others did not (Figure 13). For example, most β-Proteobacteria were oligohaline specialists independent of their inoculum source; whereas OTUs belonging to Flavobacteria were primarily oligohaline specialists (35%, 12 of 34) when they originated from the oligohaline inocula but generalists when they originated from the marine inocula (42%, 10 of 24) (Table S3). Irrespective of the inoculum source, habitat generalists spread across the dominant bacterial classes (Figure 13). However, in case of the marine inoculum, generalist OTUs tended to be more closely related than expected by chance (NTI >0; Table 2), and those close relatives were primarily found within the γ-Proteobacteria and Flavobacteria (Figure 13).

The inoculum source generally influenced the dominance of members assigned to a specific strategy at class- or family-level in terms of absolute abundance, particularly for the specialist strategies (Figure S5). Although members of the classes or the families were usually preferentially recruited from a specific inoculum source, they were assigned to a particular strategy irrespective of their inoculum in some cases. For example, β-Proteobacteria or more specifically Comamonadaceae were only found in high absolute abundances when they displayed the strategy of oligohaline specialist; while Sphingomonadaceae and Rhodobacteraceae were enriched as brackish specialists and marine specialists, respectively (Figure S5). Generalists reached only low absolute abundance

and were evenly distributed throughout bacterial taxonomic groups, particularly pronounced at the family level.

Although phylogenetic relatedness patterns suggest that traits leading specialist-behavior are highly conserved, I also observed that the life strategy of a single OTU can vary depending on the origin of its inoculum. The different life strategies assigned to the same OTU might have been caused by species interactions with the remaining community members under new abiotic and biotic conditions. However, this scenario is not well supported by the observation that the OTUs displayed a different specialist strategy depending on the inoculum source, as we detected a dominant role of habitat filtering for the assembly of specialist taxa. Alternatively, a defined OTU may harbor very close relatives with different ecological traits, including distinct salinity preferences. The 97% sequence similarity used in our OTU classification was probably insufficient to distinguish micro-niche differentiation among closely related strains or ecotypes (Hunt *et al*., 2008). For instance, OTU000004 was very abundant in our microcosms and was characterized by different specialist 461 strategies in dependence of the source inoculum (Table S4); pairwise dissimilarity comparison between the individual sequences belonging to this OTU revealed two distinct clusters that represent an oligohaline ribotype and a brackish-marine ribotype (Figure S6), thereby indicating the existence of at least two different ecotypes. However, this result contradicts the general assumption that marine-freshwater transitions among closely related microorganisms were postulated to be rare (Logares *et al*., 2010). Recent work highlights the importance of horizontal gene transfer events in driving population diversification (Kent *et al*., 2016), which could be manifested in changed salinity preference among the close relatives as observed in this study.

3.2.5 Exclusion of dispersal and concluding remarks

This transplant experiment was designed to exclude dispersal of bacterial communities among microcosms, while suppressing interactions between local taxa and immigrants that usually occur in environments where water bodies with differing salinities are mixed. However, the situation in our experiment differs from that found in most natural systems. For example, local bacteria interact with immigrants transported from different salinity conditions when responding to saltwater intrusions: immigrant species that can cope with varying environmental conditions should perform better than other immigrants in the new environment, and accordingly the proportion of generalists in the resident community should increase (Székely *et al*., 2013). Thus, natural mixing events of the Baltic Sea water may result in a less pronounced difference in the abundances between specialists and generalists than observed in my experiment. Nevertheless, the results of this study are specifically relevant for natural systems with limited dispersal, such as seepage lakes that have no surface water inputs (Jones and McMahon, 2009). To fully understand the complex interactions between assembly processes (i.e., habitat filtering, competitive exclusion and dispersal) shaping microbial communities

in natural systems is difficult, if not impossible. Thus, experimental manipulations (like in this study) are required to tease apart the effects of those processes and to investigate their partitioning.

The understanding gained from experimental systems investigating aquatic microbial assemblages extends beyond that from purely descriptive analyses. However, experimental systems often suffer from so-called 'bottle effects', where originally rare taxa overgrow the organisms that are abundant in natural habitats. Nevertheless, the revealed assembly mechanisms, associated with different life strategies (specialists vs. generalists) reflect relevant patterns that are independent of taxa's in-situ abundances. More importantly, most OTUs with assigned life strategies were closely related (> 97% sequence identity) to abundant OTUs (> 0.1% 16S rRNA gene relative abundance) of the Baltic Sea (Herlemann *et al*., 2016) (Table S4). Several very abundant Baltic Sea OTUs (> 1% 16S rRNA gene relative abundance; Herlemann *et al*., 2016) were also abundant in our microcosms. For example, Limnohabitans (Otu000010) and Microbacteriaceae (Otu000018) achieved high abundances in our microcosms as the oligohaline and brackish specialists, respectively. They also have been reported to exhibit high abundances in other natural aquatic habitats (Newton *et al*., 2011; Riemann *et al*., 2008). In addition to the justification of "bottle effects", a concern of whether the described patterns can lead to a generality of community assembly in the presence of particle-attached (PA) bacteria should be discussed. The initial inoculum for the experiment was obtained by filtering the water from the origin onto 0.8µm filters; in this case, majority of PA bacteria from the original communities were excluded. PA bacteria are an important component of the Baltic Sea communities (Rieck *et al*., 2015), and influence the community succession/assembly processes. I have, however, found that the abundance-occupancy ranks appear to be strongly consistent between the PA and FL communities, independent of the taxonomic depth (Shen *et al*., in preparation). In this regard, the presence of PA communities is unlikely to have distorted the relationship between the two ecological categories observed here.

To conclude, our study suggests that large salinity changes promote the dominance of habitat specialists. The initial abundance of taxa is less relevant to the recruitment of habitat generalists than that of specialists in a community exposed to new environments. Moreover, phylogenetic clustering revealed that habitat filtering strongly influenced the assembly of habitat specialists, thereby confirming earlier results that delineated evidence of habitat filtering from a correlation with environmental parameters (Székely and Langenheder, 2014; Liao *et al*., 2016). However, biotic interactions may be more relevant for the assembly of generalists. Altogether, our results demonstrate that, in the absence of dispersal, deterministic processes vary during community assembly for ecologically dissimilar OTUs. Future work should extend beyond investigations of assembly processes and seek to identify the physiological traits linked to the different life strategies.

3.3 Is metabolic specificity or functional plasticity as a strategy for marine-oligohaline transitions of ecologically dissimilar taxa? (Case study 2)

The main goal of this section is to characterize transcriptional activity and gene expression patterns of the habitat specialist and generalist taxa identified in the section 3.2. The results highlight the importance of energy conservation, CO_2 fixation and respiratory complex for bacteria crossing the marine-oligohaline boundaries.

3.3.1 Transcriptional activity of the selected OTUs with a consistent life strategy

I hypothesized that transcriptional activities of the specialists would be higher under their favorable environmental conditions. Unexpectedly, I found that both oligohaline and marine specialists showed overall more active gene transcription per cell in the brackish environment, although statistical significance was only detected for the latter one (*Post hoc* test, $P < 0.05$) (Figure 14). This indicates high abundance of mRNA transcripts relative to their DNA abundances for those groups in the brackish environment, in comparison with the oligohaline and marine conditions (Table S5). The group of brackish specialists also showed higher transcript abundance in the brackish environment compared to that in the two other environments (although not significant) (Figure 14). This pattern was also observed at the single OTU level (Figure S9, the panel 'Brackish specialists'), which was less pronounced for the individual oligohaline and marine specialists. However, generalists displayed no differences in the transcriptional activities across three environments (Figure 14), which was consistent with the trend delineated by their absolute abundances. I further observed that some OTUs within the same ecological group were less abundant in cell numbers (DNA based, 16S rRNA gene sequencing) but relatively more transcriptionally active (mRNA based) in dependence of their origin. For instance, the oligohaline specialist OTU000010 taxonomically assigned to *Limnohabitans* (Table 3), its

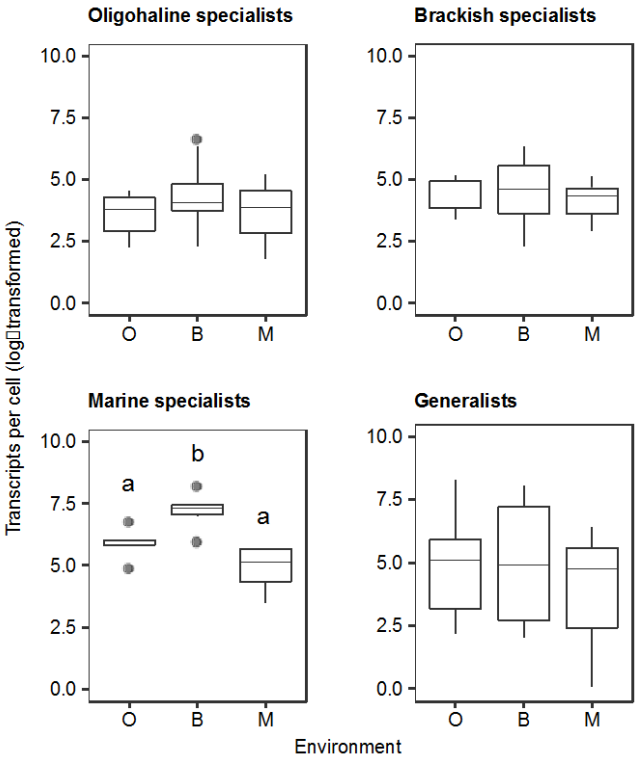

Figure 14 Transcriptional activities of the OTU life strategy groups in terms of transcripts per cell. The numbers and taxonomic affiliations of OTUs that were included in each of the four strategy groups are given in the Table 3. Significant *post hoc* among different groups are denoted by lower cases 'a' or 'b'.

absolute abundance was higher when it originated from the oligohaline source (36.35 ± 9.52 x 10^4 cells / ml) than when originated from the marine source (0.03 ± 0 x 10^4 cells / ml) (Table S5A, treatment 'Oo' and 'Om', respectively). However, the *Limnohabitans* showed the opposite trend in the transcript abundances (Figure S7, top panel; Table S5B). My data revealed that the most abundant taxa were not the most active, and this is consistent with a study on estuarine bacterioplankton 16s rRNA:DNA ratio (Campbell *et al*., 2011). Together, these results suggested that transcriptional activities of the specialist taxa were not positively correlated with their abundances (but see Moitinho-silva *et al*., 2014), and that high transcriptional activity may occur when taxa encounter the contrasting environment of their origin.

3.3.2 Significantly differential gene expression between the disparate environments

I further used transcriptomic analyses to infer the mechanisms used by ecological groups to adjust their metabolism for a preferential growth in one habitat or maintain equal growth in all habitats. Hence, pairwise gene expression analyses were performed for the specialist and generalist taxa between any of the two incubation environments. Not all representatives belonging to the same strategy (Table 3) showed significantly differential gene expression patterns between the varying environments (Tables S6-8), and no common expression pattern was observed for the individual genes within the same strategy (data not showed). This gives rise to the question on whether change in transcript composition or in transcript abundance contributes to the functional difference of the ecologically similar taxa.

Our previous study has shown that a high degree of functional redundancy occurred in the communities that were exposed to similar environmental conditions (Beier *et al*., 2017). Here, I observed that a large number of significantly expressed genes across the strategy groups was found when comparing the expression patterns between the O-M environments, followed by the B-M and the B-O (Figure 15). Hence, this extends our previous finding that functional similarity appeared to be high for a taxon experiencing the similar environmental contexts. The little overlapping of highly expressed genes for all specialist strategy groups generally support the idea that, the transition between low- and high- saline environments indeed is a most challenging physiochemical-barrier for the microbes (Logares *et al*., 2009). One exception to this trend is that the highest number of highly expressed genes (1064 genes) in the brackish specialists was found when comparing the transcriptome datasets of B and M environments (Figure 15B). None of the two generalist OTUs featured differential gene expression among the three habitats, indicating that metabolic flexibility allows them to survive in multiple environments (Székely *et al*., 2013). I detected no genes of the studied marine specialist that significantly changed their expression between B-O conditions (Figure 15C). This suggested functional similarity of marine bacteria as response to low saline waters, at least at the salinity levels studied here.

Table 3 Taxonomic affiliations of the selected OTUs with differing ecological strategies for the transcriptome exanimation in this study.

OTU ID	Life strategy	Taxonomic affiliation
Otu000010	OSpe	Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Limnohabitans
Otu000030	OSpe	Proteobacteria;Betaproteobacteria;Burkholderiales;Alcaligenaceae;unclassified
Otu000071	OSpe	Actinobacteria;Actinobacteria;Actinomycetales;unclassified;unclassified
Otu000100	OSpe	Proteobacteria;Betaproteobacteria;Burkholderiales;Oxalobacteraceae;unclassified
Otu000149	OSpe	Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Acidovorax
Otu000009	BSpe	Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas
Otu000021	BSpe	Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Sphingobium
Otu000098	BSpe	Bacteroidetes;Flavobacteria;Flavobacteriales;Cryomorphaceae;unclassified
Otu000033	MSpe	Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;unclassified
Otu000194	MSpe	Actinobacteria;Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae
Otu000045	Gener	Actinobacteria;Actinobacteria;Acidimicrobiales;Acidimicrobiaceae;Ilumatobacter
Otu000060	Gener	Proteobacteria;Gammaproteobacteria;Chromatiales;Chromatiaceae;Rheinheimera

Abbreviations: 'OSpe', 'BSpe', 'MSpe' and 'Gener' represent oligohaline, brackish, marine specialists, and generalists, respectively.

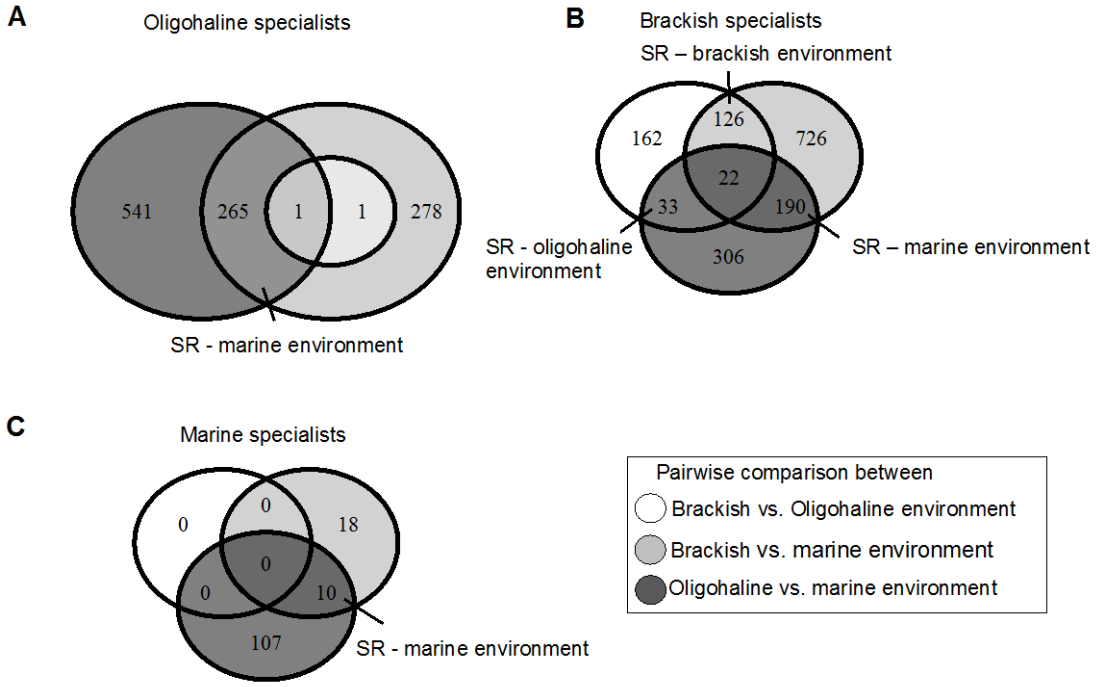

Figure 15 Venn diagrams showing the number of genes that were differentially expressed between any two of the three incubation environments, as well as the number of genes exclusively shared between / among the transcriptome comparisons for each ecological group: (**A**) oligohaline specialists, (**B**) brackish specialists, (**C**) marine specialists. Significant change in gene expression was detected at a statistical *P*-value < 0.01. Each of the three life strategy groups was compared between the brackish vs. oligohaline environment (white circle), brackish vs. marine environment (light gray circle), and oligohaline vs. marine media (dark gray circle). Abbreviation 'SR' refers to specific response to a given environment at the gene level. Specifically, the shared fraction denotes the unique set of genes expressed by the members belonging to each of the four strategies in response to that growth condition. The Venn diagrams were made using the package 'VennDiagram' (v.1.6.17) in R. The shared genes and their corresponding KEGG functional categories for the specific response of the

ecological groups to each incubation environment are listed in the Supplemental Tables 6-9; noted that only the genes belonging to functional clusters of interest are shown, as the clusters in Figure 16.

Among the differentially expressed genes by the members within the same strategy, the overlap between transcriptome profiles indicated gene-specific expression in response to a given environment. For example, 265 genes in oligohaline specialists were overlapped between F-M and B-M transcriptome datasets as specific responses to marine conditions (Figure 15A). Out of 265 genes, only 17 genes with multiple copies were significantly up-regulated in response to M environment, the remaining genes showed the opposite trend. Furthermore, a large fraction of the genes, representing 49% - 71% of the total shared genes in the individual transcriptome dataset pairs for the specialist taxa, were assigned to the 'metabolism' process according to KEGG database (data not shown). Our data on metatranscriptomics thus revealed that habitat filtering selected specialist taxa based on the remarkably different metabolic or functional traits as previously suggested (Fierer *et al*., 2007; Philippot *et al*., 2010), or inferred from the phylogenetic relatedness analyses (reference to section 3.2).

3.3.3 Functional attributes of the ecological groups

To assess functional characterization of ecological groups, significantly expressed genes were clustered into 7 categories based on KEGG orthologous groups (Figure 16). The 7 functional clusters were previously reported to show characteristics of the potential metabolic functions between freshwater- and marine-dwelling assemblages on (Eiler *et al*., 2014; Dupont *et al*., 2014), as well as transcriptional responses of a culture strain to cold and halosaline conditions (Mykytczuk *et al*., 2013). Hence, the chosen 7 functional categories broadly represent the metabolic functions and cell biological processes of the bacteria, and the differences in transcriptional patterns of bacteria differing in life strategies ware expected among them. Genes involved in cell cycle, transcription, or translation were significantly upregulated in the favored environments of habitat specialists (Figure 16), as observed at high representation of translation factors (*rplJLM*) families and DNA replication factors (*dnaQG*) (Table 4). This pointed to a robustness of classification of ecological strategy that was used to group bacteria exhibiting similar fitness optima, which is also observed in the growth-related functional of the transcripts. Differential expression ratios of all seven categories found in the oligohaline specialists were high in either oligohaline or brackish environment (Figure 16). Overall, regardless of the functional category, there is little overlapping of the top 10 highly expressed genes between the olighaline and marine specialists (Table 4). This indicates varying transcriptional effort with regards to transcript composition for the two life strategy groups in response to salinity changing. However, among the highly expressed genes, the brackish specialists appeared to exhibit an intermediate expression pattern between the two other specialist groups.

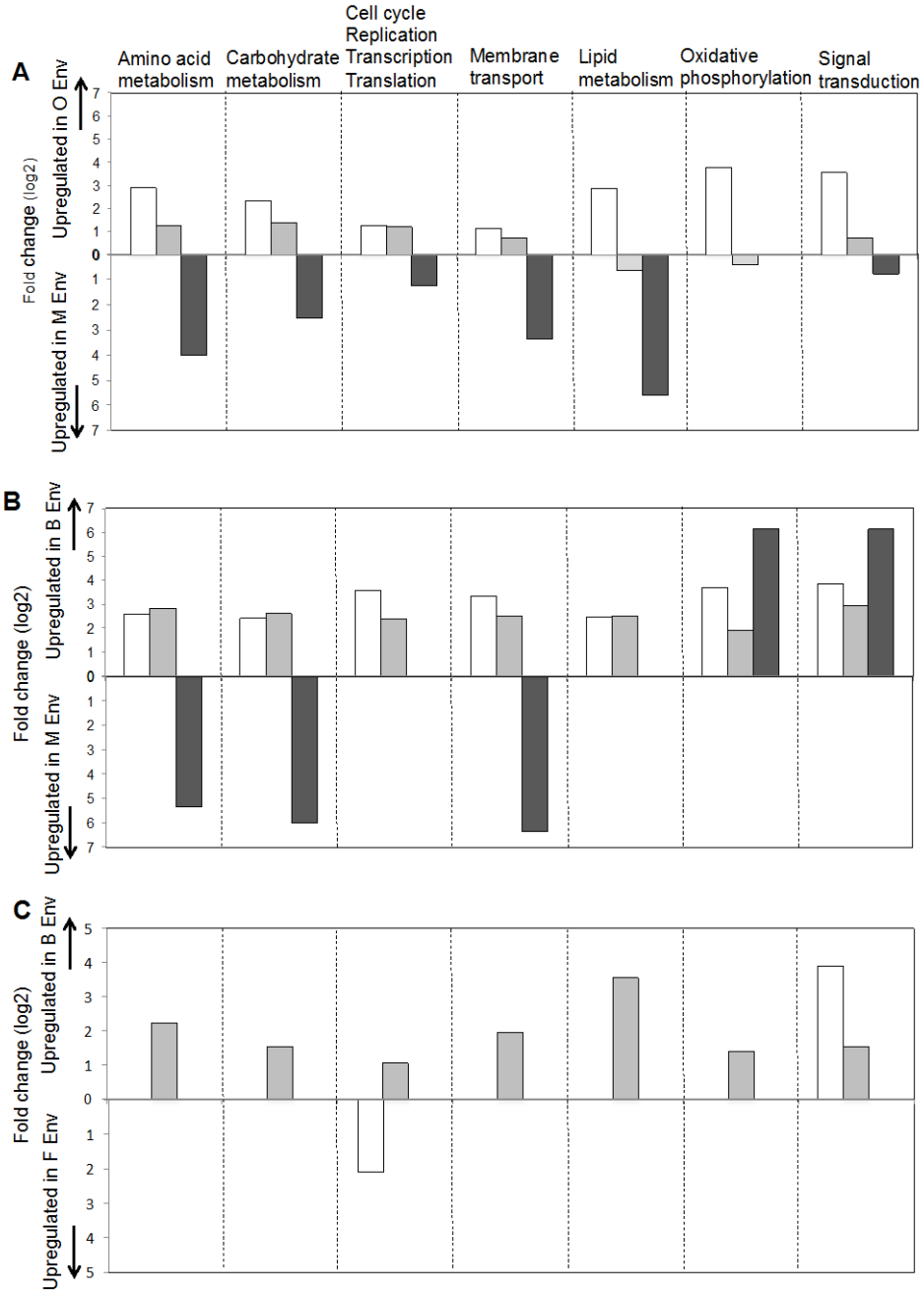

Figure 16 Transcriptional expressions of functional clusters. Colors indicate the ecological groups: oligohaline (white), brackish (light grey) and marine (dark grey) specialists. Direction of arrows indicates in which incubation environment the genes involved in the given functional cluster were significantly upregulated. Bars represent the averages of gene expression ratio of all genes in the given functional category for each ecological group.

Table 4 Top 10 highest expressed genes for each of the ecology groups between the compared environments. The capital letters 'O-M', 'B-M' and 'B-O' indicate that the two incubation environments in which the differential gene expression was detected, and that is between the oligohaline-marine, between brackish-marine, and between brackish-oligohaline environments, respectively. Colors represent that the up-regulation of the genes (red) and the down-regulation of the genes (blue) responding the first capital letter-denoted environment.

O-M environments			
KEGG identifier/gene	Osp	BSp	MSp
Amino acid metabolism			
K00605 /gcvT, AMT	-	-	-6.75
K00265 / gltB	-	-	-6.07
K00832 / tyrB	3.72	3.42	-5.58
K01626 / aroF, aroG, aroH	-	-	-5.18
K14260 / alaA	5.17	3.36	-
K01940 / argG	5.03	-	-
K01956 / carA	5.03	-	-
K00261 / gdhA	5.02	-	-
K00609 / pyrB	5.02	-	-
K01584 / adiA	5.02	-	-
Carbohydrate metabolism			
K00688 / glgP	-	-	-6.02
K01961 / accC	-	-6.42	-
K00605 / gcvT, AMT	-	-	-6.75
K03738 / aor	5.75	-	-
K01638 / aceB, glcB	-	2.94	-5.59
K01649 / leuA	5.17	-	-
K03821 / phbC, phaC	5.13	-	-
K01759 / gloA	-	2.97	-4.81
K01689 / eno	-	-1.33	-4.69
K00241 / sdhC, frdC	4.68	3.11	-
Cell cycles/replication /transcription/translation			
K02864 / rplJ	6.92	-	-
K02909 / rpmE	6.88	-	-
K02871 / rplM	6.57	0.62	-
K02906 / rplC	6.23	2.81	-
K02954 / rpsN	5.90	2.19	-
K02895 / rplX	5.83	-	-
K02879 / rplQ	5.28	-	-
K02935 / rplL	5.26	2.86	-
K02959 / rpsP	5.21	-1.81	-
K01889 / pheS	-	-	-5.17
Membrane transport			
K15580 / oppA, mppA	-	-	-7.16
K15576 / nrtA, nasF, cynA	-	-6.12	-
K11073 / potF	6.17	-	-

B-M environments			
KEGG identifier/gene	Osp	BSp	MSp
Amino acid metabolism			
K00605 / gcvT, AMT	-	-	-5.76
K10219 / ligC	-	5.41	-
K00253 / ivd	5.29	-3.39	-
K01738 / cysK	-	-	-5.20
K00166 / bkdA1	-	5.15	-
K00832 / tyrB	5.10	3.75	-
K00265 / gltB	-	1.28	-5.07
K01581 / speC, speF	-	5.03	-
K00003 / E1.1.1.3	-	4.71	-
K14267 / dapC	4.71	-	-
Carbohydrate metabolism			
K00754 / bshA	-	6.31	-
K00031 / icd	-	-	-6.23
K00605 / gcvT, AMT	-	-	-5.76
K01190 / lacZ	-	5.54	-
K00123 / fdoG, fdfH	-	5.42	-
K05973 / phaZ	3.14	5.29	-
K00241 / sdhC, frdC	4.88	4.70	-
K00975 / glgC	-	4.73	-
K00241 / sdhC, frdC	4.88	4.70	-
K18118 / aarC, cat1	-	4.67	-
Cell cycles/replication /transcription/translation			
K02342 / DPO3E, dnaQ	-	6.44	-
K02909 / rpmE	6.08	2.41	-
K02954 / rpsN	6.02	2.15	-
K02864 / rplJ	5.89	-	-
K02871 / rplM	5.36	-	-
K03531 / ftsZ	5.34	3.04	-
K01358 / clpP	-	5.10	-
K02881 / rplR	4.76	0.94	-
K02914 / rpmH	-	4.72	-
K02884 / rplS	3.37	4.52	-
Membrane transport			
K05813 / ugpB	4.08	-	-6.35
K15580 / oppA, mppA	-	-	-6.13
K11073 / potF	5.51	-	-

B-O environments			
KEGG identifier/gene	Osp	BSp	MSp
Amino acid metabolism			
K00549 / metE	-	4.53	-
K01457 / atzF	-	3.91	-
K01470 / E3.5.2.10	-	3.76	-
K00166 / bkdA1	-	3.69	-
K03781 / katE, CAT, catB, srpA	-	3.33	-
K03897 / iucD	-	3.11	-
K01941 / E6.3.4.6	-	2.95	-
K01485 / codA	-	2.40	-
K01478 / arcA	-	-2.12	-
K12256 / spuC		2.08	
Carbohydrate metabolism			
K00754 / bshA	-	4.50	-
K01190 / lacZ	-	3.89	-
K00123 / fdoG, fdhF	-	3.74	-
K03781 / katE, CAT, catB, srpA	-	3.33	-
K00971 / manC, cpsB	-	2.90	-
K00023 / phbB	-	2.48	-
K00705 / malQ	-	2.36	-
K00790 / murA	-	2.13	-
K01734 / mgsA	-	2.12	-
K00114 / exaA	-	-2.03	-
Cell cycles/replication /transcription/translation			
K02342 / DPO3E, dnaQ	-	5.17	-
K02316 / dnaG	-	4.54	-
K02876 / rplO	-	3.33	-
K02982 / rpsC	-	2.94	-
K03589 / ftsQ	-	2.55	-
K03060 / rpoZ	-	2.55	-
K02892 /rplW	-	-2.62	-
K01873 / valS	-	1.55	
K03531 / ftsZ	-	1.55	-
K02878 / rplP	-	-1.19	-
Membrane transport			
K15577 / nrtB, nasE, cynB	-	5.70	-
K15576 / nrtA, nasF, cynA	-	5.15	-
K09970 / aapQ, bztB	-	3.38	-

Table 4 Continued

O-M environments			
KEGG identifier/gene	Osp	BSp	MSp
K05813 / ugpB	5.70	-	-5.83
K02040 / pstS	5.68	-0.35	-
K02042 / phnE	5.38	-	-
K12340 / tolC	5.24	-	-
K03116 / tatA	5.13	2.38	-
K05816 / ugpC			-5.01
K03073 / secE	4.83	-	-
Lipid metabolism			
K01961 / accC	-	-6.42	-
K01897 / fadD	2.27	-	-5.93
K00507 / SCD, desC	5.74	-	-
K00864 / glpK	-	-	-4.94
K03715 / MGD	4.48	-	-
K10255 / FAD6, desA	4.44	-	-
K00208 / fabI	3.97	2.86	-
K03621 / plsX	3.63	-	-
K00019 / bdh	3.53	-	-
K09458 / fabF	3.35	-	-
Oxidative phosporylation			
K02110 / atpE	6.76	-2.78	-
K02109 / atpF	6.19	-	-
K02113 / atpH	5.95	3.26	-
K00413 / petC	5.86	-	-
K00937 / ppk	5.73	1.89	-
K00330 / nuoA	5.37	-	-
K00341 / nuoL	5.29	-	-
K00342 / nuoM	5.20	-	-
K00336 / nuoG	5.14	-	-
K02258 / COX11	5.13	-	-
Signal transduction			
K00413 / petC	5.86	-	
K00507 / SCD, desC	5.74	-	
K02040 /pstS	5.68	-0.35	
K08930 / pucA	5.56	-	
K12340 / tolC	5.24	-	
K07636 / phoR	5.20	-1.87	
K08939 / pucB	5.17	-	
K08927 / pufB	4.91	-	
K08738 / CYC	4.82	3.15	
K01759 / gloA	-	2.97	-4.81

B-M environments			
KEGG identifier/gene	Osp	BSp	MSp
K09969 / aapJ, bztA	5.11	-	-
K11960 / urtB	4.97	-	-
K03073 / secE	3.78	4.78	-
K03116 / tatA	4.68	-	-
K11959 / urtA	4.51	-	-
K09688 / ABC-2.CPSE.P	-	4.43	-
K02042 / phnE	4.38	-	-
Lipid metabolism			
K01190 / lacZ	-	5.54	-
K00507 / SCD, desC	5.13	-	-
K01613 / psd	-	4.59	-
K01782 / fadJ	3.41	4.47	-
K10255 / desA	4.22	-	-
K00208 / fabI	-	4.14	-
K03736 / eutC	-	4.06	-
K03621 / plsX	-	3.93	-
K01897 / fadD	2.11	3.79	-
K06445 / fadE	-	3.76	-
Oxidative phosporylation			
K00404 / ccoN	-	-	6.14
K02113 / atpH	5.73	4.91	-
K00336 / nuoG	5.52	-	-
K02109 / atpF	5.40	-	-
K02110 / atpE	4.92	-1.53	-
K00241 / sdhC, frdC	4.88	4.70	-
K00413 / petC	4.87	3.47	-
K02258 / COX11	4.72	-	-
K00341 / nuoL	4.53	-	-
K00937 / ppk	4.29	3.07	-
Signal transduction			
K00404 / ccoN	-	-	6.14
K08927 / pufB	5.91	-	-
K13991 / puhA	5.35	-	-
K00507 / SCD, desC	5.13	-	-
K08930 / pucA	5.01	-	-
K00413 / petC	4.87	3.47	-
K07644 / cusS, copS, silS	-	4.67	-
K08738 / CYC	4.46	4.11	-
K02556 / motA	-	4.40	-
K04771 / degP, htrA	4.29	4.27	-

B-O environments			
KEGG identifier/gene	Osp	BSp	MSp
K03217 / yidC, OXA1	-	2.76	-
K03073 / secE	-	2.67	-
K12368 / dppA	-	2.66	-
K09969 / aapJ, bztA	-	2.57	-
K15578 / nrtC, nasD	-	2.45	-
K11963 / urtE	-	2.07	-
K09971 / aapM, bztD	-	1.97	-
Lipid metabolism			
K03736 / eutC	-	4.61	-
K01190 / lacZ	-	3.89	-
K03735 / eutB	-	3.58	-
K03621 / plsX	-	3.53	-
K01897 / fadD	-	3.35	
K02372 / fabZ	-	2.55	-
Oxidative phosporylation			
K00404 / ccoN	-	1.07	-
K00405 /ccoO	-	2.42	-
K00406 / ccoP	-	1.46	-
K02111 / atpA	-	-1.03	-
K02113 / atpH	-	1.65	-
K03885 / ndh	-	2.82	-
Signal transduction			
K13991 / puhA	3.90	-	-
K03406 / mcp	-	3.59	-
K03781 /katE, CAT, catB, srpA	-	3.33	-
K04079 / htpG	-	3.08	-
K01077 / phoA, phoB	-	2.50	-
K00405 / ccoO	-	2.42	-
K02405 / filA	-	2.39	-
K07644 / cusS, copS, silS	-	2.16	-
K00406 / ccoP	-	1.46	-
K01113 / phoD	-	-1.73	-

Full gene names for the gene abbreviation are shown in Supplementary Table S9.

3.3.4 Energy, carbon and lipid metabolism between O-M environments

As many genes were differentially expressed between O-M environments, the following section mainly focuses on assessing the expression pattern of the ecological groups in these environments (Figure 15). General metabolic functions of the olighaline specialist (Out000010 *Limnohabitans*) performed well since the up-regulation of the highly expressed genes in the 7 categories was found in their own habitat (Table 4; Figure 16). Noteworthy, the presented metatranscriptomic data revealed high expression of gene subunits (*nuoA-I*) consisting of NADH-quinone oxidoreductase along with a set of genes encoding H+-transporting ATPase by the *Limnohabitans* for oxidative phosphorylation under both low saline conditions, in comparison to the marine environment (Table 4). This was consistent with a metagenomic analyses on bacterioplankton along salinity gradient of the Baltic Sea along (Dupont *et al*., 2014). Interestingly, I identified the following enzymes of one carbon pool by folate and carbon fixation: methylenetetrahydrofolate dehydrogenase ($NADP^+$)/methylenetetrahydrofolate cyclohydrolase (*folD*), glyceraldehyde 3-phosphate dehydrogenasea (*gapA*) and methylmalonyl-CoA mutases (*MUT*) (Table S6). The former enzymes in carbon fixation on folates have been shown not only in anaerobic organisms also under micro-aerobic conditions (Braakman and Smith, 2012). Increasing in the cellular $NADP^+$ has been shown to result in reduced cell sensitivity to oxidative stress (Fan *et al*., 2014). The latter gene MUT is involved in the isomerization of methylmalonyl-CoA to succinyl-CoA, which requires vitamin B12 as a cofactor to function (Young *et al*., 2015; Castelle *et al*., 2017). These results suggested that a freshwater *Limnohabitans* grown the marine environment had undergone oxidative stress induced by high salt situation, and enhanced the expression of genes invovled with CO_2 fixation and C1 metabolism as a strategy to cope with stressful conditions due to high-energy cost to respiration.

Carbohydrate metabolism has been shown to occupy a vital function in cell response to abiotic stress (e.g., salt stress, Meena *et al*., 2017). We identified several pathways and key genes in the carbohydrate metabolic process by the marine *Vibrionaceae*. Genes encoding enzymes for starch and sucrose metabolism (*glgP*), glyoxylate and dicarboxylate metabolism (*gcvT, aceB, glcB*) were down-regulated under the oligohaline conditions (Table 4; Table S9). The down-regulation of starch and sucrose metabolism in plant cells was previously observed during the response to salt stress (Zhang *et al*., 2017). Also, membrane transporters such as substrate-binding proteins (*oppA, ugpB*), ATP-binding protein (*ugpC*) as well as glycolysis (*eno*) were transcriptionally suppressed in the oligohaline environment (Table S9). These results clearly demonstrate that a much lower saline condition constrained energy production and central carbon metabolism of the marine specialist.

Interestingly, gene accC encoding the biotin acetyl-CoA carboxylase in the brackish specialists was overexpressed (~ 6-fold) in the marine compared to oligohaline environment for carbohydrate metabolism (Table 4; Table S9). This gene has been used as a functional marker to detect the chemoautotrophic Bacteria and Archaea that are capable of dark CO_2 fixation (Auguet *et*

al., 2008; Alonso-Sáez *et al*., 2010). However, some heterotrophs in the Arctic have previously been shown to have high uptake of dark bicarbonate (Alonso-Sáez *et al*., 2010). The explanation to such observation was that the major changes in lipid composition would be induced under nutrient-limiting conditions, and consequently lipid synthesis can increase the demand for CO_2 (Merlin *et al*., 2003). Accordingly, we also observed up-regulation of the gene accC by the brackish specialists for lipid metabolism in the marine environment, in comparison with that response observed in the oligohaline environment (Table 4). This gene was shared by the O-M and B-M transcriptome datasets, pointing to its specific response to marine conditions. However, the similar expression pattern of accC was only observed in Otu000021 affiliated with *Sphingobium* that was one of the three brackish specialists studied here (Table S7). Thus, we hypothesize that a brackish-adapted *Sphingobium* would simultaneously regulate gene accC involved in carbohydrate and lipid metabolisms to maintain the osmotic balance, as a response to saline conditions.

3.3.5 Strong signal transduction in the brackish environment

In addition to the differential transcriptional investment by the ecological groups between O-M environments, our analyses revealed a greater gene expression involved in signal transduction by both, freshwater and marine specialists experiencing the brackish water. In the case of the *Limnohabitans* grown under brackish conditions, multiple highly expressed genes involved in two-component systems (*pufB, pucA and puhA*), through the mechanistic strategies including light-harvesting complex 1 system, light-harvesting protein chain and photosynthetic reaction, respectively (Table 4; Table S9). Our data confirm genomic analysis on two *Limnohabitans* isolates, where a large diversity in genome size and traits indicated that these organisms exhibited capacities of bacterial photoautotroph (Zeng *et al*., 2012). However, it is still surprising, because our experiment was conducted in the dark, and those genes are the indicator of photosynthetic proton pumping and electron transfer machinery aerobically (Fortunato and Crump, 2015). Although *Limnohabitans* species have recently been reported to harbor aerobic anoxygenic phototrophs, anoxygenic photosynthesis of this genus and its relationship with light remains unresolved (review in Koblížek, 2015). Given that fewer genes were significantly differentially expressed between B-O environments for this oligohaline specialist (Figure 16), I speculate that the observed transcriptional responses in two-component regulatory-related genes were not primarily for overcoming the stress, rather facilitation of cell-cell communication or other cellular maintenance in the brackish environment. In contrast, the marine *Vibrionaceae* was expressing only one gene (*ccoN*) (~ 6 fold) under brackish conditions (Table 4). The ccoN encoding cbb_3-type cytochrome c oxidase is a core catalytic subunit of terminal oxidase in the respiratory chain of the majority aerobic organisms, such as *Pseudomonas aeruginosa* (Hirai *et al*., 2016). This process requires four-protons to reduce molecule oxygen to water through transmembrane pumping of protons. The ccoN involved in both

signal transduction and oxidative phosphorylation process showed higher expression in the brackish compared to marine environment. This observation indicated that *Vibrionaceae* invested in proton motive force across cell membrane as a respiratory strategy when sensing and responding to the brackish condition as changes in osmolality.

Concluding, this experiment study shows that at the individual level, this metatranscriptomic analysis revealed distinct metabolic pathways that the bacteria with different life strategies invoked to respond to a salinity change. In particular, the compositional changes of transcripts that mediate energy conservation and cellular carbon storage were pronounced between the olighaoline and marine specialists. Future studies including frequent sampling and longer time-periods are required to ascertain the specific transcriptional patterns observed here.

3.4 The extent of multifunctional redundancy changes as transcriptions among taxa shifts after disturbances (Case study 3)

In this study, I was able to assess the extent of functional redundancy (FR) between communities after disturbances， which is important to understand whether a community is robust to that type of disturbance, and how the degree of disturbance intensity influences the degree at which the disturbed community contains multiple species that serve similar functions. To address this issue while taking into account the multi-functionality, metatranscriptome-approach was used to quantify FR between the communities that experienced salinity changes. The variability in the transcript composition and the relative abundances of individual taxa within and between communities was calculated using Bray-Curtis dissimilarity matrix, reflecting the degree of FR between the disturbed communities. First, I found that a greater of FR occurred between-community at low disturbance intensity (3.4 psu difference) compared to those experiencing high disturbance intensity (16 / 19 psu difference), which is significant only for the marine inoculum. However, functional change between-community was significant for the brackish inoculum when comparing salinity difference in 3.4 psu with those after exposure to the 19 psu difference, while FR stay unchanged at environmental distance (disturbance intensity). The data suggest that both marine and brackish assemblages showed certain level of sensitivity to changing salinity, and that the latter one may be less sensitive with respects to functional traits. Second, I demonstrate that the differences in disturbance responses between the marine and brackish inoculum appear to relate to the community traits. FR seemed to marginally co-vary with species diversity and effective genome size for the marine inoculum, whereas was negatively related to functional diversity for the brackish inoculum. Finally, I show that the extent of FR differed depending on the phylogenetic level being considered, with higher FR found between very closely related organisms, especially under similar environmental conditions.

3.4.1 Effect of disturbance intensity on transcriptional performance of bacterial communities

Among all protein-coding metatranscriptome reads, 14%-51% could be taxonomically annotated to bacterial genome and functionally annotated to the described gene orthologs based on KEGG reference (Table S2). Similar proportion of the annotated metatranscriptome reads was presented in other metatranscriptome study (Bunse *et al*., 2016). This is because functional annotation in metatranscriptomics are challenging at the species level owing to a lack of reference genomes for aquatic bacteria. The interpretation of gene expression patterns in microbial communities would be affected if all reads are annotated, or if considering differences in copy number of protein encoding genes between closely related genotypes defined by 16S rRNA gene sequences. On the other hand, the metatranscriptome data is informative for the contribution the community-wide expression by considering the changes in transcript composition and relative abundance of individual taxa present in that community.

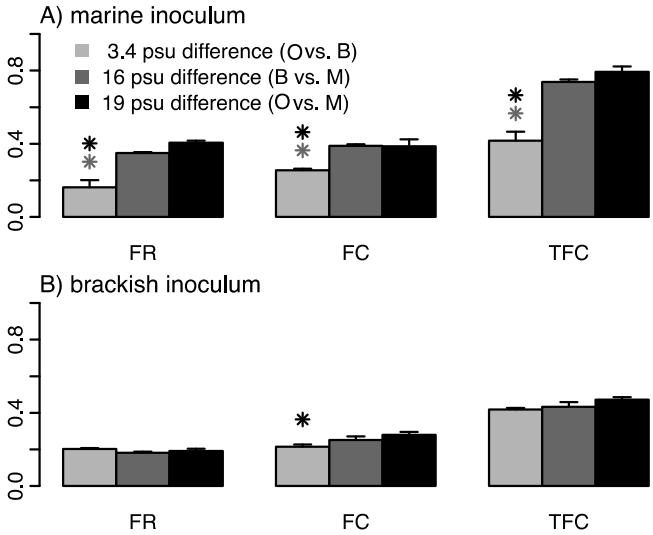

Figure 17 FR, FC, and TFC estimated among communities at increasing environmental distance. All three parameters vary between 0 and 1. Error bars indicate the standard deviation between the biological replicates. (O, B, and M represent oligohaline, brackish and marine media, respectively). Significant ($p<0.05$) differences between individual values are indicated by asterisks (one-way ANOVAs for FR, FC and TFC, separately, for incubations originating from the two inocula followed by pair-wise Tukey's post hoc tests). Slightly modified based on Beier *et al*., (2017).

I assessed the effect of disturbance intensity, that is, difference in salinity level of the incubation media on the patterns in functional redundancy (FR), functional change (FC) and theoretical functional change (TFC). In case of the marine inoculum, FR significantly increased with the increasing difference in salinity between media, with highest FR detected between the communities that experienced the conditions with a 19 psu difference (ANOVA, $P < 0.05$, Figure 17 A). However, FR between any of the two brackish communities was unaffected ($P > 0.05$, Figure 17B). Irrespective of source inoculum a significant increase in FC with increasing differences in medium salinity was observed for the compared communities, particularly pronounced for the marine inoculum ($P < 0.05$, Figure 17). The TFC between the communities originating from the marine inoculum was elevated along the salinity distance of the media, but that of the brackish inoculum remained nearly constant (Figure 17A and B, respectively). Another ANOVA further tested for the effect of incubation environment, inoculum source and their interactions on FR, FC, and TFC, individually. The results of this ANOVA showed that the both incubation environment and inoculum source had significant effects on the three functional measures (ANOVA, $P <0.001$); however, the effect of inoculum source was relatively stronger according to the outcome of *F*-value (Table 5). These results are inconsistent with an earlier assumption that environmental parameters were more relevant to the magnitude of FR than the characteristics of the compared communities and their members (Comte *et al*., 2013). Possibly, the communities studied in this earlier study were

characterized by very similar features, such as diversity indices and did not significantly influence the FR. Another explanation could be that the multiple functions were considered here, compared to limited functions measured in that earlier study, which may increase the possibility that other functions would have been driven greatly by the intrinsic traits of the community. The data presented here, still, indicate that the characteristics of inoculum source may be important in understanding of outcome of functional responses, even at the transcriptional level of individuals.

Table 5 Influence of different factors on FR, FC and TFC.

		All gene orthologs		Gene orthologs grouped in the functional categories	
Parameter	Factor	F	*P* -value	F	*P*-value
FR	Inoculum	180	***	420	***
FR	Environmental change	67	***	200	***
FC	Inoculum	100	***	182	***
FC	Environmental change	43	***	62	***
TFC	Inoculum	263	***	733	***
TFC	Environmental change	104	***	297	***

F-values and p-values were obtained from two-way analyses of variance (ANOVAs) testing for the influence of inocula (marine, brackish) and environmental change (oligohaline vs. brackish, brackish vs. marine, oligohaline vs. marine) on FR, FC and TFC between bacterial communities. The ANOVAs were performed on all gene orthologs. In the latter case, the influence of the functional categories was also tested. Significance levels are indicated by asterisks (P <0.05=*, P <0.01=**, P <0.001=***). Slightly modified based on Beier *et al*., (2017).

As hypothesized, that the disturbance intensity would influence the degree of FR being expressed between the communities, which was only supported in the case of the marine but not the brackish inoculum. My finding also suggests that functionally redundant taxa in the marine communities were sensitive to salinity disturbance, depending on the degree of intensity of such disturbance. Most unexpectedly, a greater FR was expressed between the communities that had experienced contrasting environmental conditions, that is, oligoahline vs. marine medium. Similarly, the TFC was also greater at the highest disturbance intensity (a 19 psu difference in salinity). This is the result from both shift in transcript composition (i.e., phenotypic plasticity) and changes in relative abundance (Beier *et al*., 2015), leading to varying additive-transcriptional responses of individual community members at different intensities. Additionally, changes in the taxon-specific relative transcript abundance of a gene ortholog can be caused by changes in the transcriptional levels per cell or by changes in the relative cell abundance of the taxon. For the latter case, this is reflected in the overall community composition as indicated by a large difference in composition of the marine communities grown under oligohaline and marine incubation environments (Figure S2). Environmental differences between the different media may select taxa that are either more functionally specialized or present a broader niche breadth, as noted elsewhere (Kirchman, 2002; Hahn, 2006; Comte *et al*., 2013). In this sense, taxa from marine communities that grew under

extreme environmental conditions (oligohaline and marine media) may function differently from one another, but result in the similar process when combined at the community level (for example, Allison and Martiny, 2008 and references therein). The expression of FR between any of the marine communities may depend on how similar the functions are scaled at the community-level, which is influenced by the disturbance intensity. The result of PCA showed that FR appeared to positively co-vary with the effective genome size, which is higher for the marine inoculum (Figure 18). This indicates that the greater effective genome size between the marine communities grown under oligohaline and marine conditions (19 psu) may also lead to the higher degree of FR, in comparison with that of the communities grown at the other salinity levels. In addition, FR increases with decreasing functional diversity (Figure 18), possibly, because communities with low functional diversity comprise a small fraction of specific functions performed by few taxa.

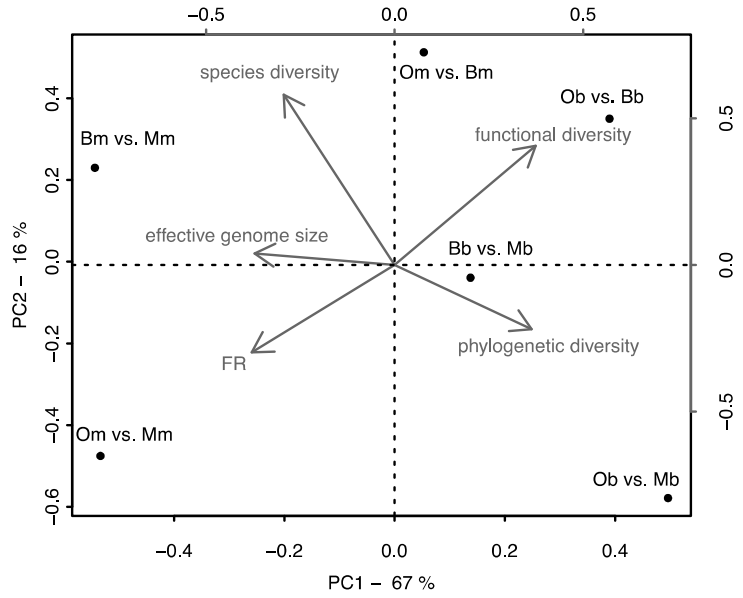

Figure 18 PCA illustrating the relationship of FR to diversity parameters and the effective genome size. Because FR is always measured between community pairs, mean values from each community pair for which FR was estimated were taken for all other parameters. For metagenome-based parameters (functional diversity, effective genome size), only one value for each incubation condition was available, and the mean of the triplicate values was built for data based on metatranscriptome (FR) or 16s rRNA gene (species diversity, phylogenetic diversity). All displayed diversity parameters are derived from abundance-weighted measures. Capital and low-case letters indicate the media and the inoculum, respectively (O/o: oligohaline, B/b: brackish, M/m: marine). Percentage values along the axes indicate the contribution of each axis to the total variability. Slightly modified based on Beier *et al*., (2017).

Contrary to my expectation, the FR between the communities originating from the brackish inoculum, was unaffected by the disturbance intensity. This implies that the expression of FR appears to be more related to community intrinsic traits of the brackish inoculum, than to factors extrinsic to the community. As evidenced in the PCA plot, the brackish inoculum was less sensitive

to the disturbance intensity and contained more phylogenetically distant related (as indicated by high phylogenetic diversity) taxa (Figure 18); that is, generalists with a wider niche breadth. Possibly, those generalists of the two communities originating from the brackish inoculum were functionally redundant to each other, independent of salinity conditions. This assumption seems to hold true, given that no change in TFC was observed for the brackish inoculum across the disturbance intensity. Taken together, higher FR compensated for the increased sensitivity of the marine inoculum to salinity compared to that of the brackish inoculum, and functional responses were the outcome of interplay between community composition and transcriptional performance of the individual community members.

3.4.2 Degree of FR at different taxonomic ranks

The metatranscriptome-based metric furthermore enabled detection of the magnitude of FR at varying taxonomic ranks. I further hypothesized that a greater FR is expressed among closely related than the distantly related organisms, especially pronounced under the similar environmental conditions. The second hypothesis was, still supported by the results that a higher proportion of FR was detected among more closely related taxa (species, genus, family), when the communities were grown under the environmental conditions with smallest difference in salinity (i.e., 3.4 psu) (Figure 19). Although this assumption was supported by the case of marine and brackish inocula, the higher FR occurred at different taxonomic levels between the two inocula. In the case of marine inoculum, FR was significantly higher between the communities that experienced the salinity conditions with a 3 psu difference, compared to that exposed to larger salinity differences; this pattern was consistent across a wide taxonomic ranks ranging from the species to class levels (Figure 19A). The reverse trend in a greater extent of FR was observed between the taxa were phylogenetically distant, that is, different classes for the same phylum (Figure 19A). In the case of brackish inoculum, taxa originating from the assigned to different genera but the same family contributed a higher degree of FR when grown at similar salinities (Figure 19B). However, at the middle difference in salinity conditions, such high FR was expressed among the high levels of taxonomy, starting from the order level to phylum level. Additionally, the contribution of different phylogenetic levels to FR at similar salinity differences (16 and 19 psu) revealed very similar patterns in the communities originating from the marine but not brackish inoculum (Figures 19A, B). Such similar pattern in FR observed at similar salinity disturbance was also evidenced in the FC and TFC only for the marine inoculum. Possibly, factors other than salinity may have been more relevant in shaping the response of taxa from the brackish inoculum. Some variations in the degree of FR being expressed at the different taxonomic ranks may depend on which factors are relatively important to the community outcome. Thus, these results highlight that brackish assemblage may not be a good model for predicting how readily bacterial community composition matters to a salinity disturbance, as well as the functional

responses to such disturbance. Rather, the expression of FR may be ecologically coherent for the marine assemblage under changing environmental conditions. However, this hypothesis should be tested in future experimental setting with a wider salinity levels and high frequency sampling. Overall, the expression of FR varies between the marine and brackish inocula at different taxonomic ranks in most cases, and it does not affect the general finding that refers to lower taxonomic ranks when considering their contribution to the high degree of FR by the two inocula (Figures 19C, D). These observations were not yet validated in oligohaline assemblages; however, it is plausible to assume that the disturbance intensity similarly influence the functional response of oligohaline as observed for the marine inoculum. This relies on an earlier suggestion that the existence of an indigenous brackish bacterioplankton that have been evolved long from originally marine and freshwater species (Herlemann *et al*., 2011; Hugerth *et al*., 2015). Brackish bacterioplankton could for this reason be less sensitive to salinity changes than its counterparts.

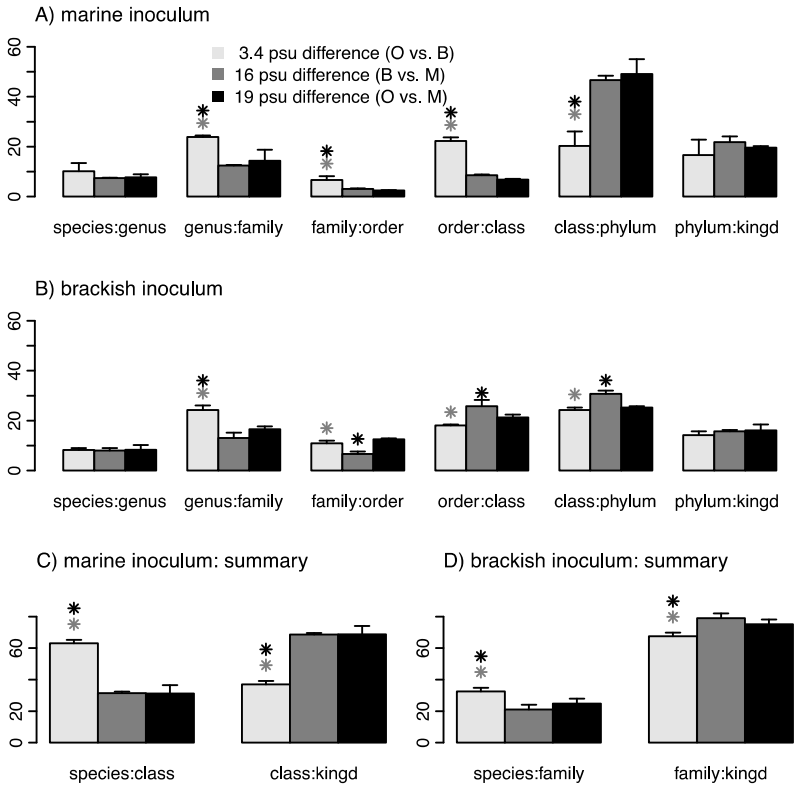

Figure 19 Percent contribution of taxa grouped at different taxonomic levels to the total species-level FR (see also Figure S8). For instance, the first three columns (species:genus) indicate the contribution of taxa that have the same genus but belong to different species to FR. Error bars indicate the standard deviation between the biological replicates; asterisks indicate significant differences ($p<0.05$) from the FR of the other community pairs at the same taxonomic level. (O oligohaline medium, B: brackish medium, M: marine medium). **A**) and **B**) illustrate the results resolved for every taxonomic level for communities originating from the marine and brackish inoculum. **C**) and **D**) illustrate summarized values, where those taxonomic levels were added, in which either community pairs with low salinity difference (O vs. B) or communities with high salinity difference (B vs. M or O vs. M) featured maximum values. Slightly modified based on Beier *et al*., 2017).

3.4.3 Methodological limitations and transferability to other ecosystems

It is essential for the developed metric that individual transcripts encoding the same gene ortholog but assigned to different reference genomes indeed derive from different taxa in the samples. FR between phylogenetically very closely related organisms in a community might be missed if the resolution of the BLAST reference database is insufficient, and the resulting transcribed sequences are incorrectly mapped to the same reference genome. Hence, FR may be underestimated, even though the bias is assumed to be similar for all pairwise comparisons. The direct link between transcript counts and corresponding functional rates has been questioned for several reasons (Prosser, 2015). For instance, post-transcriptional modifications or the mismatch between the short lifetime of mRNA and the much longer lifetime of the corresponding proteins may blur the correlation between transcript abundance and functional rates. Measured functional rates reflect the integrated activity over a longer time period, while transcriptome data—at least if measured at a single time point as here—represent a snapshot of the transcriptional activity at the moment of sampling. These problems likely contribute to the poor correlation that had been observed between gene transcription and corresponding rate measurements (Rocca *et al*., 2015). In contrast to these earlier findings reported in Rocca *et al*., (2015), bacterial production rates in this study correlated significantly with the relative abundance of ribosomal protein transcripts (Figure 20). This significant correlation indicates that the constant and controlled conditions during the incubations led to reasonably stable transcription dynamics, at least over the time period (1 hr) when bacterial productions was measured. Accordingly, production rates were reflected by the relative abundance of ribosomal protein transcripts measured at a single time point. Still, FR estimated from metatranscriptome data relates to short-term community activity processes that are relevant during the time of sampling. However, the presented metric can also be applied on metaproteomic data, which are

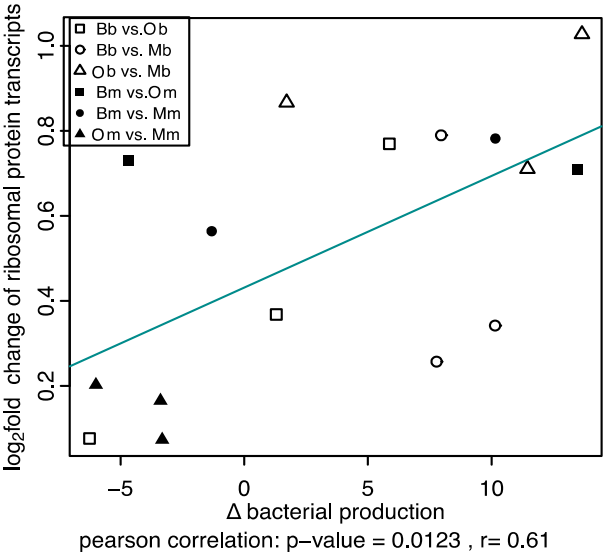

Figure 20 Linear regression of the average $\log_2$ fold transcriptional changes in gene orthologs encoding ribosomal proteins and the differences in bacterial production (µg C h^{-1} $L^{-1)}$ between community pairs also compared for the estimation of FR. Differential expression analyses were performed on functional data that were size-normalized between samples; thus, the $\log_2$ fold transcriptional changes refer to relative changes in the composition of gene orthologs between the compared communities. Slightly modified based on Beier *et al*., (2017). See also Figure S9 for bacterial production over 4 days of the experiment, as well as Appendix Text 1 for the method used to quantify transcriptional regulation of genes linked to bacterial production rate.

more directly linked to rate measurements.

This study cannot be rigorously extrapolated to natural ecosystems, given that the experimental system has limited potentials to mirror the highly complex structural and temporal context of natural communities or to populations from different domains of life. The findings, however, that the disturbance intensity strongly influences the FR of a bacterial community, the degree of which may depend on the sensitivity of the given community to the type of the disturbance, provide valuable hypotheses to be tested further with other species and in different disturbances / environments. They may also be interpreted as a general hint that bacterial composition and functional performance are altered by disturbances, and at least, FR may buffer disturbance response if not all functionally redundant taxa in the disturbed communities are sensitive to the change. The design and establishment of long-term microbial model system under natural environmental conditions would be challenge (Srivastava *et al*., 2004), if not impossible (for example, Martiny *et al*., 2017). Integrating such systems with more information on microbial genomes provides a better understanding of the mechanisms that influence how individual taxa in a community respond to environmental change. This helps determine how readily the given community composition can be used to predict the responses of ecosystem processes to such changes.

3.4.4 Concluding remarks

Metatranscriptome data have been used in many previous studies to track the presence and expression patterns of specific genes; however, in this study the utility of metatranscriptomics was extended to test ecological hypotheses by quantifying FR between two communities. Most importantly, the *in situ* transcription of multiple functions can be comprehensibly explored and the impact of FR on community functioning can be disentangled from that of transcriptional plasticity. It is not feasible to model all taxa and their responses individually (Allison and Martiny, 2008); metatranscriptome-based quantification of FR, however, enables an accurate assessment on how FR buffers disturbance responses of the community without modeling all taxa and their responses separately.

In sum, FR was more strongly affected by community properties including for instance functional diversity or the transcriptional behavior of its members than by environmental parameters. At low salinity difference, a higher proportion of the total FR in communities occurred among phylogenetically more closely related taxa. These results imply that a coherent relationship between FR and disturbance intensity can be observed at low bacterial taxonomic ranks. In the light of these observations, I propose a framework that considers the extent of FR along disturbance intensity, and that the information generated based on such framework shed light on where and when FR are most important for the stability of community in the face of disturbances.

3.5 Effects of dispersal and the interaction with contemporary environmental conditions on the diversity and community composition following a salinity change (Case study 4)

I experimentally tested how the response of estuarine bacterioplankton communities to minor changes in salinity differed depending on whether they were subjected to dispersal or not. First, I could show that dispersal significantly increased alpha diversity of the transplanted communities originating from marine but not brackish source. Second, variability between-community in the dispersal treatments was smaller than that in the non-dispersal treatments, which was driven by changes in the relative abundances rather than replacement of abundant taxa. This work suggests that dispersal could facilitate local adaption of some active community members, and that dispersal and its interaction with contemporary environments play an important role in explaining the described patterns.

3.5.1 Effect of dispersal on microbial cell abundance

Inoculum source and environment had significant effects on bacterial abundances (three-way ANOVA, source: F=224.57, P <0.001; environment: F=128.78, P <0.001), but no significant effect of dispersal was found (Tables S10 and S11). The interaction between dispersal and environment marginally affected bacterial abundance (three-way ANOVA, F= 3.14, P <0.1). This effect was most pronounced for the transplanted brackish communities under marine conditions, with higher cell abundances in the dispersal treatments (Figure 21A). The abundances of protists were significantly higher in the initial marine than brackish inocula (Figure 21B). Dispersal, environment and inoculum source individually influenced protist abundance at the end of the experiment (Table S11). Similarly, the interaction between dispersal and environment significantly affected protist abundance (three-way ANOVA, F= 6.85, P <0.05; Table S11). A higher abun-

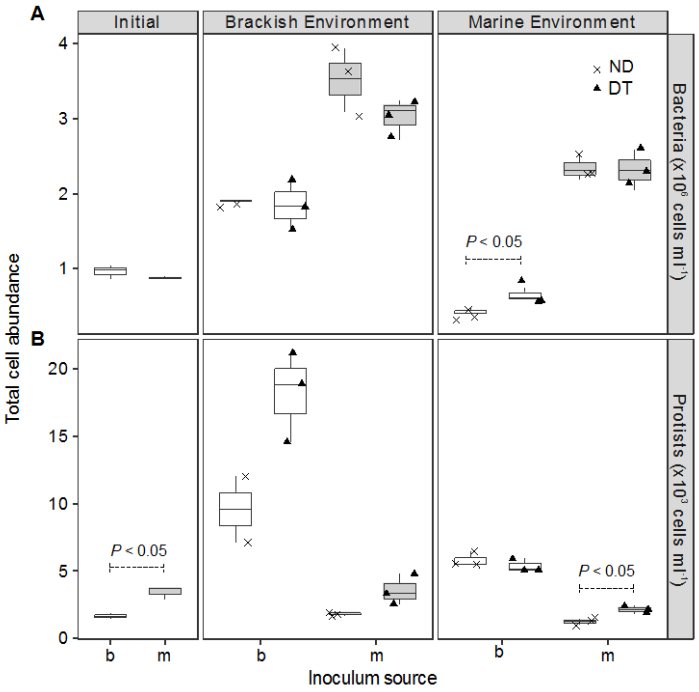

Figure 21 Cell abundances of bacteria (**A**) and protists (**B**) at the initial stages of the experiment and after incubation in the brackish and marine environments for 5 days. The source of the initial microbial inoculum (x-axis) is indicated as brackish (b, white) or marine (m, grey). Symbols represent ND, i.e., non-dispersal treatment (×), and DT, i.e., dispersal treatment (triangle). Significant differences at P <0.1 obtained from the corresponding Welch's t-tests are shown in the figures. The values of cell abundance for bacteria and protists are displayed in Supplemental Table S10.

dance was observed in the dispersal treatment for the marine communities grown in their native environment (i.e., incubation environment and inoculum source matched with regard to water origin) (Figure 21B).

3.5.2 Dispersal led to an increase in local community diversity of the marine communities

The initial brackish inocula exhibited significant greater alpha diversity (calculated as Shannon-index) compared to the initial marine inocula (Figure 22; Table S10). Results of three-way ANOVAs showed that dispersal, environment and inoculum source influenced alpha diversity, realized species richness and evenness (Table S11). However, the interactive effects of these three factors were weaker on richness and evenness (Table S11). Dispersal resulted in greater alpha diversity, richness and evenness in the brackish environment, which was, however, only significant for the transplanted marine communities (Figure 22). The overall diversity of the brackish communities was not affected by dispersal in any of the environments (Figure 22).

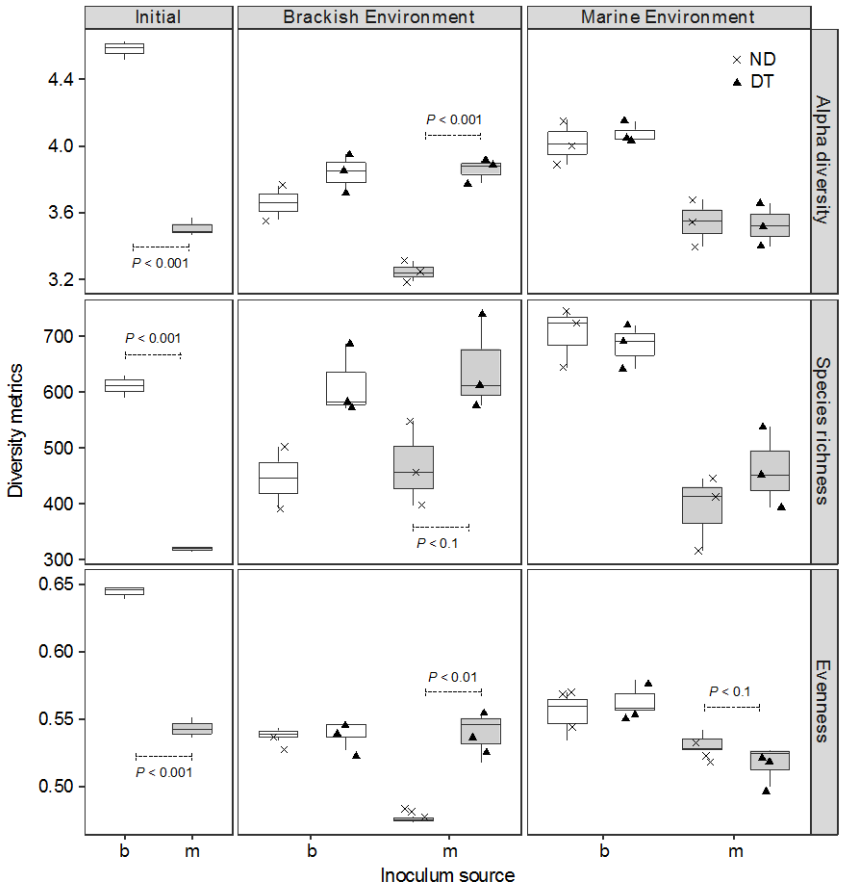

Figure 22 Alpha diversity, realized richness and evenness of the active bacterial community in the beginning and at the end of experiment (day 5). The source of the initial microbial inoculum (x-axis) is indicated as brackish (b, white) or marine (m, grey). Symbols represent ND, i.e., non-dispersal treatment (×), and DT, i.e., dispersal treatment (triangle). Significant differences at P <0.1 obtained from the corresponding Welch's t-tests are shown on the figures. The values of each diversity metric are provided in the Supplemental Table S10.

I hypothesized that dispersal increases realized species richness and evenness in response to salinity changes, even if such changes *per se* lead to a decrease in community diversity, which was only supported in the case of the marine but not the brackish community. In the marine communities, there was a small number of highly dominant OTUs when they were exposed to brackish conditions, as indicated by the low alpha diversity and low evenness. Conversely, with dispersal, alpha diversity of the transplanted marine communities increased significantly. These results support earlier suggestions based on theoretical predictions (Mouquet and Loreau, 2003) as well as findings from experimental studies (Berga *et al*., 2015; Zha *et al*., 2016) and model simulations (Evans *et al*., 2017), which also found increases in local diversity after communities experienced dispersal. Concomitantly, both realized richness and evenness increased due to dispersal; this, however, was surprising because such patterns were observed previously only at high dispersal rates (at least 25 %) as a consequence of mass effects in a simulation model (Evans *et al*., 2017). Mass effects may increase the likelihood that cells reach unfavorable habitats during or after dispersal because of continuous and large supply of immigrant cells (Lindström and Langenheder, 2012), leading to occurrence of low-abundance individuals in the communities (Loreau and Mouquet, 1999). Mass effects were unlikely to play a role in the patterns we observed because dispersal treatments were subjected to a daily exchange of only 6.6 % v/v in our experiment, i.e., they were low compared to rates at which mass effects have been shown to occur (Lindström and Östman 2011; Souffreau *et al*., 2014). Instead, frequent dispersal (exchange of cells twice per day) was enough to allow different taxa with similar competitive abilities and/or competitive dispersers to persist in the brackish environment (for example, see Matthiessen and Hillebrand, 2006). At the same time, the rate of dispersal was not so high as to result in very high densities of individuals, thereby eliminating the dominant species. Thus, the transplanted marine communities were composed of fewer abundant taxa after exposure to dispersal.

Contrary to the first hypothesis, dispersal did not increase overall diversity of brackish communities exposed to the marine environment. Given that effects of dispersal depend on initial diversity (Roy *et al*., 2012; Zha *et al*., 2016), it is likely that the higher initial diversity of the brackish compared to marine communities made them less susceptible to immigration of introduced species. Supporting this idea, we also found that the initial diversity of brackish inocula was significantly higher than that of marine inocula. To summarize, my results therefore indicate that dispersal could maintain or increase overall community diversity and that the final outcome may depend on the initial diversity of the communities.

3.5.3 Dispersal resulted in a reduction in beta diversity regardless of source inoculum

The active bacterial communities grown in the same environment became more similar when due to the dispersal manipulation although differences in community structure at the end of the experiment

still mainly depended on the inoculum source (Figure 23A). The results of the PERMANOVA test showed that variation in beta diversity among all microcosms was significantly explained by inoculum source (52.23%), followed by environment (9.32%), dispersal (3.50%) and the interactions between any of the two factors (2.92% - 6.62%) (Table S12A). When considering the microcosms with brackish and marine inoculum sources separately, the interaction between dispersal and environment was only significant for the marine but not the brackish communities and explained 14.35 % of the total variance (P <0.05; Table S12B). To explore the relationship between the variability in community composition and incubation environment, salinity, nutrient and protist concentrations were plotted in the NMDS ordination as fitting environmental variables. Variation in community composition among all microcosms correlated with differences in salinity, which were higher in the marine environment, but also appeared to be related to a set of other environmental factors, such protist abundance, DOC, PO_4^{3-}, SO_2^-, and NO_3^-, which were higher in the brackish environment (Figure 23A; Tables S10 and S13). To better understand between-community variation in dependence of dispersal for each incubation environment, we performed beta-dispersion analysis to determine the mean differences in community dissimilarity among ND and DT. Dispersal generally led to a decrease in between-community variation, which was more apparent in the marine than the brackish environment (Figure 23B).

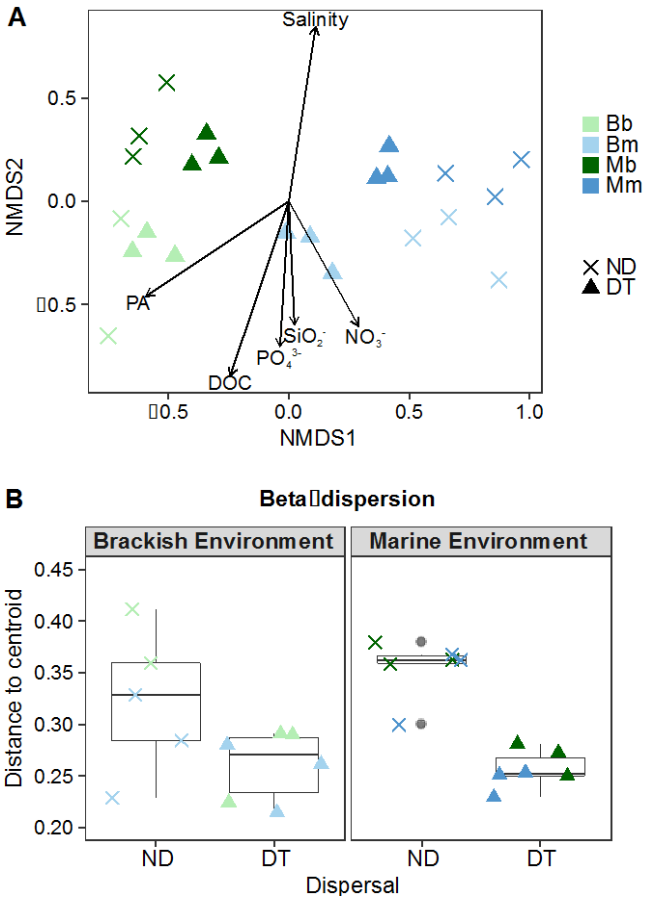

Figure 23 Beta diversity of the active bacterial communities. (**A**) NMDS ordination showing differences in the bacterial communities at the end of the experiment. Color shows the particular combination of incubation environment and inoculum source: b inoculum in B environment is Bb (light green), m inoculum in B environment is Bm (light blue), b inoculum in M environment is Mb (dark green), or m inoculum in M environment is Mm (dark blue). Symbols indicate ND, i.e., non-dispersal treatment (×), and DT, i.e., dispersal treatment (triangle). The strength of statistically significant (P <0.05) explanatory environmental variables is shown with solid arrows (for explanatory values of the environmental variables to differences in the communities see Supplemental Table S2). Abbreviations: DOC, dissolved organic carbon; PA, protist abundance (**B**) Beta-dispersion illustrating mean differences in the variation (i.e., distance to centroid) between communities in the non-dispersal and dispersal treatments for the brackish and marine environments. Significant differences at P <0.1 obtained from the corresponding Welch's t-tests are shown on the figures.

As expected, brackish and marine communities became more similar when dispersal was present regardless of the incubation environment, which is in agreement with previous studies (Lindström and Östman, 2011; Declerck *et al*., 2013; Severin *et al*., 2013). Although inoculum source and incubation environment explained a great share of the variation in community composition, dispersal and its interactions with the two factors were also significant predictors of community composition (Table S12A). This is indicative of less stochastic effects on the composition of bacterial communities compared to the deterministic effects; however, the significance of those deterministic effects (e.g., environmental effects in our case) might be weakened if dispersal occurs (Evans *et al*., 2017). It is also observed that without dispersal, rare taxa originating from the marine inoculum increased in relative abundances in response to a salinity change, while the majority of taxa from the brackish communities remained abundant. These patterns suggest that a seed bank of brackish bacteria exists within the marine species pool and that some abundant members in the brackish communities can readily grow in the marine environment. Similar findings were obtained from a transplant experiment using bacterial assemblages inhabiting the Baltic Sea (transplant experiment 1, Figure 12; Shen *et al*., Accepted).

Some empirical studies have shown that dispersal rates must be very high so that they can cause local communities to become more similar to the composition of the immigrating communities (Lindström *et al*., 2006; Logue and Lindström, 2010; Adams *et al*., 2014; but see Declerck *et al*., 2013, where this occurs at a very low rate). Still, a reduction in beta diversity by varying dispersal levels may also results from the fact that passive migration of aquatic communities is often accompanied by a change and homogenization in environmental conditions. Here we reduced such confounding effects of environmental homogenization because the dispersal manipulation was started after an equalization of salinity between the dialysis bags and incubation tank. However, I cannot exclude the possibility that DOC concentrations might not yet have been in equilibrium between dialysis bags and incubation tank at the onset of dispersal. Consequently, early-established communities associated with different DOC concentrations might have influenced the community's ultimate response (e.g., Logue *et al*., 2016; Herlemann *et al*., 2017). This was, however, relatively unimportant, since dispersal led to low mean differences in variation between communities in both brackish and marine environments at the end of the experiment (Figure 23B). Noteworthy, the compositional similarity in response to dispersal in our study seems to be more pronounced compared to earlier studies, which have included similar dispersal rates (Lindström and Östman 2011; Severin *et al*., 2013; Souffreau *et al*., 2014). One explanation could be that the metabolically active community that we examined (based on RNA) responded more to the exposure to dispersal than the total bacterial community (based on DNA) analyzed in those previous studies, as changes in the RNA would happen faster than changes in the DNA (De Vrieze *et al*., 2016). Overall, in this experiment, dispersal introduced changes in the active bacterial community composition due to the exchange of microorganisms, and not through concomitant changes in environmental conditions.

3.5.4 Occurrence patterns of abundant OTUs and identification of 'abundant dispersers'

There are two aspects that could explain increases in community similarity in response to dispersal when assessing the abundant OTU pool: replacement of OTUs or change in relative abundance. Out of the 34 abundant OTUs across all microcosms, 21 OTUs were found to have their maximal abundance in the brackish and 13 OTUs in the marine communities (Table S14). An analysis of occurrence patterns of abundant OTUs showed that regardless of the dispersal manipulation the transplanted brackish communities contained a higher fraction of abundant OTUs compared to the transplanted marine communities (Figure 24). In addition, several abundant OTUs defined as the abundant dispersers in the DT treatments were rare in the ND treatments for either brackish or marine communities (Figure 24; Table 6). If OTU replacement was more relevant, the brackish and marine communities in the dispersal treatments would share a large fraction of the abundant OTUs which were rare or absent in the ND treatments. This is not what I observed in this study because only a small fraction of the abundant dispersers was rare in the ND treatments: 14% or 31% relative to the total number of the abundant OTUs (across microcosms) for marine and brackish environments, respectively (Table 6; Figure 24). Instead, the majority of the abundant OTUs changed their relative abundances following dispersal, where the abundant OTUs of brackish and marine communities showed opposing changes in relation to their respective communities without dispersal. These patterns were evident from our data and particularly strong for OTUs originating from the marine inoculum source (Figure 24; Table S12B). Hence, our results suggest that increases in community similarity as a response to dispersal were mainly due to changes in the relative abundances rather than replacement of the abundant OTUs.

Among the OTUs that were identified as the abundant dispersers, Otu000008 affiliated with the family Rhodobacteraceae and Otu000009 affiliated with the family Colwelliaceae were detected to have good dispersal capabilities in the brackish communities, irrespective of the environment. Conversely, Otu000010 affiliated with the family Bdellovibrionaceae was detected to have good dispersal capacity in the marine communities (Table 6; Figure 24). Otu000003 affiliated with the family Campylobacteraceae was prevalent in all microcosms and exhibited its maximal relative abundance (19.85%) in the Bb_ND microcosm (Figure 24; Table S14).

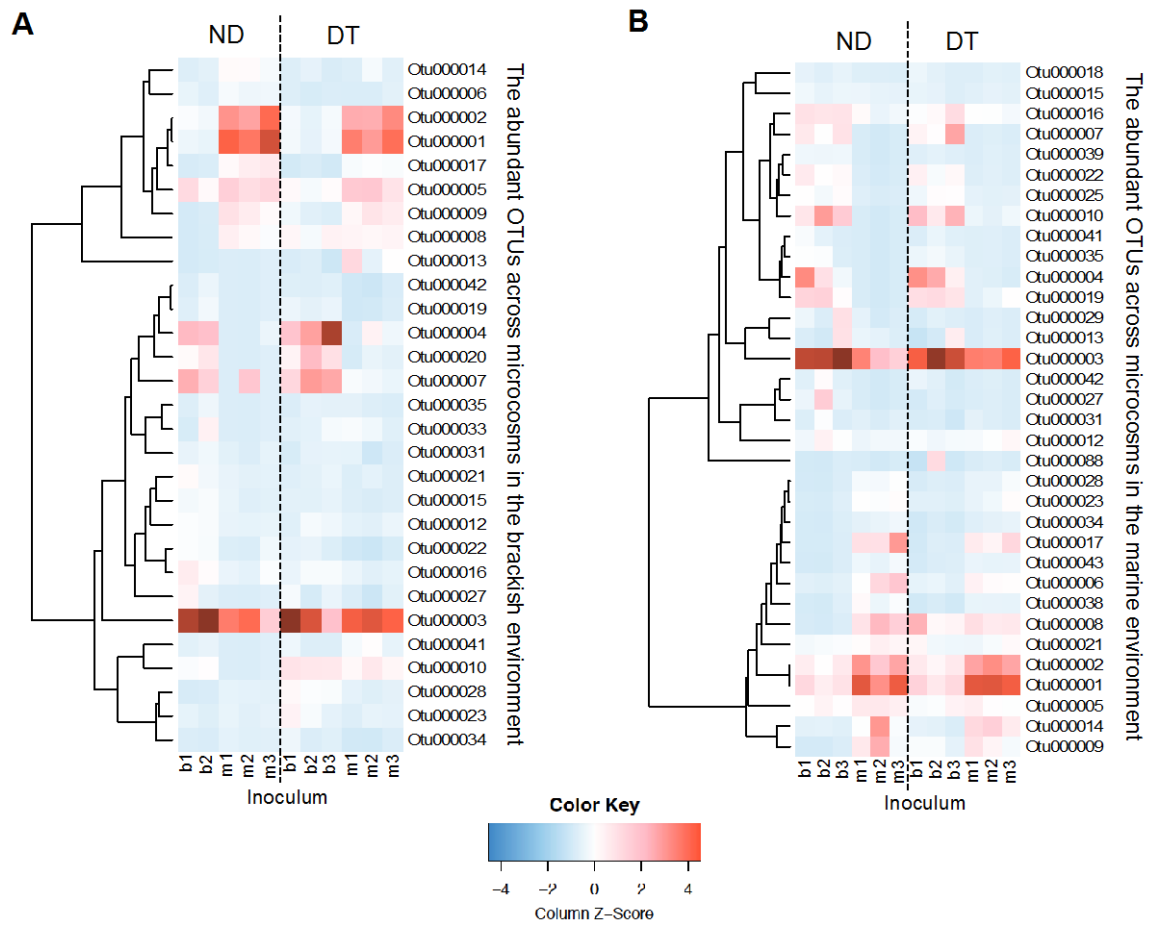

Figure 24 Heatmaps displaying relative abundances of the abundant OTUs (rows) across microcosms (columns) in the brackish environment (**A**) and in the marine environment (**B**). Color gradients indicate relative abundances of individual OTUs by column, with warm colors (towards red) indicating abundant OTUs and cold colors (towards blue) indicating rare OTUs within that sample. Column labels are sample IDs (the terminal number represents the biological replicate), while row labels are OTU IDs. The taxonomic affiliation of each of the abundant OTUs is provided in the Table S6. Dashed-lines in the heatmaps separate between ND (non-dispersal) and DT (dispersal) treatments. Side dendrograms cluster OTUs that have similar occurrence patterns.

3.5.5 Taxon-specific responses to dispersal

Bacterial communities were dominated by taxa assigned to Alpha-, Gamma-, and Deltaproteobacteria, and to Bacteroidetes (Figure 25A), together contributing ≥ 80% of total sequence reads. Three-way ANOVA tests showed significant differences in the abundance of some bacterial phyla or classes (Table S15). Dispersal led to a significant increase in the relative abundance of Deltaproteobacteria, but had weaker effects on that of Alphaproteobacteria, Epsiloprotebacteria, and Bacteroidetes depending on the inoculum source (Figure 25A; Tables S15A-C, E). However, dispersal had no effects on the relative abundance of Gammaproteobacteria (Table S15D).

Not all families / clades affiliated with particular phyla or classes had identical responses to dispersal, except for Bdellovibrionaceae (Deltaproteobacteria) and Campylobacteraceae (Epsilonproteobacteria) (Figure 25). Overall, the interactive effects of dispersal and environment were more apparent for the family-level responses than the phylum/class-level responses (Table S15). Such interactions showed a stronger effect of dispersal in the brackish environment for some families. For example, dispersal resulted in a decrease in the relative abundances of Oceanospirillaceae (although not statistically significant) and Vibrionaceae in the brackish environment, independent of inoculum source (Figure 25B; Table S15D). Interestingly, Bdellovibrionaceae in the marine communities were found at low (relative) abundances in the brackish environment, and were not present in the native environment in the absence of dispersal (Figure 25B), but were, however, significantly enriched in the dispersal treatments. The abundance of the SAR11 clade was marginally influenced by the interactions between dispersal and environment (Table S15), although no clear patterns were observed. Such interactions, however, did not significantly influence the SAR116 clade (Table S15A), Cellvibrionaceae and Colwelliaceae (Table S15D). Neither dispersal nor incubation environment had effects on the abundances of Flavobacteriaceae, whereas their interactive effect was significant (Table S15E). This suggested that at least in one of the environments, there was a unique response of both brackish and marine communities depending on dispersal. For example, members of Flavobacteriacaea showed a slight but significant increase in their abundances in response to dispersal in the brackish incubation environment (Figure 25B). In case of Rhodobacteraceae and Campylobacteraceae, the interaction between dispersal and inoculum source significantly influenced their relative abundances: the former one decreased when it originated from the marine and the latter one when it originated from the brackish inoculum (Figure 25B; Tables S15A and C, respectively).

In addition to compositional changes observed at the OTU-level, some variation in the relative abundances at higher levels of phylogenetic resolution, particularly at the family level was also observed in response to dispersal. This generally supports the third hypothesis that the importance of dispersal differs among bacterial phylogenetic groups. I further found that in the brackish environment, family-specific changes in relative abundance occurred in response to dispersal. This indicates that dispersal can influence the co-occurrence patterns of bacterial taxa under that condition, probably due to potential microbial colonization with regard to dispersal capability of microorganisms (Rime *et al*., 2016). In particular, dispersal promoted the growth of Bdellovibrionaceae, known as prokaryotic bacteriovores (Pimeiro *et al*., 2004), that were rare and/or dormant in the initial marine inocula and in the marine communities at the end of the experiment without dispersal. I speculate that the transition from dormancy to active growth of this family was most likely because dispersal substantially influenced prey-predator dynamics (Otto *et al*., 2017).

Table 6 The abundant dispersers in the brackish and marine communities for each environment.

OTU ID	Brackish environment		Marine environment		Taxonomic assignment
	Dispersal capability	Relative abundance %	Dispersal capability	Relative abundance %	
Otu000004	m	1.99%	-	-	Proteobacteria; Gammaproteobacteria; unclassified; unclassified; unclassified;
Otu000006	-	-	b	1.15%	Proteobacteria; Gammaproteobacteria; Oceanospirillales;Oceanospirillaceae; Pseudospirillum
Otu000008	b	3.12%	b	4.19%	Proteobacteria; Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae; Ascidiaceihabitans
Otu000009	b	1.24%	b	1.75%	Proteobacteria; Gammaproteobacteria; Alteromonadales; Colwelliaceae; Colwellia
Otu000010	m	3.69%	m	1.13%	Proteobacteria; Deltaproteobacteria; Bdellovibrionales; Bdellovibrionaceae; OM27 clade
Otu000012	m	1.32%	-	-	Proteobacteria; Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae; Planktomarina
Otu000019	-	-	m	1.42%	Proteobacteria; Gammaproteobacteria; Alteromonadales; Colwelliaceae; Thalassotalea
Otu000023	b / m	2.46% / 1.14%	-	-	Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; Polaribacter
Otu000028	b / m	2.74% / 1.11%	-	-	Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; Dokdonia
Otu000033	m	1.70%	-	-	Proteobacteria; Deltaproteobacteria; Desulfuromonadales; GR-WP33-58; unclassified
Otu000041	m	2.05%	-	-	Proteobacteria; Deltaproteobacteria; Desulfuromonadales; GR-WP33-58; unclassified

Abbreviations ‘b’ and ‘m’ represent the brackish and marine communities in which the abundant OTUs showed potentially high dispersal capability, respectively. For example, when the abundant OTUs in one of the environments were identified as the ‘abundant dispersers’ in the brackish communities, these OTUs must have been detected in the pool of abundant OTUs in the dispersal treatments, but were absent from the brackish communities in the non-dispersal treatments. ‘-’ indicates that the OTUs were not identified as “abundant dispersers”, that is either rare in a particular incubation environment (i.e., mean relative abundance < 1% in any microcosm), or abundant in all non-dispersal treatments for that environment. Relative abundances are presented as averages of the replicate samples.

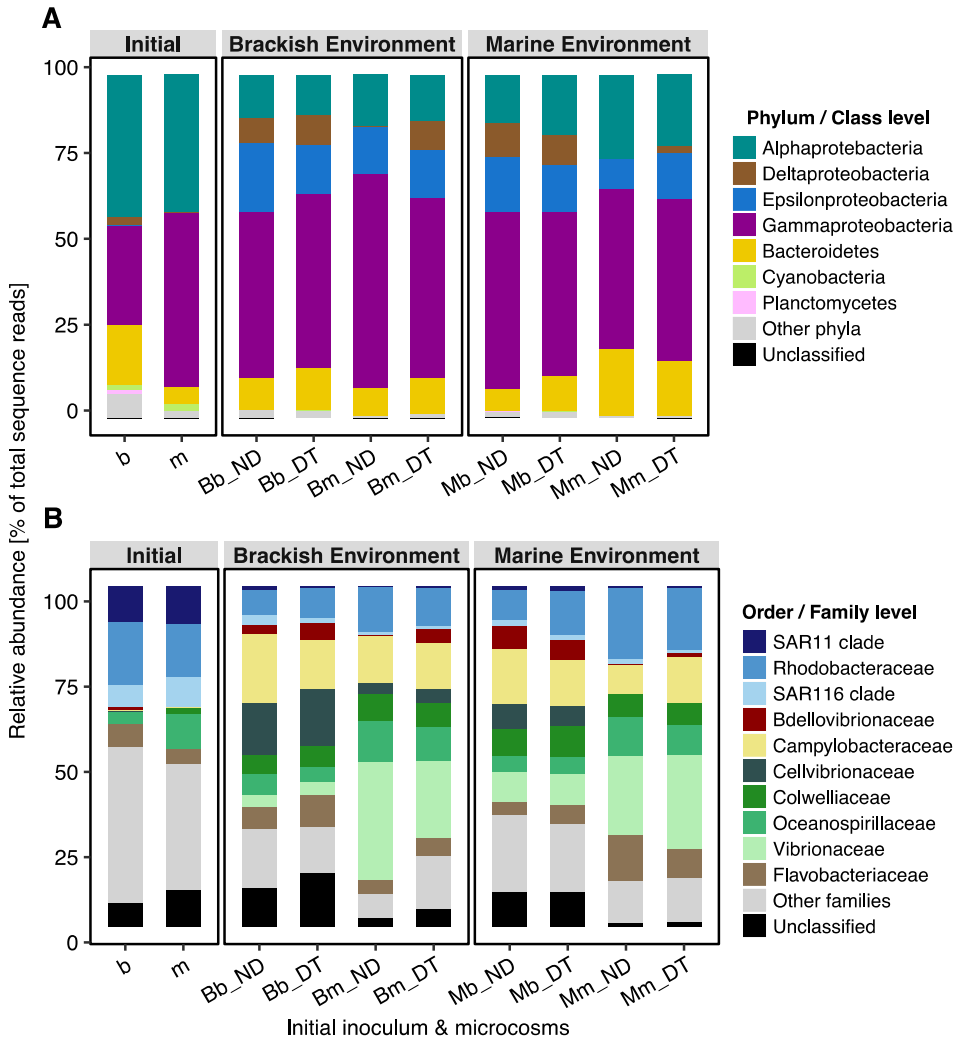

Figure 25 Taxonomic composition of bacterial communities in microcosms at the phylum/class level (**A**) and order/family level (**B**). Relative abundance was calculated from the normalized reads, i.e., percentage of total sequence reads, and is presented as the average value of triplicate samples (expect for duplicate samples from Bb_ND microcosms). 'Unclassified' indicates OTUs that could not be assigned taxonomically at the phylum/class level or order/family level.

3.5.6 Dispersal and its the interactions with environment

Several lines of evidence in this study reveal that dispersal interacted with the incubation environment to influence community assembly. However, this interaction was more apparent in the brackish than marine environment. Evans *et al*., (2017) proposed that strongly selecting conditions could lead to a small community size (total number of individuals), which is more susceptible to dispersal. This does not seem to be the case here, because the environment had significant effects on the total bacterial densities and higher cell abundances were observed under brackish conditions. Alternatively, metabolic plasticity of brackish bacteria to a salinity change may decrease the possibility of colonization by an external source, i.e., the marine species pool, thereby decoupling the

effect of dispersal from that of saline conditions. This is because community memberships of the active brackish communities did not vary substantially between two incubation conditions, and their composition was less similar to marine communities after exposure to dispersal in the marine environment (Figure 25B). This hypothesis should be tested in further studies by examining how dispersal modifies the response of brackish bacteria to a wide range of salinities. Still, our finding indicates that traits like metabolic plasticity could be important for understanding the relationship between dispersal and microbial community composition.

Some concerns need to be considered when extrapolating experimental findings to natural systems. The patterns we observed in this study were derived from two sources of microbial inocula. Consequently, this limited dispersal source would have missed functionally rich communities and/or rich pools of potential immigrants (see Comte *et al*., 2017), which could fuel natural metacommunity dynamics. Microcosm experiments have been regarded as artificial systems, where resulting communities differ from the composition of the original inoculum or where fast-growing opportunists are simply enriched (e.g. Christian and Capone, 2002; Aanderud *et al*., 2015). As the communities that established from the same inoculum still clustered together for each environment at the end of the experiment (Figure 23A), it seems, however, likely that the observed compositional differences are the consequence of the dispersal events. More importantly, our aim was to assess direct dispersal effects by passive transport of the cells on the response of communities to new environmental conditions, which was only possible to accomplish via experimentation.

In summary, I could show that a low exchange of microorganisms among local communities can alter the importance of environmental effects on bacterial communities. This response was likely driven by moderate changes in the relative abundances rather than major shifts in the identity of abundant taxa. Moreover, the interactive effects of dispersal and contemporary environmental conditions were stronger at lower taxonomic levels, and such effects facilitated local adaptation of some bacteria in brackish waters. This experimental study provides a better understanding of the fate of dispersed bacteria under saltwater inflow scenarios and enhances our ability to predict bacterial responses to environmental changes.

4. Conclusions and future perspectives

The present thesis demonstrates the feasibility of using novel experiments and integrative analyses of 16S rRNA and meta-'omic' data to address ecological aspects. These applications offer the potential to disentangle the processes contributing to bacterial community assembly (Hanson *et al*., 2012; Nemergut *et al*., 2013), and to elucidate possible links between compositional and functional changes across environmental gradients.

The two transplant experiments also reveal the importance of the careful separation of one ecological process from the others, thus enabling deeper insights into the potential impact of each process on bacterial community assembly under specific environmental conditions. I could show that, shifting salinity conditions create communities that contain a greater number of habitat specialists and fewer generalists, with the specialists being abundant. Meanwhile, the ecological importance of rare taxa was highlighted as they contributed more to the selection of habitat specialists after experiencing the disturbances, in comparison with abundant taxa. The unraveling processes that underlie the assembly of these ecological strategy groups can be inferred from the phylogenetic relatedness of taxa within the disturbed communities: environmental filtering was most influential to the assembly of specialists, while biotic interactions, that is, competitive exclusion, may be more relevant to the assembly of generalists. Dispersal, however, was unlikely to contribute to the observed patterns since the experiment 1 was designed to exclude this community assembly process. These findings have implications for the ecosystems with spatially limited dispersal: in the face of environmental changes, an array of specific taxa will fill the newly open niche, many of which are habitat-specific and become locally abundant as a result of environmental filtering. Hence, in the absence of dispersal events, deterministic processes vary during community assembly for the ecologically dissimilar bacterial taxa. Furthermore, the contribution of dispersal to the diversity and composition of the disturbed communities was explicitly investigated. It was found that dispersal could compensate the loss of the bacterial diversity after salinity exposure, in some cases, may even enhance the diversity over the initial stage. The communities experiencing dispersal became similar, resulting from change in taxa abundance rather than taxa replacement. The degree of community similarity is dependent on the interaction between dispersal and incubation environment. In other words, the relationship between dispersal (rates) and the diversity or composition may be environmental-dependent (Evans *et al*., 2016; Louca *et al*., 2016a). As such, it will be certainly interesting to examine the impact of dispersal difference, including dispersal rate and dispersal timing on community outcome in wider environmental gradients over a long period of observation. Additionally, a focused but novel framework on co-occurrence network might help explore the pattern and intensity of species

interactions under possible circumstances. This will help us ascertain the generality of environmental filtering, competitive exclusion and dispersal in natural bacterial communities observed here.

Another focus of the presented thesis was to look closer at the metabolic processes and functional performance of the bacterial communities in response to salinity changes by the means of metagenomic and metatranscriptomic analyses. The abundance patterns of transcripts per cell from the described ecological strategies revealed that, specialist taxa did not exhibit high transcriptional activity in their favored habitat, while the generalists exhibited the similar transcriptional levels across habitats (i.e., three different salinity conditions). The expression of different functional groups carried out by specialists was, however, often high in their favored habitat, particularly for the genes involved with growth and cell cycles or transcription. This finding confirms previous suggestions that habitat filtering selects specialist taxa, based on markedly different metabolic and functional roles. More specifically, a set of genes involved with respiratory complex, energy production and carbon metabolism deviated in composition from the metabolic profile of the individual taxa within each group. Expression of those genes differed significantly between the distinct specialist strategies, resulting in distinct transcriptional responses to the same environment. I believe the discussed cases of taxon-specific response to changing salinity inferred from functional gene transcript abundance, will stimulate further work in verifying and gaining more insights into the metabolic strategies of those taxa in culture or isolation. To my best knowledge, this is the first experimental study providing a more integrated view of changes in actual metabolic activity and functional attributes in response to salinity alteration at the individual taxon-level. At the community level, functional performance was also assessed and quantified using metatranscriptome data. I have shown that disturbance intensity can influence the extent of functional redundancy (FR), particularly true for the marine communities. On the other hand, the expression of FR kept stable despite variability in functional response for the brackish communities at the varying levels of salinity disturbances. FR was affected greater by community properties, including functional diversity or the transcriptional behavior of its members, than by environmental parameters. When assessing the extent of FR across taxonomic ranks, it was found that, for both brackish and marine communities, FR expressed among low taxonomic ranks (species, genus and family) was greater at low than high salinity differences. This pattern, however, skewed towards high taxonomic ranks for the brackish communities. These results imply that the relationship between the degree of FR and disturbance intensity may be dependent on how sensitive a given community is to the type of disturbance. The assessment of FR based on metatranscriptome-data presented here for the first time highlights mechanisms in transcriptional machinery through which FR may contribute to community response under shifting environmental conditions. An exciting extension using such metric would be possible if and how the extent of FR differs among different functional groups categorized for the community before and

after disturbances (Fanin *et al*., 2018), as well as its relationship with taxonomic variability within each functional guild (Louca *et al*., 2016a).

Overall, the present thesis is very informative in regards to the response of bacterial communities to environmental shifts, specifically salinity changes. Several ideas raised in this thesis shed light on ecology and potential evolution of bacterial community during marine-freshwater transitions: bacteria can cross marine-freshwater boundaries with respect to their taxon identity despite potential constraints in some metabolic functions and large investments in energy tradeoff. I hope that this collection of studies will stimulate further perspectives and inspire valuable hypotheses to follow up on a research topic "underlying mechanisms of microbial compositional and functional responses to environmental changes or gradients" in aquatic ecosystems. Last but not least, future studies can focus on press disturbance that imposes a continuous disturbance on microbes and their surroundings (Shade *et al*., 2012; Konopka *et al*., 2015) rather than pulse disturbance studied here, in order to test the robustness of microbial community and their functional stability.

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-- Helen Keller

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My thanks also go to those from other departments of the IOW. **Jenny**, my thanks also go to you. I guess I can say you are 'DOC queen', thank you for all DOC measurements of this work. And I am grateful that you always tried to help me out and provide me with the DOC glass bottles and some filters for my experiments from the two cruises. **Monika, Herbert, Ines**, thank you for your lovely/funny conversations and our nice time together during MSM cruise, as well as thank your for your encouragement to my work.

Here, my gratitude express towards those close colleagues who are also my nice friends outside of the work. **Mercè**, you are the brightest person who has been very close to me and will be. You always can sense when I am in happy or strange mood. Well, our connection started in 2012 when I was doing my Master thesis in Uppsala. I always found you a very calm and nice girl to talk to, and I still remember that I bothered you several times for questions of my master thesis, and you are the one that showed me how to analyses TRFLP results!! I was sooo happy after you came to IOW, finally, I found that I was not alone anymore. In the past 4 years, you have showed me the importance in appreciating friendships. My living in Germany could have not been as bright as it would be without you. You are the one, who always laid your hands on me when I am blue, and who always open your ears when I desperately want to express any emotions, and you are also the one who reminds me of many fun things after work. You always remember me at many occasions, thank you for company all the way. I am also grateful that you patiently taught me a lot statistical tools, and certainly, I could not havt achieved so far without your helpful discussions and suggestions, and I have learnt a lot from you scientifically and personally. **Kerstin**, you have brought me many nice and relaxing moments; we hanged out together for cocktails and dinning. Thank you for being as my good friend, your company is really necessary. I truly appreciate our friendship; you are the one whom I can talk basically everything with. Surely, this will continue, even though I am not in Rostock anymore. I remember the little tips you provided for my life and work, they are helpful when I make some decisions. Also, thank you for checking the grammar in my German abstract. **Christian. S**, wow, you are the nicest officemate I have ever had. I am grateful for your support and many office talks that are very helpful. When I have big laugh, sadness, anxiety and happiness, you always are the one in our office who I share first with, and you are the one who provide me with sweets, coffee and refreshments at work. **Sonja**, oh I will not forget our drinking time together ☺, you always said towards me "this girl is so funny when she is drinking (of course, alcohol)! And I am thankful for many our heart-to-heart talks about life and work, various aspects. And you are very talent on what you are doing, so please keep it up. And I will not forget our great time together in Zagreb; hope that such occasions will come more. **Kati**, thank you for having you as my lovely friend, you listen to my story whenever I am down and happy. Your encouragement has helped me move on during this journey, and you let me know that in this long tough tunnel, I am not alone. We all will make it eventually and successfully. **Daniel**, thank you for the kindness and patience in answering many of my questions, as well as your suggestions. I am also grateful that you appreciate my knowledge, and some nice discussion about science, you told me that "Science is hard, but lots fun in it too". **Jan**, thank you for being a very nice officemate, I really like our working environment. As well as many our office talks, coffee and cakes sharing moments. **Christian. M**, thank you for

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My sincere gratitude goes to you **René**, I can't imagine how my time living in Germany would have been without your significant help, support and giving. Every time when I was sick, you are the one next to me and took care of me; when I am sad, you are the one that listen to my story and cheer me up. I deeply cherish for all what you have provided and have done for me. I have never felt lonely, because I have you around, telling me plenty of German jokes and cultures. I appreciate that a lot stickers on the furniture in our apartment have been labeled in German, that is for me to learn. There are many wonderful things and moments we have done and shared together, I will never forget.

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Oh, some words have been saved for myself here. Thanks to myself perseverance, strength and self-motivation during these years. I am proud of the earlier, present myself, and to future myself: focus on making little but constant progresses in your life, rather than evaluating if a big goal has been achieved or not!!

7. Appendix

Text 1 Meta-omic analyses

The raw sequences with adaptors generated from the Illimina HiSeq for the meta-omic approaches were trimmed using the cutadapt software (Martin, 2011). The reads were subsequently quality-filtered using 'sickle' (Joshi and Fass, 2011), in the following setting: quality cut-off < 20, minimum read length metagenomic data < 50 nt, minimum read length metatranscriptomic data < 75 nt were removed. The 'sortmerna' software (Kopylova *et al*., 2012) was carried out to separate nonprotein-coding RNA rom protein-coding reads.

The R package DeSeq2 (Love *et al*., 2014) was used to quantify the transcriptional regulation of genes linked to bacterial production rates by detecting the $\log_2$ fold change of gene orthologs encoding ribosomal proteins (KEGG path: ko03010) between two communities. The comparisons were peformed in the direction that provided a positive $\log_2$ fold changes.

Text 2 Calculation of functional change (FC) and theoretical community functional change (TFC)

The calculation of FC/TFC and FR can be described by the following equations:

FC = BCcommunity (1)

$$TFC = \sum_{i=1}^{n} BC_i \times \frac{t_i}{t} \quad (2)$$

FR = TFC - FC (3)

where BC is the Bray-Curtis dissimilarity of functional profiles derived from the count data of gene orthologs in two compared communities (Equation 1) or in a specific taxon bin i detected in at least one of the two compared communities (Equation 2), and n is the number of taxon bins detected in two compared communities, including taxa present in only one community. If a taxon is detected in only one of the compared communities, counts for all genes equal 0 in the other community and the taxon-specific Bray-Curtis distance BCi in this case is 1. *t* represents the number of protein-coding transcripts in the two compared communities.

The count data for the community were subsampled to the same number of reads in each sample. Individual taxon bins, however, were not normalized prior to the calculation of TFC, with considering the changes in both transcript composition (i.e., phenotypic plasticity) and the relative transcript abundance of each taxon bin (Beier *et al*., 2015). This was necessary because the bulk community parameters FC and FR depend on both the changes in transcript composition and the transcript abundance of individual community members.

TFC, FC, and FR can vary between 0 and 1. The value of 0 indicates that no FR will be detected, in which two cases would occur: i) communities with functionally and taxonomically identical metatranscriptomes and ii) communities where individual taxa do not share any transcribed gene. The value of 1 indicates that FR reach maximum, in which functionally identical metatranscriptomes of two communities that do not share any taxon for a theoretical case.

Text 3 Transplant experiment 2 of dispersal manipulation: experimental conditions

Conductivity was measured and samples for inorganic nutrients and dissolved organic carbon (DOC) were taken from the initial inocula and all microcosms at the end of the experiment. Briefly, 15 mL samples were filtered through GF/F filters (Whatman, Dassel, Germany) for measurements of NO_3^-, NO_2^-, PO_4^{3-}, NH_4^+, and SiO_2^-. Concentrations of these nutrients were determined colorometrically according to Grasshoff *et al*., (1999) by means of a Seal Analytical QuAAtro automated nutrient analyzer (SEAL Analytical GmbH, Norderstedt, Germany). DOC was measured by filtering 20 mL of the samples through combusted GF/F filters (Whatman, Dassel, Germamy), which were analyzed on TOC-VCPH TOC Analysator (Shimadzu Europe GmbH, Duisburg, Germany).

Text 4 Transplant experiment 2: sequence processing

Paired-end sequences were merged. Sequenced then were quality filtered in the following settings: any reads with length < 400 nt, ambiguous bases > 0, and homopolymer length > 8 were removed for further analysis. The remaining sequences were aligned to the SILVA v123 reference database and those sequences that did not align to the correct region were eliminated. To further reduce the noise in our sequences, we used pre-clustering and the resulting sequences were screened for chimeras using UCHIME (Edgar *et al*., 2011). We then used Bayesian classifier to classify those sequences against the Ribosomal Database Project (Wang *et al*., 2007), and only classifications above an 80% bootstrap cutoff value were included in the analyses. All Archaea, Eukaryota, chloroplasts, mitochondria and unknown sequences were removed from the sequence dataset. Finally, sequences were clustered according to their taxonomy and assigned to operational taxonomic units (OTUs) at a 3% dissimilarity level using the average neighbor method. Singletons (OTUs with only one sequence across all libraries) were also discarded. For downstream analyses, sequences were subsampled to 11,074 sequences (the size of smallest library; see Table S11 for details) across 29 samples using the R script described in Zha *et al*., 2016.

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Supplemental figures

Figure S1 Principal component analysis (PCA) based on all the measured salinity and nutrient concentrations in the water of the initial inocula and of all microcosms after 4 days incubations. The following colors indicate the different incubation environments after incubation: oligohaline habitat (red), brackish habitat (yellow), or marine habitat (blue). Different symbols indicate the inocula from which the microcosms were established: oligohaline inocula (circles), brackish inocula (squares) or marine inoculum (triangles). Black symbols represent the initial inocula of the three sources at day 0. Upper-case letters refer to the incubation environment and lower-case letters to the inoculum. The two first axes explain 79% of the variance. Abbreviation: [DOC] Dissolved organic carbon. (Shen *et al*., accepted; doi: 10.1111/1462-2920.14059; the article is protected by copyright. All rights reserved)

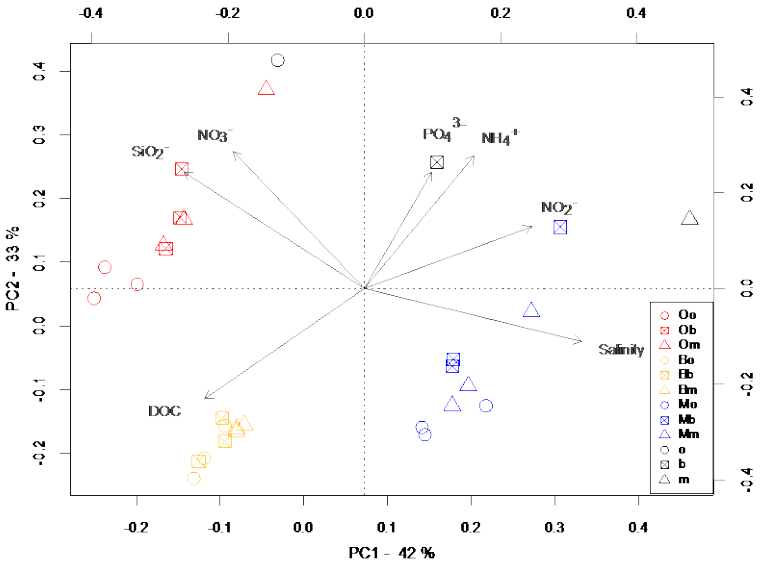


Figure S2 NMDS plot showing the differences in community structure among the initial oligohaline, brackish, and marine inocula (black symbols), and among the microcosms in which the same starting community was exposed to the three environments after 4 days. Upper-case letters refer to the incubation environment and lower-case letters to the inoculum source. (Shen *et al*., accepted; doi: 10.1111/1462-2920.14059; the article is protected by copyright. All rights reserved)

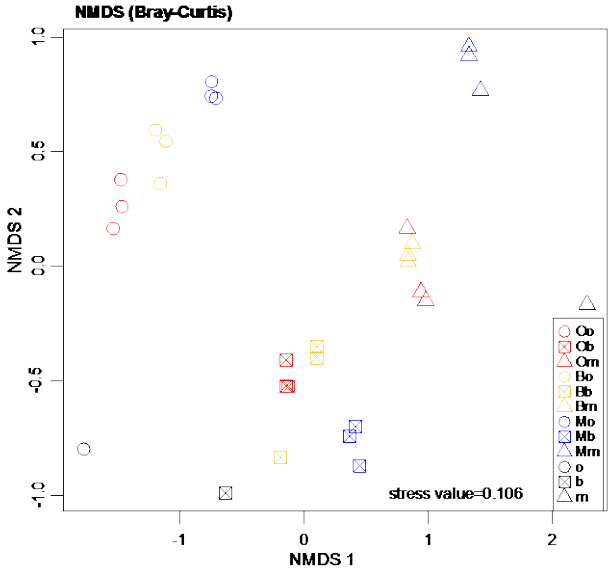


Figure S3 Mean absolute abundance of OTUs assigned to a specific life strategy in all microcosms at the end of the experiment. The data were plotted after log-transformation. In each panel, only the specialists that were specialized to the respective habitat are shown and compared with generalists found in the same microcosms. The significance of this comparison was tested for all 9 microcosms together using a repeated measures t-test on log-transformed data ($P = 4.7 \times 10^{-5}$), followed by independent two-sample t-tests for each microcosm. *P*-values of the individual two-sample t-tests were corrected using the Bonferroni correction procedure. Significance codes: $P < 0.05$ *, $P < 0.01$ **, $P < 0.005$ ***, and error bars represent the standard deviation of the triplicate microcosms. (Shen *et al.*, accepted; doi: 10.1111/1462-2920.14059; the article is protected by copyright. All rights reserved)

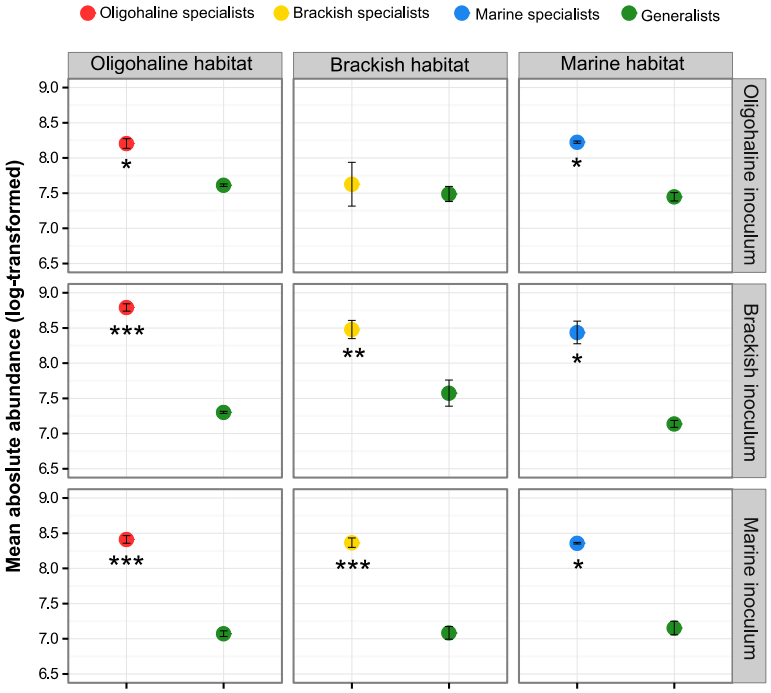


Figure S4 Venn diagrams (**A**) display exclusive and shared OTUs that were assigned to life strategies in the communities originating from oligohaline inocula, brackish inocula or marine inocula. A total of 304 OTUs were present across samples. Piecharts (**B**) illustrate the percentage (number) of OTUs with different life strategies (purple) and OTUs that displayed a unique strategy (white), considering 112 OTUs shared by at least two inoculum sources. (Shen *et al.*, accepted; doi: 10.1111/1462-2920.14059; the article is protected by copyright. All rights reserved)

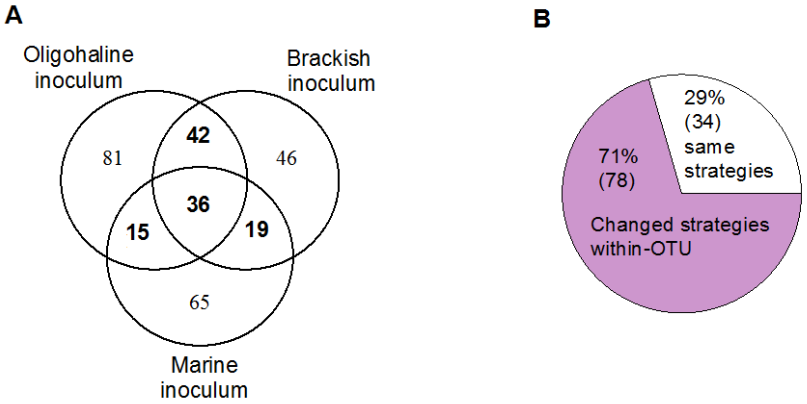


Figure S5 Absolute abundances of the dominant classes and families of bacteria with different life strategies. For habitat specialists, the size of the circles indicates the average absolute abundance detected in their preferred habitat (averages for triplicate microcosms in one habitat). For generalists, the size of the circles indicates the average absolute abundance detected in three habitats (averages for nine microcosms across habitats). 'Other' indicates a collection of OTUs assigned to a different taxonomy at the class or family level from the ones above, whereas 'Unclassified' indicates OTUs that could not be assigned taxonomically at the class or family level. (Shen *et al*., accepted; doi: 10.1111/1462-2920.14059; the article is protected by copyright. All rights reserved)

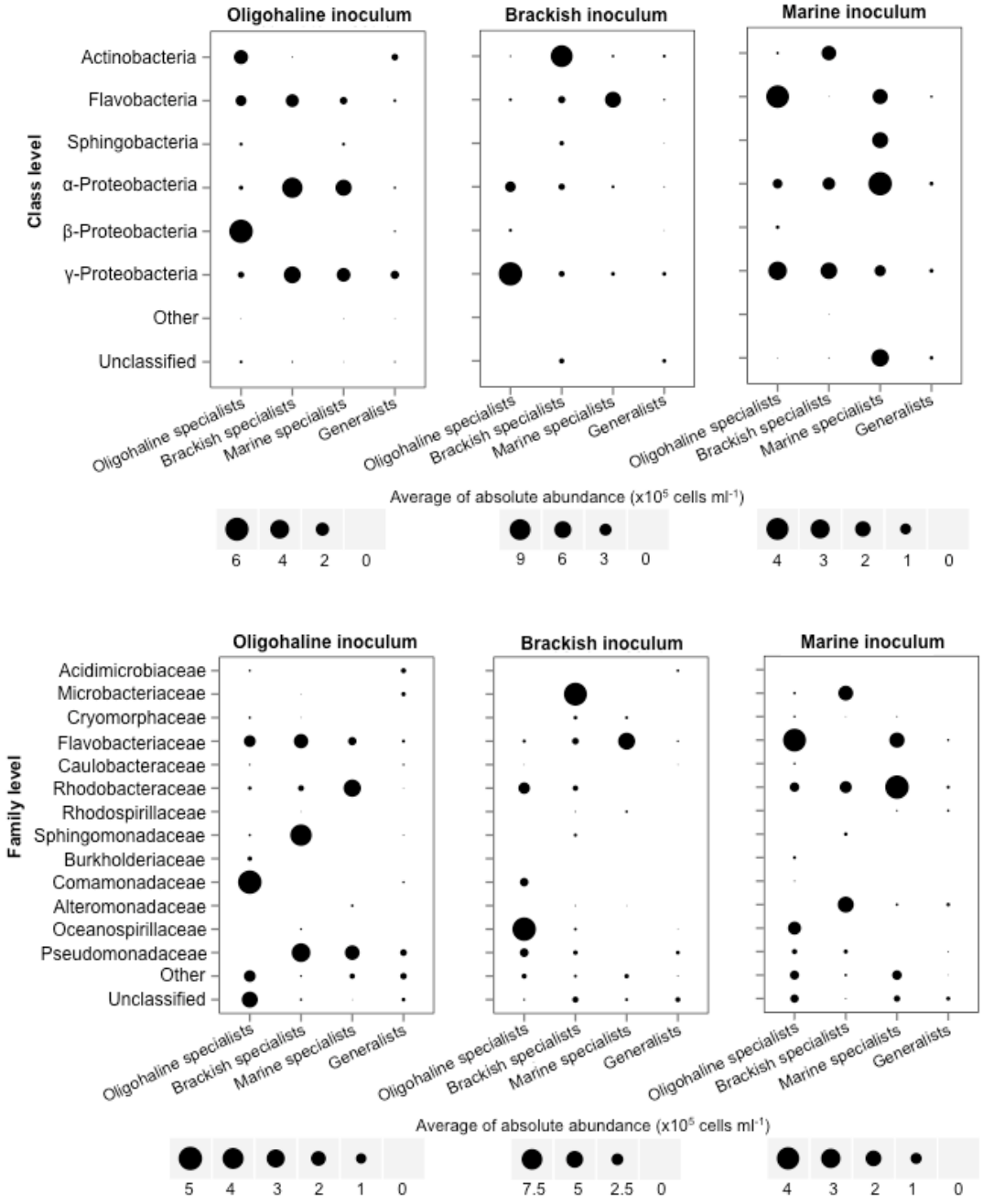


Figure S6 Principal coordinates analysis (PCoA) illustrating pairwise dissimilarity among the total of 5105 sequences that belong to OTU000004 (Flavobacteriaceae). The results demonstrate the existence of two ribotypes: one contains reads originating from the brackish-or marine inoculum, while the other contains reads originating from the oligohaline inoculum. This indicates that the distinct specialist behavior found for OTU000004 originating from the oligohaline inoculum was likely due to a genetically different ecotype of this OTU at the oligohaline sample site. Obviously, it was not possible to discriminate different ribotypes of this OTU originating from the brackish or marine sample site. In this case, the detected distinct specialist strategies between the brackish and marine representatives for OTU000004 may still be due to either the appearance of genetically different ecotypes that could not be resolved by the 16s rRNA gene or species interactions.

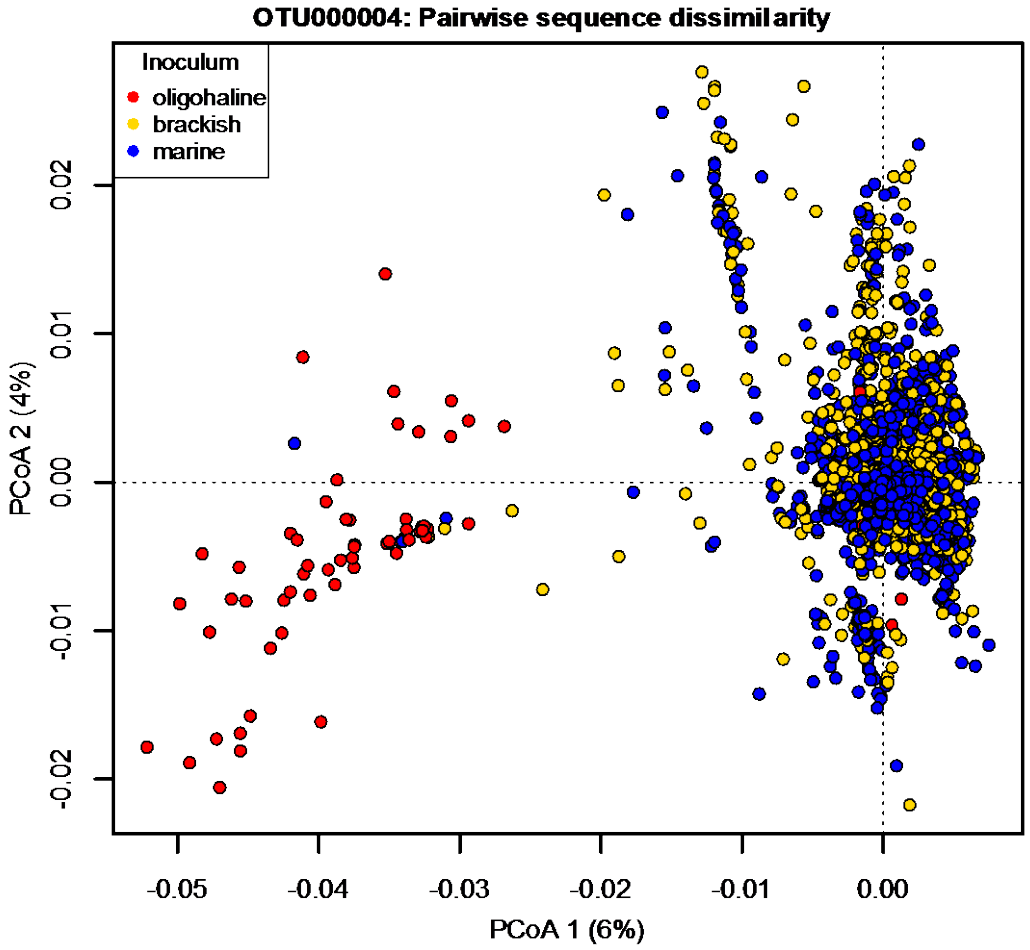


Figure S7 Cell-specific mRNA transcript quantities contributed by individual representative OTUs for each of the four strategy groups in all treatments (except for the treatment "Mo"). No metatranscriptomic libraries could be constructed from the treatment 'Mo'. Different OTUs within each strategy group are represented by different grey shades. Abbreviations were used throughout the manuscript to indicate treatments, i.e., capital letter for incubation environment and small letter for source inoculum.

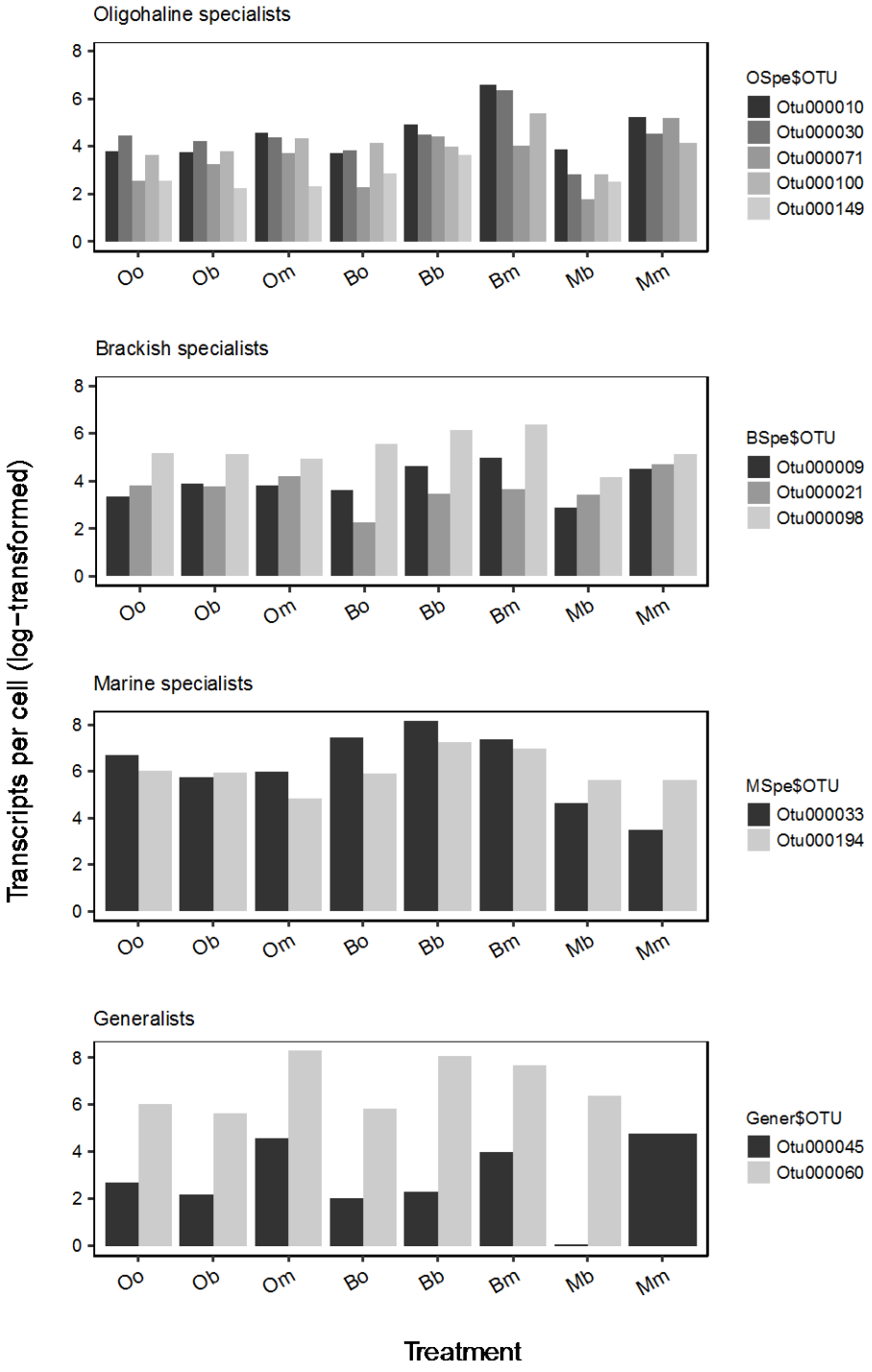


Figure S8 Decrease in FR among community members grouped by taxonomic level with decreasing degree of phylogenetic relatedness between communities originating from the marine inoculum and grown in media of differing salinity. **A)** FR of oligohaline (O), brackish (B), and marine (M) treatments; **B)** To compare the shape of the curves in **A**), all FR values were scaled up to 100 for the total species-level FR; **C)** For the example of the community pair O vs. B, the red bars indicate the percent decrease in FR with the decreasing degree of phylogenetic relatedness, which reflects the percent contribution to FR of the taxa at different taxonomic levels as shown in Figure 17. Slightly modified based on Beier *et al*., (2017).

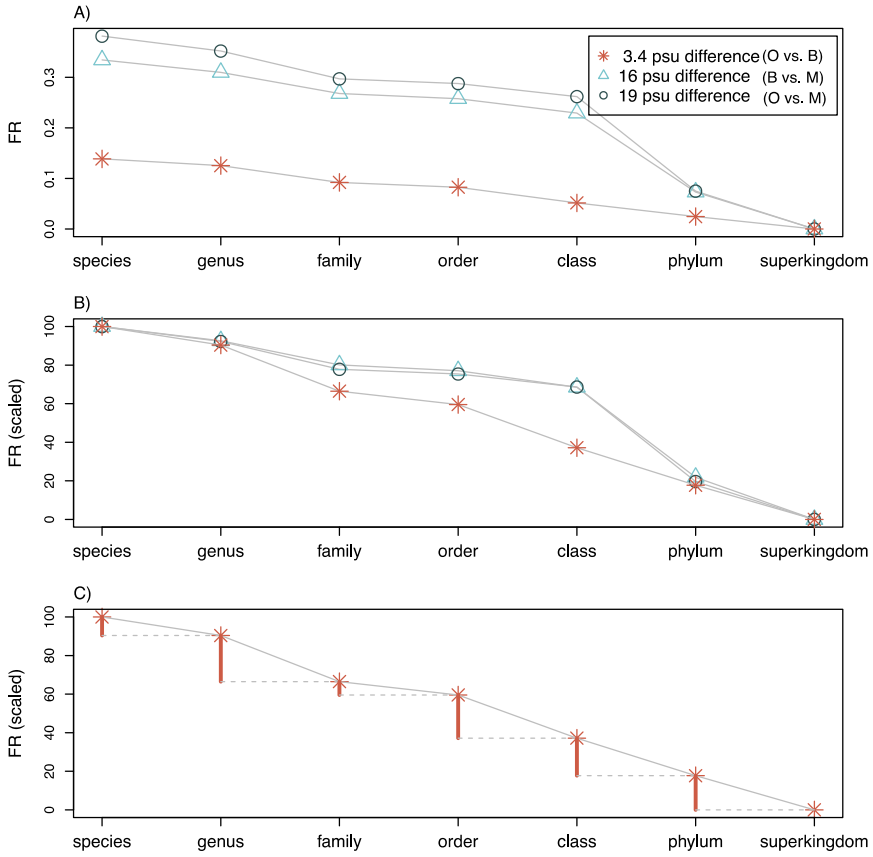
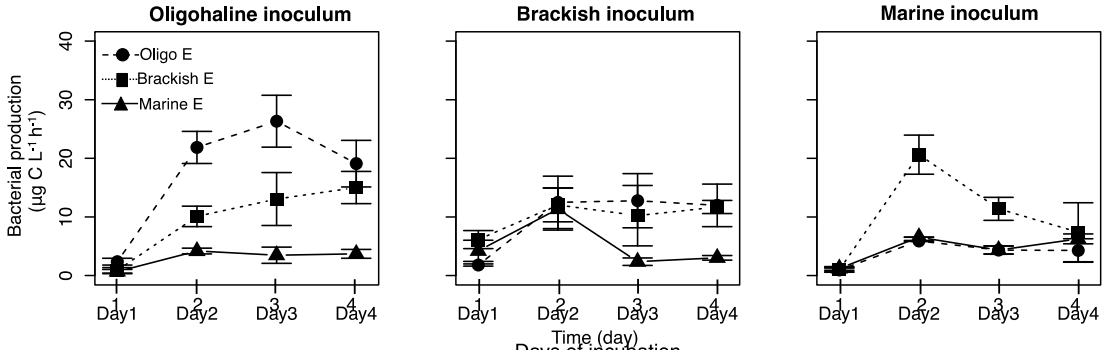


Figure S9 Heterotrophic bacterial production during the course of experiment. Data represent the averages of triplicate samples ± s.d. Abbreviations: 'Oligo E', oligohaline environment; 'Brackish E', brackish environment; 'Marine E', marine environment. The bacterial communities from three origins of the Baltic Sea showed varying trends in heterotrophic productions over time.



Supplementary Tables

Table S1 Biological characteristics, the nutrient profiles of the initial inocula (Day 0) and of the microcosms at the end of the experiment (Day 4), as well as numbers of the 16S amplicon reads obtained from all samples. Capital letters refer to the incubation environment, lower case letters refer to the inoculum source (O/o: oligohaline; B/b: brackish; M/m: marine), and the terminal number represents the biological replicate. psu: practical salinity unit. DOC: dissolved organic carbon. Data obtained from DOC measurements represent averages of technical triplicates of each of the samples ± s.d. "NA" indicates that no data were available for the measurement. (Shen *et al*., accepted; doi: 10.1111/1462-2920.14059; the article is protected by copyright. All rights reserved)

Sample	Salinity (psu)	pH	NO_3^- (μM)	NO_2^- (μM)	PO_4^{3-} (μM)	NH_4^+ (μM)	SiO_2^- (μM)	DOC (μM)	Bacteria ($x10^6$ cells ml^{-1})	No. amplicon reads	No. amplicon reads (singletons removed)
Oligohaline inoculum	2.55	7.73	4.43	0.2	0.16	1.89	46.8	468.94±1.88	1.31	18065	17502
Brackish inoculum	6.89	7.83	0.49	0.05	0.34	1.49	13.37	340.63±2.95	1.23	11021	10608
Marine inoculum	28	7.54	0.55	0.37	0.13	1.32	2.04	156.57±1.93	0.56	34165	33238
Supplement	NA	NA	4.87	0.1	4.53	34.18	45.52	184.93±3.96	NA	NA	NA
Oo1	4.04	7.73	4.71	0.11	0.06	0.45	43.65	647.79±8.50	3.86	21997	21325
Oo2	4.07	7.73	4.78	0.09	0.13	0.36	45.08	599.55±1.90	4.47	16933	16341
Oo3	4.09	7.73	4.48	0.16	0.06	0.4	42.09	569.30±2.80	4.28	35103	33770
Ob1	4.04	7.73	6.35	0.21	0.16	0.62	41.52	500.47±1.34	5.45	37886	36797
Ob2	4.11	7.73	4.41	0.15	0.08	0.5	43.39	494.45±2.50	5.17	30332	29539
Ob3	4.11	7.73	4.74	0.19	0.12	0.49	44.45	483.30±2.40	4.64	33766	32760
Om1	4.17	7.73	5.03	0.27	0.31	0.96	43.47	496.35±7.50	1.9	12912	12652
Om2	4.12	7.73	4.79	0.18	0.12	0.47	42.87	466.21±3.42	1.85	33815	32804
Om3	4.12	7.73	4.92	0.15	0.09	0.41	41.35	479.75±2.10	2.2	15773	15309
Bo1	7.53	7.83	0.49	0.07	0.1	0.5	17.71	674.80±7.60	4.79	21124	20666
Bo2	7.51	7.83	0.2	0.03	0.05	0.33	16.57	693.25±5.30	4.63	7913	7658
Bo3	7.49	7.83	0.23	0.04	0.07	0.4	16.96	692.30±1.40	4.67	25024	24308

Appendix

Sample	Salinity (psu)	pH	NO_3^- (μM)	NO_2^- (μM)	PO_4^{3-} (μM)	NH_4^+ (μM)	SiO_2^- (μM)	DOC (μM)	Bacteria ($x10^6$ cells ml^{-1})	No. amplicon reads	No. amplicon reads (singletons removed)
Bb1	7.52	7.83	0.97	0.05	0.12	0.38	15.95	645.60±1.20	5.75	16111	15546
Bb2	7.48	7.83	0.27	0.05	0.1	0.37	15.71	659.85±7.50	6.28	38042	36967
Bb3	7.49	7.83	0.48	0.05	0.09	0.34	15.98	720.50±2.80	6.13	35847	34864
Bm1	7.52	7.83	0.38	0.04	0.11	0.32	15.06	601.95±1.90	1.14	47428	46159
Bm2	7.5	7.83	0.27	0.05	0.1	0.37	15.71	595.85±0.90	1.15	49455	47986
Bm3	7.46	7.83	0.48	0.05	0.09	0.34	15.98	595.85±1.50	1.1	24751	24197
Mo1	23.6	7.54	0.45	0.42	0.04	0.48	7.48	530.50±1.00	3.09	22005	21460
Mo2	23.6	7.54	0.43	0.24	0.04	0.46	7.8	529.15±1.10	2.8	35373	34500
Mo3	23.6	7.54	0.53	0.22	0.06	0.5	7.49	532.19±1.38	3.04	17836	17335
Mb1	23.6	7.54	0.75	0.34	0.37	1.02	7.19	492.00±3.00	4.83	25013	24460
Mb2	23.6	7.54	0.62	0.18	0.14	0.69	7.35	474.70±3.80	4.77	30516	29645
Mb3	23.5	7.54	0.65	0.15	0.14	0.74	7.16	449.55±2.90	4.89	21018	20540
Mm1	23.6	7.54	1.1	0.29	0.03	0.45	6.57	464.58±2.76	2.3	5950	5748
Mm2	23.5	7.54	0.8	0.38	0.18	0.71	7	458.20±5.60	2.27	32580	31782
Mm3	23.5	7.54	0.93	0.29	0.06	0.5	6.84	445.60±3.00	2.43	23970	23341

Table S2 Contextual parameters and meta-'omic' reads from the transplant experiment 1. Metag/metatr: metagenome/metatranscriptome; EGS: effective genome size; PDaw: abundance-weighted phylogenetic diversity. For all metagenome-derived parameters only one number per sample is given, since the replicates were pooled before metagenome library preparation. The number of amplicon reads includes only the reads remaining after quality trimming and the removal of singleton sequences. The number of sequence read data includes reads after quality trimming, while for the amplicon reads, numbers before and after singleton removal are given. The number of annotated (bacterial) reads classified according to the KEGG onthology in general are indicated by ALL. For the replicate sample Bm.1, metatranscriptome library preparation was not successful. The percent values shown in parentheses after the number of annotated reads indicate the fraction of protein-coding read that could be annotated. (n.d.: not determined). The capital letter in the sample abbreviations indicates the medium, and the lower-case letter indicates the inoculum (O/o: oligohaline, B/b: brackish; M/m: marine). The data in this table were previously reported by Beier *et al*., (2017).

Sample	#nr metag. reads	#nr metag. protein coding reads	Functional diversity	EGS [Mb]	#nr amplicon reads	#nr amplicon reads (singletons removed)	Species diversity	PDaw	#nr metatr. reads	#nr metatr. protein coding reads	#nr annotated reads (ALL)
Ob1	28,652,432	28,223,620	7.6	1.61	37,886	36,797	2.846	31.105	58,983,264	24,383,932	6,198,527 (25.4%)
Ob2					30,332	29,539	2.856	19.634	44,977,944	20,525,984	5,888,891 (28.7%)
Ob3					33,766	32,760	2.989	18.487	29,649,750	2,016,292	466,529 (23.1%)
Om1	27,960,441	27,389,564	7.55	1.68	12,912	12,652	2.983	12.778	36,655,650	20,930,146	8,911,582 (42.6%)
Om2					33,815	32,804	2.972	17.799	45,443,130	22,353,888	10,217,551 (45.7%)
Om3					15,773	15,309	2.723	13.551	30,905,338	22,247,576	11,363,715 (51.1%)
Bb1	30,001,172	29,517,798	7.56	1.52	16,111	15,546	3.034	9.963	28,189,254	5,091,744	724,604 (14.2%)
Bb2					49,455	36,967	3.182	16.516	30,810,442	3,772,362	1,390,754 (36.9%)
Bb3					35,847	34,864	3.113	13.907	21,626,480	2,210,382	640,227 (29.0%)
Bm1	28,565,150	28,161,274	7.6	1.77	47,428	46,159	3.071	15.218	n.d.	n.d.	n.d.

Appendix

Sample	#nr metag. reads	#nr metag. protein coding reads	Functional diversity	EGS [Mb]	#nr amplicon reads	#nr amplicon reads (singletons removed)	Species diversity	PDaw	#nr metatr. reads	#nr metatr. protein coding reads	#nr annotated reads (ALL)
Bm2					38,042	47,986	3.147	15.036	31,486,916	2,034,640	857,607 (42.2%)
Bm3					24,751	24,197	2.983	11.477	47,276,364	21,292,008	7,935,909 (37.3%)
Mb1	28,251,661	27,804,206	7.53	1.65	25,013	24,460	2.777	14.575	63,731,146	23,429,610	7,088,895 (30.3%)
Mb2					30,516	29,645	2.66	17.616	31,998,660	19,401,258	4,837,141 (24.9%)
Mb3					21,018	20,540	2.804	12.955	22,620,428	1,617,184	542,434 (33.5%)
Mm1	31,493,044	30,828,824	7.47	1.81	5,950	5,748	2.902	9.922	34,542,166	18,675,808	4,287,224 (23.0%)
Mm2					32,580	31,782	2.976	13.549	29,954,694	20,935,822	3,859,753 (18.4%)
Mm3					23,970	23,341	3.328	15.798	38,932,448	27,815,234	5,236,256 (18.8%)

Appendix

Table S3 Number of OTUs affiliated with the dominant classes of bacteria in each of four strategy groups. (Shen *et al*., accepted; doi: 10.1111/1462-2920.14059; the article is protected by copyright. All rights reserved)

Class level	Life strategy			
Oligohaline inoculum				
	Oligohaline specialists	Brackish specialists	Marine specialists	Generalists
Actinobacteria	11	3	0	8
α-Proteobacteria	6	10	12	11
β-Proteobacteria	20	0	0	5
γ-Proteobacteria	2	4	10	8
Flavobacteria	12	4	9	9
Sphingobacteria	4	0	1	1
Brackish inoculum				
	Oligohaline specialists	Brackish specialists	Marine specialists	Generalists
Actinobacteria	2	10	3	7
α-Proteobacteria	4	13	3	7
β-Proteobacteria	7	0	0	5
γ-Proteobacteria	11	8	7	11
Flavobacteria	6	9	5	8
Sphingobacteria	0	1	1	2
Marine inoculum				
	Oligohaline specialists	Brackish specialists	Marine specialists	Generalists
Actinobacteria	1	3	1	1
α-Proteobacteria	4	9	13	8
β-Proteobacteria	7	0	0	0
γ-Proteobacteria	14	7	7	12
Flavobacteria	4	1	9	10
Sphingobacteria	0	0	2	2

Table S4 Information for top 20 abundant OTUs detected in this study (S:otu) after the removal of singletons and after subsampling, including their life strategy dependent on the inocula from which they originated (Strategy.o/b/m), taxonomic affiliation (S:phylogeny, the percentage in brackets indicates proportion of the sequences that were classified as members of the given taxonomy for an OTU), and maximal relative sequence abundance of individual OTUs in one of the microcosms (S:max.abundance %). The reference sequences of all OTUs (S:otu) in this study were blasted against reference sequences of the OTUs detected from a field study of the Baltic Sea (Herlemann *et al.*, 2016). The percent identity (Identity %) and the e-value of the closest Hit (E-value) are given. The columns H:max.abundance % and H:phylogeny represent the maximal sequence abundance of the reference OTUs in the Baltic Sea environments and taxonomic affiliation as detailed in Herlemann *et al*., 2016. We considered OTUs exhibiting maximal sequence abundance > 0.1% as abundant OTUs, and OTUs exhibiting maximal sequence abundance > 1% as highly abundant OTUs in their respective data sets. Abbreviations 'OSpe', 'BSpe', 'MSpe' and 'Gener' represent oligohaline specialists, brackish specialists, marine specialists and generalists, respectively. "NA" indicates that no specific life strategies were identified. See more information on other OTUs in (Shen *et al*., accepted; doi: 10.1111/1462-2920.14059; the article is protected by copyright. All rights reserved)

S:otu	Strategy.o	Strategy.b	Strategy.m	S:phylogeny	S:max. abundance %
Otu000001	BSpe	OSpe	OSpe	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas	32.57
Otu000002	NA	OSpe	OSpe	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;unclassified	40.36
Otu000003	Gener	BSpe	BSpe	Actinobacteria;Actinobacteria;Actinomycetales;Microbacteriaceae;Microbacterium	29.57
Otu000004	BSpe	MSpe	OSpe	Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;unclassified	39.61
Otu000005	BSpe	NA	BSpe	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter	25.18
Otu000006	MSpe	BSpe	MSpe	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;unclassified	17.25
Otu000007	NA	Gener	BSpe	Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;unclassified	20.72
Otu000008	NA	NA	OSpe	Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Limnobacter	36.1
Otu000009	NA	BSpe	BSpe	Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas	12.53
Otu000010	OSpe	OSpe	OSpe	Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Limnohabitans	17.54
Otu000011	NA	NA	MSpe	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;unclassified	16.04
Otu000012	MSpe	OSpe	OSpe	Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas	12.69
Otu000013	Gener	NA	OSpe	Proteobacteria;Gammaproteobacteria;Oceanospirillales;Alcanivoracaceae;Alcanivorax	4.57
Otu000014	Gener	OSpe	OSpe	Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas	3.18

S:otu	Strategy.o	Strategy.b	Strategy.m	S:phylogeny	S:max. abundance %
Otu000015	NA	OSpe	Gener	unclassified;unclassified;unclassified;unclassified;unclassified	17.09
Otu000016	NA	NA	MSpe	Proteobacteria;unclassified;unclassified;unclassified;unclassified	24.84
Otu000017	Gener	NA	OSpe	Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;unclassified	6.05
Otu000018	NA	BSpe	OSpe	Actinobacteria;Actinobacteria;Actinomycetales;Microbacteriaceae;unclassified	3.16
Otu000019	BSpe	OSpe	OSpe	Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas	13.75
Otu000020	NA	NA	OSpe	Proteobacteria;Gammaproteobacteria;unclassified;unclassified;unclassified	6.29

Table S4 continued

Identity %	E-value	H:otu	H:max. abundance %	H:phylogeny
97.86	0	OTU99_021980	0.08	Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas
99.73	0	OTU99_002564	0.37	Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
97.33	0	OTU99_013995	0.1	Bacteria;Actinobacteria;Actinobacteria;Micrococcales;Microbacteriaceae
100	0	OTU99_000012	16.77	Bacteria;Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;NS3a_marine_group
100	0	OTU99_000335	2.35	Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter
98.13	0	OTU99_007218	0.09	Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
95.99	3.00E-174	OTU99_010595	0.08	Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Glaciecola
99.73	0	OTU99_002260	0.38	Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales
99.73	0	OTU99_001041	0.55	Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae
99.47	0	OTU99_004591	0.19	Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Limnohabitans
99.47	0	OTU99_003145	0.28	Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
99.73	0	OTU99_003793	0.27	Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas

Identity %	E-value	H:otu	H:max. abundance %	H:phylogeny
97.34	0	OTU99_000624	0.94	Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Alcanivoracaceae;Alcanivorax
100	0	OTU99_000558	1.08	Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas
87.8	2.00E-122	OTU99_004767	0.16	Bacteria;BD1-5
98.66	0	OTU99_000840	0.79	Bacteria;Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;OM27_clade
96.26	2.00E-176	OTU99_000779	0.76	Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Marivita
99.47	0	OTU99_000110	4.38	Bacteria;Actinobacteria;Actinobacteria;Micrococcales;Microbacteriaceae;Candidatus_Aquiluna
98.66	0	OTU99_007897	0.09	Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas
99.2	0	OTU99_004135	0.19	Bacteria;Proteobacteria

Table S5 (**A**) Absolute abundance (DNA based, 16Sr rRNA genes) and (**B**) transcript abundance (mRNA based, metatranscriptomics) of the individual OTUs included in this study. '-' indicates that the given OTU was deemed as 'absent' according to the filtering of the OTUs datasets prior to the detection of life strategy.

A)

OTU ID	Life strategy	Aboslute abundance (x 10^4 cells / ml)								
		Oo	Ob	Om	*Bo*	*Bb*	*Bm*	*Mo*	*Mb*	*Mm*
Otu000010	OSpe	36.35 ± 9.52	1.23 ± 0.27	0.03 ± 0	12.44 ± 4.55	0.42 ± 0.40	0.02 ± 0	0.08 ± 0.03	0.05 ± 0	0.01 ± 0
Otu000030	OSpe	1.61 ± 0.07	2.22 ± 0.31	-	1.19 ± 0.04	1.92 ± 0.04	-	0.2 ± 0.09	1.07 ± 0.10	-
Otu000071	OSpe	1.52 ± 0.39	0.53 ± 0.05	-	0.62 ± 0.16	0.48 ± 0.04	-	0.06 ± 0.02	0.27 ± 0.02	-
Otu000100	OSpe	0.16 ± 0.01	0.08 ± 0.02	-	0.04 ± 0.04	0.04 ± 0	-	0.002 ± 0.002	0.004 ± 0.007	-
Otu000149	OSpe	0.49 ± 0.13	0.27 ± 0.11	-	0.02 ± 0.01	0.27 ± 0.05	-	0	0.07 ± 0.01	-
Otu000009	BSpe	-	2.15 ± 0.34	0.45 ± 0.1	-	2.63 ± 0.81	1.03 ± 0.19	-	0.59 ± 0.19	0.09 ± 0.06
Otu000021	BSpe	0.17 ± 0.04	0.27 ± 0.06	0.01 ± 0.01	42.14 ± 7.10	1.65 ± 0.19	1.12 ± 0.15	0.03 ± 0.02	0.24 ± 0.09	0.02 ± 0.01

Appendix

A)

OTU ID	Life strategy	Aboslute abundance (x 10^4 cells / ml)								
		Oo	Ob	Om	*Bo*	*Bb*	*Bm*	*Mo*	*Mb*	*Mm*
Otu000098	BSpe	0.002 ± 0.004	0.11 ± 0.04	0.002 ± 0.01	0.08 ± 0.05	1.07 ± 1.60	0.08 ± 0.02	0.01 ± 0.01	0.79 ± 0.10	0.002 ± 0.004
Otu000033	MSpe	0.006 ± 0.006	-	0.002 ± 0.003	0.01 ± 0.01	0.01 ± 0.02	0.006 ± 0.005	0.52 ± 0.08	0.06 ± 0.03	6.38 ± 2.12
Otu000194	MSpe	0	-	0.007 ± 0.006	0	-	0.003 ± 0.006	0.57 ± 0.04	-	0.05 ± 0.03
Otu000045	Gener	1.57 ± 4.78	0.76 ± 0.71	-	1.25 ± 0.16	1.41 ±0.46	-	0.79 ± 0.16	1.50 ± 0.27	-
Otu000060	Gener	1.38 ± 0.52	0.29 ± 0.11	0.02 ± 0.01	0.60 ± 0.39	0.29 ± 0.12	0.005 ± 0.008	0.76 ± 0.08	0.14 ± 0.04	0

B)

OTU ID	Life strategy	Transcript abundance per cell							
		Oo	Ob	*Om*	*Bo*	Bb	Bm	Mb	Mm
Otu000010	OSpe	45.417	43.81	95.324	40.983	137.139	731.911	47.923	185.62
Otu000030	OSpe	85.484	67.804	79.852	46.505	88.946	574.676	16.917	93.17
Otu000071	OSpe	13.084	26.118	41.203	10.009	82.993	56.686	5.879	179.305
Otu000100	OSpe	38.246	44.818	77.739	62.254	53.922	217.91	17.057	62.887
Otu000149	OSpe	13.017	9.428	10.327	17.559	38.117	-	12.226	-
Otu000009	BSpe	29	48.4	46.4	37	102	147	18.2	91.8
Otu000021	BSpe	46.1	43.8	67.8	9.75	32.6	39.7	30.7	109
Otu000098	BSpe	175	173	138	257	470	578	64.4	169
Otu000033	MSpe	833.023	320.903	400.636	1753.372	3577.215	1607.129	102.992	32.587
Otu000194	MSpe	419.715	386.403	126.23	370.972	1429.026	1080.74	285.662	283.826
Otu000045	Gener	15.123	8.747	98.445	7.47	10.073	53.682	1.07	117.873
Otu000060	Gener	419.659	276.204	4034.486	348.505	3191.517	2175.088	606.154	-

Table S6 The specific response of the oligohaline specialists to each of the three incubation environments by illustrating the numbers of genes that were shared between the transcriptome dataset pairs. Each transriptome dataset pair contains the genes that were significantly differential expressed under any of the two incubation environments. For instance, to detect specific transcriptional responses to the brackish environment, the differentially expressed genes between the brackish and marine environments for each OTU were compared with that between the brackish and freshwater environments. The capital letters following the OTU ID indicate the two environments between which the gene expressions were compared, and the first capital letter indicates that the environment to which the given gene was significantly regulated as a response. The data were presented in fold change of the gene (relative) abundance for the comparisons and the positive and negative fold change indicate the up- or down-regulation of the given genes. Only the genes belonging to the 7 functional clusters are listed.

Oligohaline specialists

Specific response to brackish environment

ko	KEGG ortholog	sub-functional category	pathway	gene description	Otu000010_BO	Otu000010_BM
K13991	ko02020	Signal transduction	Two-component system	puhA; photosynthetic reaction center H subunit	3.901	5.354091174

Specific response to marine environment

ko	KEGG ortholog	sub-functional category	pathway	gene description	Otu000010_BM	Otu000010_OM
K18661	ko00280	Amino acid metabolism	Valine, leucine and isoleucine degradation	matB; malonyl-CoA/methylmalonyl-CoA synthetase [EC:6.2.1.-]	4.16	3.95
K16165	ko00350	Amino acid metabolism	Tyrosine metabolism	nagK; fumarylpyruvate hydrolase [EC:3.7.1.20]	3.87	3.82
K14267	ko00300	Amino acid metabolism	Lysine biosynthesis	dapC; N-succinyldiaminopimelate aminotransferase [EC:2.6.1.17]	4.71	3.49
K14260	ko00220	Amino acid metabolism	Arginine biosynthesis	alaA; alanine-synthesizing transaminase [EC:2.6.1.66 2.6.1.2]	2.79	3.48
K14260	ko00250	Amino acid metabolism	Alanine, aspartate and glutamate metabolism	alaA; alanine-synthesizing transaminase [EC:2.6.1.66 2.6.1.2]	2.79	3.48
K14260	ko00290	Amino acid metabolism	Valine, leucine and isoleucine biosynthesis	alaA; alanine-synthesizing transaminase [EC:2.6.1.66 2.6.1.2]	2.79	3.48
K01956	ko00250	Amino acid metabolism	Alanine, aspartate and glutamate metabolism	carA, CPA1; carbamoyl-phosphate synthase small subunit [EC:6.3.5.5]	2.16	1.65
K01847	ko00280	Amino acid metabolism	Valine, leucine and isoleucine degradation	MUT; methylmalonyl-CoA mutase [EC:5.4.99.2]	-1.85	-2.49
K01834	ko00260	Amino acid metabolism	Glycine, serine and threonine metabolism	PGAM, gpmA; 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase [EC:5.4.2.11]	3.07	2.78
K01782	ko00280	Amino acid metabolism	Valine, leucine and isoleucine degradation	fadJ; 3-hydroxyacyl-CoA dehydrogenase / enoyl-CoA hydratase / 3-hydroxybutyryl-CoA epimerase [EC:1.1.1.35 4.2.1.17 5.1.2.3]	3.41	2.99
K01782	ko00310	Amino acid metabolism	Lysine degradation	fadJ; 3-hydroxyacyl-CoA dehydrogenase / enoyl-CoA hydratase / 3-hydroxybutyryl-CoA epimerase [EC:1.1.1.35 4.2.1.17 5.1.2.3]	3.41	2.99
K01782	ko00380	Amino acid metabolism	Tryptophan metabolism	fadJ; 3-hydroxyacyl-CoA dehydrogenase / enoyl-CoA hydratase / 3-hydroxybutyryl-CoA epimerase [EC:1.1.1.35 4.2.1.17 5.1.2.3]	3.41	2.99
K01696	ko00260	Amino acid metabolism	Glycine, serine and threonine metabolism	trpB; tryptophan synthase beta chain [EC:4.2.1.20]	4.19	5.03
K01696	ko00400	Amino acid metabolism	Phenylalanine, tyrosine and tryptophan biosynthesis	trpB; tryptophan synthase beta chain [EC:4.2.1.20]	4.19	5.03
K01692	ko00280	Amino acid metabolism	Valine, leucine and isoleucine degradation	paaF, echA; enoyl-CoA hydratase [EC:4.2.1.17]	1.95	1.96
K01692	ko00310	Amino acid metabolism	Lysine degradation	paaF, echA; enoyl-CoA hydratase [EC:4.2.1.17]	1.95	1.96
K01692	ko00360	Amino acid metabolism	Phenylalanine metabolism	paaF, echA; enoyl-CoA hydratase [EC:4.2.1.17]	1.95	1.96
K01692	ko00380	Amino acid metabolism	Tryptophan metabolism	paaF, echA; enoyl-CoA hydratase [EC:4.2.1.17]	1.95	1.96
K01652	ko00290	Amino acid metabolism	Valine, leucine and isoleucine biosynthesis	E2.2.1.6L, ilvB, ilvG, ilvI; acetolactate synthase I/II/III large subunit [EC:2.2.1.6]	1.46	2.26

Oligohaline specialists

Specific response to marine environment

ko	KEGG ortholog	sub-functional category	pathway	gene description	Otu000010_BM	Otu000010_OM
K01649	ko00290	Amino acid metabolism	Valine, leucine and isoleucine biosynthesis	leuA; 2-isopropylmalate synthase [EC:2.3.3.13]	4.29	5.17
K01586	ko00300	Amino acid metabolism	Lysine biosynthesis	lysA; diaminopimelate decarboxylase [EC:4.1.1.20]	-1.34	-1.78
K01584	ko00330	Amino acid metabolism	Arginine and proline metabolism	adiA; arginine decarboxylase [EC:4.1.1.19]	3.67	3.83
K01251	ko00270	Amino acid metabolism	Cysteine and methionine metabolism	E3.3.1.1, ahcY; adenosylhomocysteinase [EC:3.3.1.1]	-1.60	-1.66
K00930	ko00220	Amino acid metabolism	Arginine biosynthesis	argB; acetylglutamate kinase [EC:2.7.2.8]	3.49	3.53
K00832	ko00270	Amino acid metabolism	Cysteine and methionine metabolism	tyrB; aromatic-amino-acid transaminase [EC:2.6.1.57]	5.10	5.02
K00832	ko00350	Amino acid metabolism	Tyrosine metabolism	tyrB; aromatic-amino-acid transaminase [EC:2.6.1.57]	5.10	5.02
K00832	ko00360	Amino acid metabolism	Phenylalanine metabolism	tyrB; aromatic-amino-acid transaminase [EC:2.6.1.57]	5.10	5.02
K00832	ko00400	Amino acid metabolism	Phenylalanine, tyrosine and tryptophan biosynthesis	tyrB; aromatic-amino-acid transaminase [EC:2.6.1.57]	5.10	5.02
K00831	ko00260	Amino acid metabolism	Glycine, serine and threonine metabolism	serC, PSAT1; phosphoserine aminotransferase [EC:2.6.1.52]	3.57	3.86
K00818	ko00220	Amino acid metabolism	Arginine biosynthesis	E2.6.1.11, argD; acetylornithine aminotransferase [EC:2.6.1.11]	3.48	4.09
K00450	ko00350	Amino acid metabolism	Tyrosine metabolism	E1.13.11.4; gentisate 1,2-dioxygenase [EC:1.13.11.4]	-1.60	-1.97
K00382	ko00260	Amino acid metabolism	Glycine, serine and threonine metabolism	DLD, lpd, pdhD; dihydrolipoamide dehydrogenase [EC:1.8.1.4]	2.35	2.66
K00382	ko00280	Amino acid metabolism	Valine, leucine and isoleucine degradation	DLD, lpd, pdhD; dihydrolipoamide dehydrogenase [EC:1.8.1.4]	2.35	2.66
K00253	ko00280	Amino acid metabolism	Valine, leucine and isoleucine degradation	IVD, ivd; isovaleryl-CoA dehydrogenase [EC:1.3.8.4]	5.29	4.37
K00248	ko00280	Amino acid metabolism	Valine, leucine and isoleucine degradation	ACADS, bcd; butyryl-CoA dehydrogenase [EC:1.3.8.1]	-4.28	-2.92
K00058	ko00260	Amino acid metabolism	Glycine, serine and threonine metabolism	serA, PHGDH; D-3-phosphoglycerate dehydrogenase [EC:1.1.1.95]	3.85	3.97
K00053	ko00290	Amino acid metabolism	Valine, leucine and isoleucine biosynthesis	ilvC; ketol-acid reductoisomerase [EC:1.1.1.86]	1.41	1.47
K00052	ko00290	Amino acid metabolism	Valine, leucine and isoleucine biosynthesis	leuB; 3-isopropylmalate dehydrogenase [EC:1.1.1.85]	4.19	3.96
K05973	ko00650	Carbohydrate metabolism	Butanoate metabolism	E3.1.1.75, phaZ; poly(3-hydroxybutyrate) depolymerase [EC:3.1.1.75]	3.14	3.67
K03821	ko00650	Carbohydrate metabolism	Butanoate metabolism	phbC, phaC; polyhydroxyalkanoate synthase [EC:2.3.1.-]	4.31	5.13
K03738	ko00030	Carbohydrate metabolism	Pentose phosphate pathway	aor; aldehyde:ferredoxin oxidoreductase [EC:1.2.7.5]	4.62	5.75
K01908	ko00640	Carbohydrate metabolism	Propanoate metabolism	E6.2.1.17, prpE; propionyl-CoA synthetase [EC:6.2.1.17]	3.70	3.12
K01895	ko00010	Carbohydrate metabolism	Glycolysis / Gluconeogenesis	ACSS, acs; acetyl-CoA synthetase [EC:6.2.1.1]	2.26	1.95
K01895	ko00620	Carbohydrate metabolism	Pyruvate metabolism	ACSS, acs; acetyl-CoA synthetase [EC:6.2.1.1]	2.26	1.95
K01895	ko00640	Carbohydrate metabolism	Propanoate metabolism	ACSS, acs; acetyl-CoA synthetase [EC:6.2.1.1]	2.26	1.95

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ko	KEGG ortholog	sub-functional category	pathway	gene description	Otu000010_BM	Otu000010_OM
K01847	ko00630	Carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism	MUT; methylmalonyl-CoA mutase [EC:5.4.99.2]	-1.85	-2.49
K01847	ko00640	Carbohydrate metabolism	Propanoate metabolism	MUT; methylmalonyl-CoA mutase [EC:5.4.99.2]	-1.85	-2.49
K01834	ko00010	Carbohydrate metabolism	Glycolysis / Gluconeogenesis	PGAM, gpmA; 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase [EC:5.4.2.11]	3.07	2.78
K01783	ko00030	Carbohydrate metabolism	Pentose phosphate pathway	rpe, RPE; ribulose-phosphate 3-epimerase [EC:5.1.3.1]	3.35	3.61
K01783	ko00040	Carbohydrate metabolism	Pentose and glucuronate interconversions	rpe, RPE; ribulose-phosphate 3-epimerase [EC:5.1.3.1]	3.35	3.61
K01782	ko00640	Carbohydrate metabolism	Propanoate metabolism	fadJ; 3-hydroxyacyl-CoA dehydrogenase / enoyl-CoA hydratase / 3-hydroxybutyryl-CoA epimerase [EC:1.1.1.35 4.2.1.17 5.1.2.3]	3.41	2.99
K01782	ko00650	Carbohydrate metabolism	Butanoate metabolism	fadJ; 3-hydroxyacyl-CoA dehydrogenase / enoyl-CoA hydratase / 3-hydroxybutyryl-CoA epimerase [EC:1.1.1.35 4.2.1.17 5.1.2.3]	3.41	2.99
K01692	ko00640	Carbohydrate metabolism	Propanoate metabolism	paaF, echA; enoyl-CoA hydratase [EC:4.2.1.17]	1.95	1.96
K01692	ko00650	Carbohydrate metabolism	Butanoate metabolism	paaF, echA; enoyl-CoA hydratase [EC:4.2.1.17]	1.95	1.96
K01682	ko00020	Carbohydrate metabolism	Citrate cycle (TCA cycle)	acnB; aconitate hydratase 2 / 2-methylisocitrate dehydratase [EC:4.2.1.3 4.2.1.99]	3.29	2.54
K01682	ko00630	Carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism	acnB; aconitate hydratase 2 / 2-methylisocitrate dehydratase [EC:4.2.1.3 4.2.1.99]	3.29	2.54
K01682	ko00640	Carbohydrate metabolism	Propanoate metabolism	acnB; aconitate hydratase 2 / 2-methylisocitrate dehydratase [EC:4.2.1.3 4.2.1.99]	3.29	2.54
K01676	ko00020	Carbohydrate metabolism	Citrate cycle (TCA cycle)	E4.2.1.2A, fumA, fumB; fumarate hydratase, class I [EC:4.2.1.2]	3.45	4.12
K01676	ko00620	Carbohydrate metabolism	Pyruvate metabolism	E4.2.1.2A, fumA, fumB; fumarate hydratase, class I [EC:4.2.1.2]	3.45	4.12
K01652	ko00650	Carbohydrate metabolism	Butanoate metabolism	E2.2.1.6L, ilvB, ilvG, ilvI; acetolactate synthase I/II/III large subunit [EC:2.2.1.6]	1.46	2.26
K01652	ko00660	Carbohydrate metabolism	C5-Branched dibasic acid metabolism	E2.2.1.6L, ilvB, ilvG, ilvI; acetolactate synthase I/II/III large subunit [EC:2.2.1.6]	1.46	2.26
K01649	ko00620	Carbohydrate metabolism	Pyruvate metabolism	leuA; 2-isopropylmalate synthase [EC:2.3.3.13]	4.29	5.17
K01638	ko00620	Carbohydrate metabolism	Pyruvate metabolism	E2.3.3.9, aceB, glcB; malate synthase [EC:2.3.3.9]	2.86	2.23
K01638	ko00630	Carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism	E2.3.3.9, aceB, glcB; malate synthase [EC:2.3.3.9]	2.86	2.23
K01596	ko00010	Carbohydrate metabolism	Glycolysis / Gluconeogenesis	E4.1.1.32, pckA, PEPCK; phosphoenolpyruvate carboxykinase (GTP) [EC:4.1.1.32]	2.81	2.68
K01596	ko00020	Carbohydrate metabolism	Citrate cycle (TCA cycle)	E4.1.1.32, pckA, PEPCK; phosphoenolpyruvate carboxykinase (GTP) [EC:4.1.1.32]	2.81	2.68
K01596	ko00620	Carbohydrate metabolism	Pyruvate metabolism	E4.1.1.32, pckA, PEPCK; phosphoenolpyruvate carboxykinase (GTP) [EC:4.1.1.32]	2.81	2.68
K01092	ko00562	Carbohydrate metabolism	Inositol phosphate metabolism	E3.1.3.25, IMPA, suhB; myo-inositol-1(or 4)-monophosphatase [EC:3.1.3.25]	3.86	2.94
K00627	ko00010	Carbohydrate metabolism	Glycolysis / Gluconeogenesis	DLAT, aceF, pdhC; pyruvate dehydrogenase E2 component (dihydrolipoamide acetyltransferase) [EC:2.3.1.12]	3.07	3.96
K00627	ko00020	Carbohydrate metabolism	Citrate cycle (TCA cycle)	DLAT, aceF, pdhC; pyruvate dehydrogenase E2 component (dihydrolipoamide acetyltransferase) [EC:2.3.1.12]	3.07	3.96
K00627	ko00620	Carbohydrate metabolism	Pyruvate metabolism	DLAT, aceF, pdhC; pyruvate dehydrogenase E2 component (dihydrolipoamide acetyltransferase) [EC:2.3.1.12]	3.07	3.96
K00382	ko00010	Carbohydrate metabolism	Glycolysis / Gluconeogenesis	DLD, lpd, pdhD; dihydrolipoamide dehydrogenase [EC:1.8.1.4]	2.35	2.66
K00382	ko00020	Carbohydrate metabolism	Citrate cycle (TCA cycle)	DLD, lpd, pdhD; dihydrolipoamide dehydrogenase [EC:1.8.1.4]	2.35	2.66

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ko	KEGG ortholog	sub-functional category	pathway	gene description	Otu000010_BM	Otu000010_OM
K00382	ko00620	Carbohydrate metabolism	Pyruvate metabolism	DLD, lpd, pdhD; dihydrolipoamide dehydrogenase [EC:1.8.1.4]	2.35	2.66
K00382	ko00630	Carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism	DLD, lpd, pdhD; dihydrolipoamide dehydrogenase [EC:1.8.1.4]	2.35	2.66
K00284	ko00630	Carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism	E1.4.7.1; glutamate synthase (ferredoxin) [EC:1.4.7.1]	2.07	1.90
K00248	ko00650	Carbohydrate metabolism	Butanoate metabolism	ACADS, bcd; butyryl-CoA dehydrogenase [EC:1.3.8.1]	-4.28	-2.92
K00241	ko00020	Carbohydrate metabolism	Citrate cycle (TCA cycle)	sdhC, frdC; succinate dehydrogenase / fumarate reductase, cytochrome b subunit	4.88	4.68
K00241	ko00650	Carbohydrate metabolism	Butanoate metabolism	sdhC, frdC; succinate dehydrogenase / fumarate reductase, cytochrome b subunit	4.88	4.68
K00239	ko00020	Carbohydrate metabolism	Citrate cycle (TCA cycle)	sdhA, frdA; succinate dehydrogenase / fumarate reductase, flavoprotein subunit [EC:1.3.5.1 1.3.5.4]	2.95	3.54
K00239	ko00650	Carbohydrate metabolism	Butanoate metabolism	sdhA, frdA; succinate dehydrogenase / fumarate reductase, flavoprotein subunit [EC:1.3.5.1 1.3.5.4]	2.95	3.54
K00138	ko00010	Carbohydrate metabolism	Glycolysis / Gluconeogenesis	aldB; aldehyde dehydrogenase [EC:1.2.1.-]	-4.64	-4.61
K00138	ko00620	Carbohydrate metabolism	Pyruvate metabolism	aldB; aldehyde dehydrogenase [EC:1.2.1.-]	-4.64	-4.61
K00134	ko00010	Carbohydrate metabolism	Glycolysis / Gluconeogenesis	GAPDH, gapA; glyceraldehyde 3-phosphate dehydrogenase [EC:1.2.1.12]	-1.48	-1.46
K00127	ko00630	Carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism	fdoI; formate dehydrogenase subunit gamma	4.04	3.61
K00104	ko00630	Carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism	glcD; glycolate oxidase [EC:1.1.3.15]	4.11	4.24
K00052	ko00660	Carbohydrate metabolism	C5-Branched dibasic acid metabolism	leuB; 3-isopropylmalate dehydrogenase [EC:1.1.1.85]	4.19	3.96
K00019	ko00650	Carbohydrate metabolism	Butanoate metabolism	E1.1.1.30, bdh; 3-hydroxybutyrate dehydrogenase [EC:1.1.1.30]	3.61	3.53
K08738	ko04115	Cell growth and death	p53 signaling pathway	CYC; cytochrome c	4.46	4.82
K08738	ko04210	Cell growth and death	Apoptosis	CYC; cytochrome c	4.46	4.82
K03531	ko04112	Cell growth and death	Cell cycle - Caulobacter	ftsZ; cell division protein FtsZ	5.34	4.91
K03364	ko04110	Cell growth and death	Cell cycle	CDH1; cell division cycle 20-like protein 1, cofactor of APC complex	2.94	3.87
K03364	ko04111	Cell growth and death	Cell cycle - yeast	CDH1; cell division cycle 20-like protein 1, cofactor of APC complex	2.94	3.87
K17227	ko00920	Energy metabolism	Sulfur metabolism	soxZ; sulfur-oxidizing protein SoxZ	4.10	4.28
K01783	ko00710	Energy metabolism	Carbon fixation in photosynthetic organisms	rpe, RPE; ribulose-phosphate 3-epimerase [EC:5.1.3.1]	3.35	3.61
K00134	ko00710	Energy metabolism	Carbon fixation in photosynthetic organisms	GAPDH, gapA; glyceraldehyde 3-phosphate dehydrogenase [EC:1.2.1.12]	-1.48	-1.46
K01895	ko00720	Energy metabolism	Carbon fixation pathways in prokaryotes	ACSS, acs; acetyl-CoA synthetase [EC:6.2.1.1]	2.26	1.95
K01847	ko00720	Energy metabolism	Carbon fixation pathways in prokaryotes	MUT; methylmalonyl-CoA mutase [EC:5.4.99.2]	-1.85	-2.49
K01682	ko00720	Energy metabolism	Carbon fixation pathways in prokaryotes	acnB; aconitate hydratase 2 / 2-methylisocitrate dehydratase [EC:4.2.1.3 4.2.1.99]	3.29	2.54
K01676	ko00720	Energy metabolism	Carbon fixation pathways in prokaryotes	E4.2.1.2A, fumA, fumB; fumarate hydratase, class I [EC:4.2.1.2]	3.45	4.12
K01491	ko00720	Energy metabolism	Carbon fixation pathways in prokaryotes	folD; methylenetetrahydrofolate dehydrogenase (NADP+) / methenyltetrahydrofolate cyclohydrolase [EC:1.5.1.5 3.5.4.9]	-4.45	-4.08
K00241	ko00720	Energy metabolism	Carbon fixation pathways in prokaryotes	sdhC, frdC; succinate dehydrogenase / fumarate reductase, cytochrome b subunit	4.88	4.68
K00239	ko00720	Energy metabolism	Carbon fixation pathways in prokaryotes	sdhA, frdA; succinate dehydrogenase / fumarate reductase, flavoprotein subunit [EC:1.3.5.1 1.3.5.4]	2.95	3.54
K01895	ko00680	Energy metabolism	Methane metabolism	ACSS, acs; acetyl-CoA synthetase [EC:6.2.1.1]	2.26	1.95
K01834	ko00680	Energy metabolism	Methane metabolism	PGAM, gpmA; 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase [EC:5.4.2.11]	3.07	2.78
K00831	ko00680	Energy metabolism	Methane metabolism	serC, PSAT1; phosphoserine aminotransferase [EC:2.6.1.52]	3.57	3.86
K00127	ko00680	Energy metabolism	Methane metabolism	fdoI; formate dehydrogenase subunit gamma	4.04	3.61
K00058	ko00680	Energy metabolism	Methane metabolism	serA, PHGDH; D-3-phosphoglycerate dehydrogenase [EC:1.1.1.95]	3.85	3.97
K15577	ko00910	Energy metabolism	Nitrogen metabolism	nrtB, nasE, cynB; nitrate/nitrite transport system permease protein	3.10	2.93
K01725	ko00910	Energy metabolism	Nitrogen metabolism	cynS; cyanate lyase [EC:4.2.1.104]	3.49	3.13

Appendix

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Specific response to marine environment

ko	KEGG ortholog	sub-functional category	pathway	gene description	Otu000010_BM	Otu000010_OM
K00362	ko00910	Energy metabolism	Nitrogen metabolism	nirB; nitrite reductase (NADH) large subunit [EC:1.7.1.15]	4.34	3.95
K00284	ko00910	Energy metabolism	Nitrogen metabolism	E1.4.7.1; glutamate synthase (ferredoxin) [EC:1.4.7.1]	2.07	1.90
K02275	ko00190	Energy metabolism	Oxidative phosphorylation	coxB; cytochrome c oxidase subunit II [EC:1.9.3.1]	3.72	4.53
K02274	ko00190	Energy metabolism	Oxidative phosphorylation	coxA; cytochrome c oxidase subunit I [EC:1.9.3.1]	2.30	3.16
K02258	ko00190	Energy metabolism	Oxidative phosphorylation	COX11; cytochrome c oxidase assembly protein subunit 11	4.72	5.13
K02115	ko00190	Energy metabolism	Oxidative phosphorylation	ATPF1G, atpG; F-type H+-transporting ATPase subunit gamma	4.20	4.96
K02114	ko00190	Energy metabolism	Oxidative phosphorylation	ATPF1E, atpC; F-type H+-transporting ATPase subunit epsilon	3.06	4.47
K02113	ko00190	Energy metabolism	Oxidative phosphorylation	ATPF1D, atpH; F-type H+-transporting ATPase subunit delta	5.73	5.95
K02111	ko00190	Energy metabolism	Oxidative phosphorylation	ATPF1A, atpA; F-type H+-transporting ATPase subunit alpha [EC:3.6.3.14]	1.77	2.62
K02110	ko00190	Energy metabolism	Oxidative phosphorylation	ATPF0C, atpE; F-type H+-transporting ATPase subunit c	4.92	6.76
K02109	ko00190	Energy metabolism	Oxidative phosphorylation	ATPF0B, atpF; F-type H+-transporting ATPase subunit b	5.40	6.19
K00937	ko00190	Energy metabolism	Oxidative phosphorylation	ppk; polyphosphate kinase [EC:2.7.4.1]	4.29	5.73
K00413	ko00190	Energy metabolism	Oxidative phosphorylation	CYC1, CYT1, petC; ubiquinol-cytochrome c reductase cytochrome c1 subunit	4.87	5.86
K00411	ko00190	Energy metabolism	Oxidative phosphorylation	UQCRFS1, RIP1, petA; ubiquinol-cytochrome c reductase iron-sulfur subunit [EC:1.10.2.2]	3.68	4.28
K00341	ko00190	Energy metabolism	Oxidative phosphorylation	nuoL; NADH-quinone oxidoreductase subunit L [EC:1.6.5.3]	4.53	5.29
K00338	ko00190	Energy metabolism	Oxidative phosphorylation	nuoI; NADH-quinone oxidoreductase subunit I [EC:1.6.5.3]	3.27	3.27
K00337	ko00190	Energy metabolism	Oxidative phosphorylation	nuoH; NADH-quinone oxidoreductase subunit H [EC:1.6.5.3]	4.25	4.45
K00336	ko00190	Energy metabolism	Oxidative phosphorylation	nuoG; NADH-quinone oxidoreductase subunit G [EC:1.6.5.3]	5.52	5.14
K00335	ko00190	Energy metabolism	Oxidative phosphorylation	nuoF; NADH-quinone oxidoreductase subunit F [EC:1.6.5.3]	2.32	3.31
K00334	ko00190	Energy metabolism	Oxidative phosphorylation	nuoE; NADH-quinone oxidoreductase subunit E [EC:1.6.5.3]	3.80	4.40
K00333	ko00190	Energy metabolism	Oxidative phosphorylation	nuoD; NADH-quinone oxidoreductase subunit D [EC:1.6.5.3]	3.55	4.48
K00332	ko00190	Energy metabolism	Oxidative phosphorylation	nuoC; NADH-quinone oxidoreductase subunit C [EC:1.6.5.3]	3.68	4.34
K00331	ko00190	Energy metabolism	Oxidative phosphorylation	nuoB; NADH-quinone oxidoreductase subunit B [EC:1.6.5.3]	-2.76	-3.20
K00330	ko00190	Energy metabolism	Oxidative phosphorylation	nuoA; NADH-quinone oxidoreductase subunit A [EC:1.6.5.3]	4.15	5.37
K00241	ko00190	Energy metabolism	Oxidative phosphorylation	sdhC, frdC; succinate dehydrogenase / fumarate reductase, cytochrome b subunit	4.88	4.68
K00239	ko00190	Energy metabolism	Oxidative phosphorylation	sdhA, frdA; succinate dehydrogenase / fumarate reductase, flavoprotein subunit [EC:1.3.5.1 1.3.5.4]	2.95	3.54
K02115	ko00195	Energy metabolism	Photosynthesis	ATPF1G, atpG; F-type H+-transporting ATPase subunit gamma	4.20	4.96
K02114	ko00195	Energy metabolism	Photosynthesis	ATPF1E, atpC; F-type H+-transporting ATPase subunit epsilon	3.06	4.47
K02113	ko00195	Energy metabolism	Photosynthesis	ATPF1D, atpH; F-type H+-transporting ATPase subunit delta	5.73	5.95
K02111	ko00195	Energy metabolism	Photosynthesis	ATPF1A, atpA; F-type H+-transporting ATPase subunit alpha [EC:3.6.3.14]	1.77	2.62
K02110	ko00195	Energy metabolism	Photosynthesis	ATPF0C, atpE; F-type H+-transporting ATPase subunit c	4.92	6.76
K02109	ko00195	Energy metabolism	Photosynthesis	ATPF0B, atpF; F-type H+-transporting ATPase subunit b	5.40	6.19
K17223	ko00920	Energy metabolism	Sulfur metabolism	soxX; sulfur-oxidizing protein SoxX	3.61	4.21
K17222	ko00920	Energy metabolism	Sulfur metabolism	soxA; sulfur-oxidizing protein SoxA	3.39	3.13
K08738	ko00920	Energy metabolism	Sulfur metabolism	CYC; cytochrome c	4.46	4.82
K02045	ko00920	Energy metabolism	Sulfur metabolism	cysA; sulfate transport system ATP-binding protein [EC:3.6.3.25]	-4.28	-4.70
K10255	ko01040	Lipid metabolism	Biosynthesis of unsaturated fatty acids	FAD6, desA; omega-6 fatty acid desaturase (delta-12 desaturase) [EC:1.14.19.-]	4.22	4.44
K09458	ko00061	Lipid metabolism	Fatty acid biosynthesis	fabF; 3-oxoacyl-[acyl-carrier-protein] synthase II [EC:2.3.1.179]	3.14	3.35
K01897	ko00061	Lipid metabolism	Fatty acid biosynthesis	ACSL, fadD; long-chain acyl-CoA synthetase [EC:6.2.1.3]	2.11	2.27
K01897	ko00071	Lipid metabolism	Fatty acid degradation	ACSL, fadD; long-chain acyl-CoA synthetase [EC:6.2.1.3]	2.11	2.27
K01782	ko00071	Lipid metabolism	Fatty acid degradation	fadJ; 3-hydroxyacyl-CoA dehydrogenase / enoyl-CoA hydratase / 3-hydroxybutyryl-CoA epimerase [EC:1.1.1.35 4.2.1.17 5.1.2.3]	3.41	2.99
K01782	ko01040	Lipid metabolism	Biosynthesis of unsaturated fatty acids	fadJ; 3-hydroxyacyl-CoA dehydrogenase / enoyl-CoA hydratase / 3-hydroxybutyryl-CoA epimerase [EC:1.1.1.35 4.2.1.17 5.1.2.3]	3.41	2.99
K01692	ko00071	Lipid metabolism	Fatty acid degradation	paaF, echA; enoyl-CoA hydratase [EC:4.2.1.17]	1.95	1.96
K00507	ko01040	Lipid metabolism	Biosynthesis of unsaturated fatty acids	SCD, desC; stearoyl-CoA desaturase (delta-9 desaturase) [EC:1.14.19.1]	5.13	5.74
K00248	ko00071	Lipid metabolism	Fatty acid degradation	ACADS, bcd; butyryl-CoA dehydrogenase [EC:1.3.8.1]	-4.28	-2.92

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ko	KEGG ortholog	sub-functional category	pathway	gene description	Otu000010_BM	Otu000010_OM
K00019	ko00072	Lipid metabolism	Synthesis and degradation of ketone bodies	E1.1.1.30, bdh; 3-hydroxybutyrate dehydrogenase [EC:1.1.1.30]	3.61	3.53
K15577	ko02010	Membrane transport	ABC transporters	nrtB, nasE, cynB; nitrate/nitrite transport system permease protein	3.10	2.93
K12340	ko03070	Membrane transport	Bacterial secretion system	tolC; outer membrane protein	4.12	5.24
K11963	ko02010	Membrane transport	ABC transporters	urtE; urea transport system ATP-binding protein	3.62	2.65
K11961	ko02010	Membrane transport	ABC transporters	urtC; urea transport system permease protein	4.13	3.81
K11960	ko02010	Membrane transport	ABC transporters	urtB; urea transport system permease protein	4.97	4.73
K11959	ko02010	Membrane transport	ABC transporters	urtA; urea transport system substrate-binding protein	4.51	4.18
K11073	ko02010	Membrane transport	ABC transporters	potF; putrescine transport system substrate-binding protein	5.51	6.17
K09969	ko02010	Membrane transport	ABC transporters	aapJ, bztA; general L-amino acid transport system substrate-binding protein	5.11	4.77
K08483	ko02060	Membrane transport	Phosphotransferase system (PTS)	PTS-EI.PTSI, ptsI; phosphotransferase system, enzyme I, PtsI [EC:2.7.3.9]	-1.26	-1.09
K07323	ko02010	Membrane transport	ABC transporters	mlaC; phospholipid transport system substrate-binding protein	3.34	3.07
K05813	ko02010	Membrane transport	ABC transporters	ugpB; sn-glycerol 3-phosphate transport system substrate-binding protein	4.08	5.70
K03217	ko03070	Membrane transport	Bacterial secretion system	yidC, spoIIIJ, OXA1; YidC/Oxa1 family membrane protein insertase	2.90	3.36
K03117	ko03070	Membrane transport	Bacterial secretion system	tatB; sec-independent protein translocase protein TatB	3.65	4.19
K03116	ko03070	Membrane transport	Bacterial secretion system	tatA; sec-independent protein translocase protein TatA	4.68	5.13
K03076	ko03070	Membrane transport	Bacterial secretion system	secY; preprotein translocase subunit SecY	3.06	4.28
K03073	ko03070	Membrane transport	Bacterial secretion system	secE; preprotein translocase subunit SecE	3.78	4.83
K03071	ko03070	Membrane transport	Bacterial secretion system	secB; preprotein translocase subunit SecB	4.21	3.70
K02045	ko02010	Membrane transport	ABC transporters	cysA; sulfate transport system ATP-binding protein [EC:3.6.3.25]	-4.28	-4.70
K02044	ko02010	Membrane transport	ABC transporters	phnD; phosphonate transport system substrate-binding protein	1.57	2.05
K02042	ko02010	Membrane transport	ABC transporters	phnE; phosphonate transport system permease protein	4.38	5.38
K02040	ko02010	Membrane transport	ABC transporters	pstS; phosphate transport system substrate-binding protein	4.08	5.68
K02037	ko02010	Membrane transport	ABC transporters	pstC; phosphate transport system permease protein	2.92	4.08
K02016	ko02010	Membrane transport	ABC transporters	ABC.FEV.S; iron complex transport system substrate-binding protein	3.51	3.77
K01999	ko02010	Membrane transport	ABC transporters	livK; branched-chain amino acid transport system substrate-binding protein	3.46	4.11
K01998	ko02010	Membrane transport	ABC transporters	livM; branched-chain amino acid transport system permease protein	4.14	4.00
K03657	ko03420	Replication and repair	Nucleotide excision repair	uvrD, pcrA; DNA helicase II / ATP-dependent DNA helicase PcrA [EC:3.6.4.12]	3.86	3.43
K03657	ko03430	Replication and repair	Mismatch repair	uvrD, pcrA; DNA helicase II / ATP-dependent DNA helicase PcrA [EC:3.6.4.12]	3.86	3.43
K02686	ko03440	Replication and repair	Homologous recombination	priB; primosomal replication protein N	4.78	5.85
K02337	ko03030	Replication and repair	DNA replication	DPO3A1, dnaE; DNA polymerase III subunit alpha [EC:2.7.7.7]	3.73	4.72
K02337	ko03430	Replication and repair	Mismatch repair	DPO3A1, dnaE; DNA polymerase III subunit alpha [EC:2.7.7.7]	3.73	4.72
K02337	ko03440	Replication and repair	Homologous recombination	DPO3A1, dnaE; DNA polymerase III subunit alpha [EC:2.7.7.7]	3.73	4.72
K12340	ko02020	Signal transduction	Two-component system	tolC; outer membrane protein	4.12	5.24
K10255	ko02020	Signal transduction	Two-component system	FAD6, desA; omega-6 fatty acid desaturase (delta-12 desaturase) [EC:1.14.19.-]	4.22	4.44
K08939	ko02020	Signal transduction	Two-component system	pucB; light-harvesting protein B-800-850 beta chain	3.89	5.17
K08930	ko02020	Signal transduction	Two-component system	pucA; light-harvesting protein B-800-850 alpha chain	5.01	5.56
K08927	ko02020	Signal transduction	Two-component system	pufB; light-harvesting complex 1 beta chain	5.91	4.91
K08738	ko02020	Signal transduction	Two-component system	CYC; cytochrome c	4.46	4.82
K04771	ko02020	Signal transduction	Two-component system	degP, htrA; serine protease Do [EC:3.4.21.107]	4.29	4.04
K04079	ko04151	Signal transduction	PI3K-Akt signaling pathway	htpG, HSP90A; molecular chaperone HtpG	3.56	3.02
K02040	ko02020	Signal transduction	Two-component system	pstS; phosphate transport system substrate-binding protein	4.08	5.68
K01596	ko04068	Signal transduction	FoxO signaling pathway	E4.1.1.32, pckA, PEPCK; phosphoenolpyruvate carboxykinase (GTP) [EC:4.1.1.32]	2.81	2.68
K01596	ko04151	Signal transduction	PI3K-Akt signaling pathway	E4.1.1.32, pckA, PEPCK; phosphoenolpyruvate carboxykinase (GTP) [EC:4.1.1.32]	2.81	2.68
K01596	ko04152	Signal transduction	AMPK signaling pathway	E4.1.1.32, pckA, PEPCK; phosphoenolpyruvate carboxykinase (GTP) [EC:4.1.1.32]	2.81	2.68
K01092	ko04070	Signal transduction	Phosphatidylinositol signaling system	E3.1.3.25, IMPA, suhB; myo-inositol-1(or 4)-monophosphatase [EC:3.1.3.25]	3.86	2.94
K00507	ko04152	Signal transduction	AMPK signaling pathway	SCD, desC; stearoyl-CoA desaturase (delta-9 desaturase) [EC:1.14.19.1]	5.13	5.74
K00413	ko02020	Signal transduction	Two-component system	CYC1, CYT1, petC; ubiquinol-cytochrome c reductase cytochrome c1 subunit	4.87	5.86

Appendix

Oligohaline specialists

Specific response to marine environment

ko	KEGG ortholog	sub-functional category	pathway	gene description	Otu000010_BM	Otu000010_OM
K00411	ko02020	Signal transduction	Two-component system	UQCRFS1, RIP1, petA; ubiquinol-cytochrome c reductase iron-sulfur subunit [EC:1.10.2.2]	3.68	4.28
K00134	ko04066	Signal transduction	HIF-1 signaling pathway	GAPDH, gapA; glyceraldehyde 3-phosphate dehydrogenase [EC:1.2.1.12]	-1.48	-1.46
K03046	ko03020	Transcription	RNA polymerase	rpoC; DNA-directed RNA polymerase subunit beta' [EC:2.7.7.6]	1.95	2.52
K03043	ko03020	Transcription	RNA polymerase	rpoB; DNA-directed RNA polymerase subunit beta [EC:2.7.7.6]	2.10	2.63
K03040	ko03020	Transcription	RNA polymerase	rpoA; DNA-directed RNA polymerase subunit alpha [EC:2.7.7.6]	3.10	3.86
K13288	ko03008	Translation	Ribosome biogenesis in eukaryotes	orn, REX2, REXO2; oligoribonuclease [EC:3.1.-.-]	3.38	3.07
K02996	ko03010	Translation	Ribosome	RP-S9, MRPS9, rpsI; small subunit ribosomal protein S9	3.40	4.73
K02994	ko03010	Translation	Ribosome	RP-S8, rpsH; small subunit ribosomal protein S8	2.35	4.52
K02990	ko03010	Translation	Ribosome	RP-S6, MRPS6, rpsF; small subunit ribosomal protein S6	1.87	2.48
K02988	ko03010	Translation	Ribosome	RP-S5, MRPS5, rpsE; small subunit ribosomal protein S5	3.77	4.43
K02986	ko03010	Translation	Ribosome	RP-S4, rpsD; small subunit ribosomal protein S4	2.33	3.75
K02968	ko03010	Translation	Ribosome	RP-S20, rpsT; small subunit ribosomal protein S20	2.77	3.15
K02959	ko03010	Translation	Ribosome	RP-S16, MRPS16, rpsP; small subunit ribosomal protein S16	3.85	5.21
K02954	ko03010	Translation	Ribosome	RP-S14, MRPS14, rpsN; small subunit ribosomal protein S14	6.02	5.90
K02952	ko03010	Translation	Ribosome	RP-S13, rpsM; small subunit ribosomal protein S13	2.32	3.57
K02948	ko03010	Translation	Ribosome	RP-S11, MRPS11, rpsK; small subunit ribosomal protein S11	2.80	2.75
K02935	ko03010	Translation	Ribosome	RP-L7, MRPL12, rplL; large subunit ribosomal protein L7/L12	3.96	5.26
K02931	ko03010	Translation	Ribosome	RP-L5, MRPL5, rplE; large subunit ribosomal protein L5	2.45	3.52
K02909	ko03010	Translation	Ribosome	RP-L31, rpmE; large subunit ribosomal protein L31	6.08	6.88
K02904	ko03010	Translation	Ribosome	RP-L29, rpmC; large subunit ribosomal protein L29	3.04	4.00
K02902	ko03010	Translation	Ribosome	RP-L28, MRPL28, rpmB; large subunit ribosomal protein L28	3.65	4.80
K02897	ko03010	Translation	Ribosome	RP-L25, rplY; large subunit ribosomal protein L25	2.36	2.73
K02895	ko03010	Translation	Ribosome	RP-L24, MRPL24, rplX; large subunit ribosomal protein L24	3.68	5.83
K02892	ko03010	Translation	Ribosome	RP-L23, MRPL23, rplW; large subunit ribosomal protein L23	3.05	4.15
K02888	ko03010	Translation	Ribosome	RP-L21, MRPL21, rplU; large subunit ribosomal protein L21	4.47	4.88
K02887	ko03010	Translation	Ribosome	RP-L20, MRPL20, rplT; large subunit ribosomal protein L20	3.24	4.52
K02886	ko03010	Translation	Ribosome	RP-L2, MRPL2, rplB; large subunit ribosomal protein L2	3.37	4.12
K02884	ko03010	Translation	Ribosome	RP-L19, MRPL19, rplS; large subunit ribosomal protein L19	3.37	4.72
K02881	ko03010	Translation	Ribosome	RP-L18, MRPL18, rplR; large subunit ribosomal protein L18	4.76	4.78
K02879	ko03010	Translation	Ribosome	RP-L17, MRPL17, rplQ; large subunit ribosomal protein L17	3.87	5.28
K02876	ko03010	Translation	Ribosome	RP-L15, MRPL15, rplO; large subunit ribosomal protein L15	2.41	3.09
K02874	ko03010	Translation	Ribosome	RP-L14, MRPL14, rplN; large subunit ribosomal protein L14	2.88	2.99
K02871	ko03010	Translation	Ribosome	RP-L13, MRPL13, rplM; large subunit ribosomal protein L13	5.36	6.57
K02864	ko03010	Translation	Ribosome	RP-L10, MRPL10, rplJ; large subunit ribosomal protein L10	5.89	6.92
K02434	ko00970	Translation	Aminoacyl-tRNA biosynthesis	gatB, PET112; aspartyl-tRNA(Asn)/glutamyl-tRNA(Gln) amidotransferase subunit B [EC:6.3.5.6 6.3.5.7]	3.69	2.89
K01890	ko00970	Translation	Aminoacyl-tRNA biosynthesis	FARSB, pheT; phenylalanyl-tRNA synthetase beta chain [EC:6.1.1.20]	4.06	4.32
K01883	ko00970	Translation	Aminoacyl-tRNA biosynthesis	CARS, cysS; cysteinyl-tRNA synthetase [EC:6.1.1.16]	4.05	4.86
K01879	ko00970	Translation	Aminoacyl-tRNA biosynthesis	glyS; glycyl-tRNA synthetase beta chain [EC:6.1.1.14]	3.88	3.93
K01874	ko00970	Translation	Aminoacyl-tRNA biosynthesis	MARS, metG; methionyl-tRNA synthetase [EC:6.1.1.10]	3.75	5.05
K01873	ko00970	Translation	Aminoacyl-tRNA biosynthesis	VARS, valS; valyl-tRNA synthetase [EC:6.1.1.9]	4.47	5.11
K01869	ko00970	Translation	Aminoacyl-tRNA biosynthesis	LARS, leuS; leucyl-tRNA synthetase [EC:6.1.1.4]	3.10	3.50

Table S7 The specific response of the brackish specialists to each of the three incubation environments by illustrating the numbers of genes that were shared between the transcriptome dataset pairs. The capital letters following the OTU ID indicate the two environments between which the gene expressions were compared, and the first capital letter indicates that the environment to which the given gene was significantly regulated as a response. The data were presented in fold change of the gene (relative) abundance for the comparisons and the positive and negative fold change indicate the up- or down-regulation of the given genes. Only the genes belonging to the 7 functional clusters are listed.

Brackish specialists

Specific to oligohaline enviornment

ko	KEGG ortholog	sub-functional category	pathway	gene description	Otu000009_BO	Otu000009_OM
K01478	ko00220	Amino acid metabolism	Arginine biosynthesis	arcA; arginine deiminase [EC:3.5.3.6]	-2.12	1.76
K03897	ko00310	Amino acid metabolism	Lysine degradation	iucD; lysine N6-hydroxylase [EC:1.14.13.59]	3.11	-4.05
K00114	ko00010	Carbohydrate metabolism	Glycolysis / Gluconeogenesis	exaA; alcohol dehydrogenase (cytochrome c) [EC:1.1.2.8]	-2.03	3.38
K00134	ko00010	Carbohydrate metabolism	Glycolysis / Gluconeogenesis	GAPDH, gapA; glyceraldehyde 3-phosphate dehydrogenase [EC:1.2.1.12]	0.65	-1.01
K17716	ko00052	Carbohydrate metabolism	Galactose metabolism	capD; UDP-glucose 4-epimerase [EC:5.1.3.2]	1.63	-2.08
K17716	ko00520	Carbohydrate metabolism	Amino sugar and nucleotide sugar metabolism	capD; UDP-glucose 4-epimerase [EC:5.1.3.2]	1.63	-2.08
K00134	ko00710	Energy metabolism	Carbon fixation in photosynthetic organisms	GAPDH, gapA; glyceraldehyde 3-phosphate dehydrogenase [EC:1.2.1.12]	0.65	-1.01
K00405	ko00190	Energy metabolism	Oxidative phosphorylation	ccoO; cytochrome c oxidase cbb3-type subunit II	2.42	-1.61
K02575	ko00910	Energy metabolism	Nitrogen metabolism	NRT, narK, nrtP, nasA; MFS transporter, NNP family, nitrate/nitrite transporter	3.86	-4.34
K03885	ko00190	Energy metabolism	Oxidative phosphorylation	ndh; NADH dehydrogenase [EC:1.6.99.3]	2.82	-3.45
K15576	ko00910	Energy metabolism	Nitrogen metabolism	nrtA, nasF, cynA; nitrate/nitrite transport system substrate-binding protein	5.15	-6.12
K03735	ko00564	Lipid metabolism	Glycerophospholipid metabolism	eutB; ethanolamine ammonia-lyase large subunit [EC:4.3.1.7]	3.58	-3.26
K03116	ko03070	Membrane transport	Bacterial secretion system	tatA; sec-independent protein translocase protein TatA	-1.22	2.38
K15576	ko02010	Membrane transport	ABC transporters	nrtA, nasF, cynA; nitrate/nitrite transport system substrate-binding protein	5.15	-6.12
K00134	ko04066	Signal transduction	HIF-1 signaling pathway	GAPDH, gapA; glyceraldehyde 3-phosphate dehydrogenase [EC:1.2.1.12]	0.65	-1.01
K00405	ko02020	Signal transduction	Two-component system	ccoO; cytochrome c oxidase cbb3-type subunit II	2.42	-1.61
K01873	ko00970	Translation	Aminoacyl-tRNA biosynthesis	VARS, valS; valyl-tRNA synthetase [EC:6.1.1.9]	1.55	-2.23
K02874	ko03010	Translation	Ribosome	RP-L14, MRPL14, rplN; large subunit ribosomal protein L14	-1.03	1.34
K02878	ko03010	Translation	Ribosome	RP-L16, MRPL16, rplP; large subunit ribosomal protein L16	-1.19	1.23
K02916	ko03010	Translation	Ribosome	RP-L35, MRPL35, rpmI; large subunit ribosomal protein L35	-1.13	1.52
K02956	ko03010	Translation	Ribosome	RP-S15, MRPS15, rpsO; small subunit ribosomal protein S15	-1.73	1.79

Brackish specialists

Specific response to brackish environment

ko	KEGG ortholog	sub-functional category	pathway	gene description	Otu000009_BO	Otu000021_BO	Otu000009_BM	Otu000021_BM
K00166	ko00280	Amino acid metabolism	Valine, leucine and isoleucine degradation	BCKDHA, bkdA1; 2-oxoisovalerate dehydrogenase E1 component alpha subunit [EC:1.2.4.4]	NA	3.69	NA	5.15
K00265	ko00250	Amino acid metabolism	Alanine, aspartate and glutamate metabolism	gltB; glutamate synthase (NADPH/NADH) large chain [EC:1.4.1.13 1.4.1.14]	1.23	NA	1.28	NA

Brackish specialists

Specific response to brackish environment

ko	KEGG ortholog	sub-functional category	pathway	gene description	Otu000009_BO	Otu000021_BO	Otu000009_BM	Otu000021_BM
K00549	ko00270	Amino acid metabolism	Cysteine and methionine metabolism	metE; 5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase [EC:2.1.1.14]	4.53	NA	4.33	NA
K01457	ko00220	Amino acid metabolism	Arginine biosynthesis	E3.5.1.54; allophanate hydrolase [EC:3.5.1.54]	3.91	NA	2.14	NA
K01470	ko00330	Amino acid metabolism	Arginine and proline metabolism	E3.5.2.10; creatinine amidohydrolase [EC:3.5.2.10]	3.76	NA	3.40	NA
K01485	ko00330	Amino acid metabolism	Arginine and proline metabolism	codA; cytosine deaminase [EC:3.5.4.1]	2.40	NA	2.55	NA
K01915	ko00220	Amino acid metabolism	Arginine biosynthesis	glnA, GLUL; glutamine synthetase [EC:6.3.1.2]	0.83	NA	0.77	NA
K01915	ko00250	Amino acid metabolism	Alanine, aspartate and glutamate metabolism	glnA, GLUL; glutamine synthetase [EC:6.3.1.2]	0.83	NA	0.77	NA
K03781	ko00380	Amino acid metabolism	Tryptophan metabolism	katE, CAT, catB, srpA; catalase [EC:1.11.1.6]	NA	3.33	2.02	2.71
K12256	ko00330	Amino acid metabolism	Arginine and proline metabolism	spuC; putrescine aminotransferase [EC:2.6.1.-]	2.08	NA	2.97	NA
K00012	ko00040	Carbohydrate metabolism	Pentose and glucuronate interconversions	UGDH, ugd; UDPglucose 6-dehydrogenase [EC:1.1.1.22]	2.03	NA	1.55	NA
K00012	ko00053	Carbohydrate metabolism	Ascorbate and aldarate metabolism	UGDH, ugd; UDPglucose 6-dehydrogenase [EC:1.1.1.22]	2.03	NA	1.55	NA
K00012	ko00500	Carbohydrate metabolism	Starch and sucrose metabolism	UGDH, ugd; UDPglucose 6-dehydrogenase [EC:1.1.1.22]	2.03	NA	1.55	NA
K00012	ko00520	Carbohydrate metabolism	Amino sugar and nucleotide sugar metabolism	UGDH, ugd; UDPglucose 6-dehydrogenase [EC:1.1.1.22]	2.03	NA	1.55	NA
K00023	ko00630	Carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism	E1.1.1.36, phbB; acetoacetyl-CoA reductase [EC:1.1.1.36]	NA	2.48	NA	2.47
K00023	ko00650	Carbohydrate metabolism	Butanoate metabolism	E1.1.1.36, phbB; acetoacetyl-CoA reductase [EC:1.1.1.36]	NA	2.48	NA	2.47
K00123	ko00630	Carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism	fdoG, fdfH; formate dehydrogenase major subunit [EC:1.2.1.2]	NA	3.74	NA	5.42
K00138	ko00010	Carbohydrate metabolism	Glycolysis / Gluconeogenesis	aldB; aldehyde dehydrogenase [EC:1.2.1.-]	-1.32	NA	-1.73	NA
K00138	ko00620	Carbohydrate metabolism	Pyruvate metabolism	aldB; aldehyde dehydrogenase [EC:1.2.1.-]	-1.32	NA	-1.73	NA
K00688	ko00500	Carbohydrate metabolism	Starch and sucrose metabolism	E2.4.1.1, glgP, PYG; starch phosphorylase [EC:2.4.1.1]	1.16	NA	1.28	NA
K00705	ko00500	Carbohydrate metabolism	Starch and sucrose metabolism	malQ; 4-alpha-glucanotransferase [EC:2.4.1.25]	2.36	NA	2.91	NA
K00754	ko00051	Carbohydrate metabolism	Fructose and mannose metabolism	E2.4.1.-	NA	4.50	NA	6.31
K00963	ko00040	Carbohydrate metabolism	Pentose and glucuronate interconversions	UGP2, galU, galF; UTP--glucose-1-phosphate uridylyltransferase [EC:2.7.7.9]	1.16	NA	1.65	NA
K00963	ko00052	Carbohydrate metabolism	Galactose metabolism	UGP2, galU, galF; UTP--glucose-1-phosphate uridylyltransferase [EC:2.7.7.9]	1.16	NA	1.65	NA
K00963	ko00500	Carbohydrate metabolism	Starch and sucrose metabolism	UGP2, galU, galF; UTP--glucose-1-phosphate uridylyltransferase [EC:2.7.7.9]	1.16	NA	1.65	NA
K00963	ko00520	Carbohydrate metabolism	Amino sugar and nucleotide sugar metabolism	UGP2, galU, galF; UTP--glucose-1-phosphate uridylyltransferase [EC:2.7.7.9]	1.16	NA	1.65	NA
K00971	ko00051	Carbohydrate metabolism	Fructose and mannose metabolism	manC, cpsB; mannose-1-phosphate guanylyltransferase [EC:2.7.7.13]	2.90	NA	3.73	4.74
K00971	ko00520	Carbohydrate metabolism	Amino sugar and nucleotide sugar metabolism	manC, cpsB; mannose-1-phosphate guanylyltransferase [EC:2.7.7.13]	2.90	NA	3.73	4.74
K01069	ko00620	Carbohydrate metabolism	Pyruvate metabolism	E3.1.2.6, gloB; hydroxyacylglutathione hydrolase [EC:3.1.2.6]	1.31	NA	1.67	NA
K01190	ko00052	Carbohydrate metabolism	Galactose metabolism	lacZ; beta-galactosidase [EC:3.2.1.23]	NA	3.89	NA	5.54

Brackish specialists

Specific response to brackish environment

ko	KEGG ortholog	sub-functional category	pathway	gene description	Otu000009_BO	Otu000021_BO	Otu000009_BM	Otu000021_BM
K01647	ko00020	Carbohydrate metabolism	Citrate cycle (TCA cycle)	CS, gltA; citrate synthase [EC:2.3.3.1]	0.71	NA	1.46	NA
K01647	ko00630	Carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism	CS, gltA; citrate synthase [EC:2.3.3.1]	0.71	NA	1.46	NA
K01734	ko00640	Carbohydrate metabolism	Propanoate metabolism	mgsA; methylglyoxal synthase [EC:4.2.3.3]	2.12	NA	4.43	NA
K01903	ko00020	Carbohydrate metabolism	Citrate cycle (TCA cycle)	sucC; succinyl-CoA synthetase beta subunit [EC:6.2.1.5]	1.02	NA	0.93	NA
K01903	ko00640	Carbohydrate metabolism	Propanoate metabolism	sucC; succinyl-CoA synthetase beta subunit [EC:6.2.1.5]	1.02	NA	0.93	NA
K01903	ko00660	Carbohydrate metabolism	C5-Branched dibasic acid metabolism	sucC; succinyl-CoA synthetase beta subunit [EC:6.2.1.5]	1.02	NA	0.93	NA
K01915	ko00630	Carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism	glnA, GLUL; glutamine synthetase [EC:6.3.1.2]	0.83	NA	0.77	NA
K03781	ko00630	Carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism	katE, CAT, catB, srpA; catalase [EC:1.11.1.6]	NA	3.33	2.02	2.71
K06044	ko00500	Carbohydrate metabolism	Starch and sucrose metabolism	treY, glgY; (1->4)-alpha-D-glucan 1-alpha-D-glucosylmutase [EC:5.4.99.15]	1.08	NA	1.83	NA
K01338	ko04112	Cell growth and death	Cell cycle - Caulobacter	lon; ATP-dependent Lon protease [EC:3.4.21.53]	1.14	NA	1.99	NA
K03531	ko04112	Cell growth and death	Cell cycle - Caulobacter	ftsZ; cell division protein FtsZ	1.55	NA	3.04	NA
K00123	ko00680	Energy metabolism	Methane metabolism	fdoG, fdfH; formate dehydrogenase major subunit [EC:1.2.1.2]	NA	3.74	NA	5.42
K00265	ko00910	Energy metabolism	Nitrogen metabolism	gltB; glutamate synthase (NADPH/NADH) large chain [EC:1.4.1.13 1.4.1.14]	1.23	NA	1.28	NA
K00362	ko00910	Energy metabolism	Nitrogen metabolism	nirB; nitrite reductase (NADH) large subunit [EC:1.7.1.15]	3.22	3.69	1.66	5.71
K00363	ko00910	Energy metabolism	Nitrogen metabolism	nirD; nitrite reductase (NADH) small subunit [EC:1.7.1.15]	3.09	NA	1.99	NA
K00372	ko00910	Energy metabolism	Nitrogen metabolism	nasA; assimilatory nitrate reductase catalytic subunit [EC:1.7.99.4]	4.10	5.76	2.53	5.08
K00381	ko00920	Energy metabolism	Sulfur metabolism	cysI; sulfite reductase (NADPH) hemoprotein beta-component [EC:1.8.1.2]	1.87	NA	2.93	NA
K00390	ko00920	Energy metabolism	Sulfur metabolism	cysH; phosphoadenosine phosphosulfate reductase [EC:1.8.4.8]	2.84	NA	2.82	NA
K00406	ko00190	Energy metabolism	Oxidative phosphorylation	ccoP; cytochrome c oxidase cbb3-type subunit III	1.46	NA	2.16	NA
K00957	ko00920	Energy metabolism	Sulfur metabolism	cysD; sulfate adenylyltransferase subunit 2 [EC:2.7.7.4]	1.47	NA	1.72	NA
K01673	ko00910	Energy metabolism	Nitrogen metabolism	cynT, can; carbonic anhydrase [EC:4.2.1.1]	NA	2.75	NA	6.12
K01903	ko00720	Energy metabolism	Carbon fixation pathways in prokaryotes	sucC; succinyl-CoA synthetase beta subunit [EC:6.2.1.5]	1.02	NA	0.93	NA
K01915	ko00910	Energy metabolism	Nitrogen metabolism	glnA, GLUL; glutamine synthetase [EC:6.3.1.2]	0.83	NA	0.77	NA
K15577	ko00910	Energy metabolism	Nitrogen metabolism	nrtB, nasE, cynB; nitrate/nitrite transport system permease protein	5.70	NA	3.86	NA
K18277	ko00680	Energy metabolism	Methane metabolism	tmm; trimethylamine monooxygenase [EC:1.14.13.148]	4.69	NA	2.99	NA
K01190	ko00600	Lipid metabolism	Sphingolipid metabolism	lacZ; beta-galactosidase [EC:3.2.1.23]	NA	3.89	NA	5.54
K01897	ko00061	Lipid metabolism	Fatty acid biosynthesis	ACSL, fadD; long-chain acyl-CoA synthetase [EC:6.2.1.3]	NA	3.35	NA	3.79
K01897	ko00071	Lipid metabolism	Fatty acid degradation	ACSL, fadD; long-chain acyl-CoA synthetase [EC:6.2.1.3]	NA	3.35	NA	3.79
K02372	ko00061	Lipid metabolism	Fatty acid biosynthesis	fabZ; 3-hydroxyacyl-[acyl-carrier-protein] dehydratase [EC:4.2.1.59]	2.55	NA	3.72	NA
K03621	ko00561	Lipid metabolism	Glycerolipid metabolism	plsX; glycerol-3-phosphate acyltransferase PlsX [EC:2.3.1.15]	3.53	NA	3.93	NA
K03621	ko00564	Lipid metabolism	Glycerophospholipid metabolism	plsX; glycerol-3-phosphate acyltransferase PlsX [EC:2.3.1.15]	3.53	NA	3.93	NA
K03736	ko00564	Lipid metabolism	Glycerophospholipid metabolism	eutC; ethanolamine ammonia-lyase small subunit [EC:4.3.1.7]	4.61	NA	4.06	NA

Brackish specialists

Specific response to brackish environment

ko	KEGG ortholog	sub-functional category	pathway	gene description	Otu000009_BO	Otu000021_BO	Otu000009_BM	Otu000021_BM
K01999	ko02010	Membrane transport	ABC transporters	livK; branched-chain amino acid transport system substrate-binding protein	1.39	NA	1.60	NA
K02044	ko02010	Membrane transport	ABC transporters	phnD; phosphonate transport system substrate-binding protein	-1.54	NA	-1.61	NA
K03073	ko03070	Membrane transport	Bacterial secretion system	secE; preprotein translocase subunit SecE	2.67	NA	4.78	NA
K09970	ko02010	Membrane transport	ABC transporters	aapQ, bztB; general L-amino acid transport system permease protein	3.38	NA	4.10	NA
K09971	ko02010	Membrane transport	ABC transporters	aapM, bztC; general L-amino acid transport system permease protein	1.97	NA	2.98	NA
K09972	ko02010	Membrane transport	ABC transporters	aapP, bztD; general L-amino acid transport system ATP-binding protein [EC:3.6.3.-]	1.85	NA	2.18	NA
K11073	ko02010	Membrane transport	ABC transporters	potF; putrescine transport system substrate-binding protein	1.30	NA	1.92	NA
K12368	ko02010	Membrane transport	ABC transporters	dppA; dipeptide transport system substrate-binding protein	2.66	NA	2.82	NA
K15577	ko02010	Membrane transport	ABC transporters	nrtB, nasE, cynB; nitrate/nitrite transport system permease protein	5.70	NA	3.86	NA
K02342	ko03430	Replication and repair	Mismatch repair	DPO3E, dnaQ; DNA polymerase III subunit epsilon [EC:2.7.7.7]	NA	5.17	NA	6.44
K02342	ko03440	Replication and repair	Homologous recombination	DPO3E, dnaQ; DNA polymerase III subunit epsilon [EC:2.7.7.7]	NA	5.17	NA	6.44
K00406	ko02020	Signal transduction	Two-component system	ccoP; cytochrome c oxidase cbb3-type subunit III	1.46	NA	2.16	NA
K01077	ko02020	Signal transduction	Two-component system	E3.1.3.1, phoA, phoB; alkaline phosphatase [EC:3.1.3.1]	NA	2.50	NA	3.82
K01113	ko02020	Signal transduction	Two-component system	phoD; alkaline phosphatase D [EC:3.1.3.1]	-1.73	NA	-0.95	NA
K01915	ko02020	Signal transduction	Two-component system	glnA, GLUL; glutamine synthetase [EC:6.3.1.2]	0.83	NA	0.77	NA
K02405	ko02020	Signal transduction	Two-component system	fliA; RNA polymerase sigma factor for flagellar operon FliA	2.39	NA	4.09	NA
K03406	ko02020	Signal transduction	Two-component system	mcp; methyl-accepting chemotaxis protein	NA	3.59	NA	2.84
K03781	ko04068	Signal transduction	FoxO signaling pathway	katE, CAT, catB, srpA; catalase [EC:1.11.1.6]	NA	3.33	2.02	2.71
K07644	ko02020	Signal transduction	Two-component system	cusS, copS, silS; two-component system, OmpR family, heavy metal sensor histidine kinase CusS [EC:2.7.13.3]	2.16	NA	4.67	NA
K03040	ko03020	Transcription	RNA polymerase	rpoA; DNA-directed RNA polymerase subunit alpha [EC:2.7.7.6]	1.05	NA	1.49	NA
K03060	ko03020	Transcription	RNA polymerase	rpoZ; DNA-directed RNA polymerase subunit omega [EC:2.7.7.6]	1.80	3.31	3.19	5.34
K02886	ko03010	Translation	Ribosome	RP-L2, MRPL2, rplB; large subunit ribosomal protein L2	NA	-1.43	NA	-1.51
K01897	ko04146	Transport and catabolism	Peroxisome	ACSL, fadD; long-chain acyl-CoA synthetase [EC:6.2.1.3]	NA	3.35	NA	3.79
K03781	ko04146	Transport and catabolism	Peroxisome	katE, CAT, catB, srpA; catalase [EC:1.11.1.6]	NA	3.33	2.02	2.71
K01971	ko03450	Replication and repair	Non-homologous end-joining	ligD; bifunctional non-homologous end joining protein LigD [EC:6.5.1.1]	NA	4.54	NA	4.98
K02342	ko03030	Replication and repair	DNA replication	DPO3E, dnaQ; DNA polymerase III subunit epsilon [EC:2.7.7.7]	NA	5.17	NA	6.44

Brackish specialists

Specific response to marine environment

ko	KEGG ortholog	sub-functional category	pathway	gene description	Otu000009_BM	Otu000021_BM	Otu000009_OM	Otu000021_OM
K00133	ko00260	Amino acid metabolism	Glycine, serine and threonine metabolism	asd; aspartate-semialdehyde dehydrogenase [EC:1.2.1.11]	2.31	3.67	1.91	NA
K00133	ko00270	Amino acid metabolism	Cysteine and methionine metabolism	asd; aspartate-semialdehyde dehydrogenase [EC:1.2.1.11]	2.31	3.67	1.91	NA
K00133	ko00300	Amino acid metabolism	Lysine biosynthesis	asd; aspartate-semialdehyde dehydrogenase [EC:1.2.1.11]	2.31	3.67	1.91	NA
K00140	ko00280	Amino acid metabolism	Valine, leucine and isoleucine degradation	mmsA, iolA, ALDH6A1; malonate-semialdehyde dehydrogenase (acetylating) / methylmalonate-semialdehyde dehydrogenase [EC:1.2.1.18 1.2.1.27]	NA	4.20	-2.04	NA
K00145	ko00220	Amino acid metabolism	Arginine biosynthesis	argC; N-acetyl-gamma-glutamyl-phosphate reductase [EC:1.2.1.38]	3.05	NA	2.76	NA
K00164	ko00310	Amino acid metabolism	Lysine degradation	OGDH, sucA; 2-oxoglutarate dehydrogenase E1 component [EC:1.2.4.2]	1.29	NA	NA	NA
K00164	ko00380	Amino acid metabolism	Tryptophan metabolism	OGDH, sucA; 2-oxoglutarate dehydrogenase E1 component [EC:1.2.4.2]	1.29	NA	NA	NA
K00253	ko00280	Amino acid metabolism	Valine, leucine and isoleucine degradation	IVD, ivd; isovaleryl-CoA dehydrogenase [EC:1.3.8.4]	-3.39	NA	-2.23	NA
K00263	ko00280	Amino acid metabolism	Valine, leucine and isoleucine degradation	E1.4.1.9; leucine dehydrogenase [EC:1.4.1.9]	-3.52	NA	-4.57	NA
K00263	ko00290	Amino acid metabolism	Valine, leucine and isoleucine biosynthesis	E1.4.1.9; leucine dehydrogenase [EC:1.4.1.9]	-3.52	NA	-4.57	NA
K00281	ko00260	Amino acid metabolism	Glycine, serine and threonine metabolism	GLDC, gcvP; glycine dehydrogenase [EC:1.4.4.2]	2.65	NA	1.87	NA
K00658	ko00310	Amino acid metabolism	Lysine degradation	DLST, sucB; 2-oxoglutarate dehydrogenase E2 component (dihydrolipoamide succinyltransferase) [EC:2.3.1.61]	2.30	NA	2.16	NA
K00797	ko00270	Amino acid metabolism	Cysteine and methionine metabolism	speE, SRM; spermidine synthase [EC:2.5.1.16]	2.46	NA	3.23	NA
K00797	ko00330	Amino acid metabolism	Arginine and proline metabolism	speE, SRM; spermidine synthase [EC:2.5.1.16]	2.46	NA	3.23	NA
K00817	ko00340	Amino acid metabolism	Histidine metabolism	hisC; histidinol-phosphate aminotransferase [EC:2.6.1.9]	4.25	NA	2.96	NA
K00817	ko00350	Amino acid metabolism	Tyrosine metabolism	hisC; histidinol-phosphate aminotransferase [EC:2.6.1.9]	4.25	NA	2.96	NA
K00817	ko00360	Amino acid metabolism	Phenylalanine metabolism	hisC; histidinol-phosphate aminotransferase [EC:2.6.1.9]	4.25	NA	2.96	NA
K00817	ko00400	Amino acid metabolism	Phenylalanine, tyrosine and tryptophan biosynthesis	hisC; histidinol-phosphate aminotransferase [EC:2.6.1.9]	4.25	NA	2.96	NA
K00821	ko00220	Amino acid metabolism	Arginine biosynthesis	argD; acetylornithine/N-succinyldiaminopimelate aminotransferase [EC:2.6.1.11 2.6.1.17]	3.59	NA	3.07	NA
K00821	ko00300	Amino acid metabolism	Lysine biosynthesis	argD; acetylornithine/N-succinyldiaminopimelate aminotransferase [EC:2.6.1.11 2.6.1.17]	3.59	NA	3.07	NA
K00831	ko00260	Amino acid metabolism	Glycine, serine and threonine metabolism	serC, PSAT1; phosphoserine aminotransferase [EC:2.6.1.52]	4.14	NA	3.27	NA
K00832	ko00270	Amino acid metabolism	Cysteine and methionine metabolism	tyrB; aromatic-amino-acid transaminase [EC:2.6.1.57]	3.75	NA	3.42	NA
K00832	ko00350	Amino acid metabolism	Tyrosine metabolism	tyrB; aromatic-amino-acid transaminase [EC:2.6.1.57]	3.75	NA	3.42	NA
K00832	ko00360	Amino acid metabolism	Phenylalanine metabolism	tyrB; aromatic-amino-acid transaminase [EC:2.6.1.57]	3.75	NA	3.42	NA
K00832	ko00400	Amino acid metabolism	Phenylalanine, tyrosine and tryptophan biosynthesis	tyrB; aromatic-amino-acid transaminase [EC:2.6.1.57]	3.75	NA	3.42	NA
K00928	ko00260	Amino acid metabolism	Glycine, serine and threonine metabolism	lysC; aspartate kinase [EC:2.7.2.4]	3.05	NA	2.91	NA

Brackish specialists

Specific response to marine environment

ko	KEGG ortholog	sub-functional category	pathway	gene description	Otu000009_BM	Otu000021_BM	Otu000009_OM	Otu000021_OM
K00928	ko00270	Amino acid metabolism	Cysteine and methionine metabolism	lysC; aspartate kinase [EC:2.7.2.4]	3.05	NA	2.91	NA
K00928	ko00300	Amino acid metabolism	Lysine biosynthesis	lysC; aspartate kinase [EC:2.7.2.4]	3.05	NA	2.91	NA
K00930	ko00220	Amino acid metabolism	Arginine biosynthesis	argB; acetylglutamate kinase [EC:2.7.2.8]	3.75	NA	3.31	NA
K01028	ko00280	Amino acid metabolism	Valine, leucine and isoleucine degradation	E2.8.3.5A, scoA; 3-oxoacid CoA-transferase subunit A [EC:2.8.3.5]	-3.64	NA	-2.31	NA
K01496	ko00340	Amino acid metabolism	Histidine metabolism	hisI; phosphoribosyl-AMP cyclohydrolase [EC:3.5.4.19]	-2.04	NA	-2.43	NA
K01581	ko00330	Amino acid metabolism	Arginine and proline metabolism	E4.1.1.17, ODC1, speC, speF; ornithine decarboxylase [EC:4.1.1.17]	NA	5.03	NA	4.32
K01652	ko00290	Amino acid metabolism	Valine, leucine and isoleucine biosynthesis	E2.2.1.6L, ilvB, ilvG, ilvI; acetolactate synthase I/II/III large subunit [EC:2.2.1.6]	1.83	NA	NA	NA
K01658	ko00400	Amino acid metabolism	Phenylalanine, tyrosine and tryptophan biosynthesis	trpG; anthranilate synthase component II [EC:4.1.3.27]	3.44	NA	3.02	NA
K01704	ko00290	Amino acid metabolism	Valine, leucine and isoleucine biosynthesis	leuD; 3-isopropylmalate/(R)-2-methylmalate dehydratase small subunit [EC:4.2.1.33 4.2.1.35]	3.50	NA	2.91	NA
K01735	ko00400	Amino acid metabolism	Phenylalanine, tyrosine and tryptophan biosynthesis	aroB; 3-dehydroquinate synthase [EC:4.2.3.4]	3.88	NA	2.97	NA
K01755	ko00220	Amino acid metabolism	Arginine biosynthesis	argH, ASL; argininosuccinate lyase [EC:4.3.2.1]	3.85	NA	3.47	NA
K01755	ko00250	Amino acid metabolism	Alanine, aspartate and glutamate metabolism	argH, ASL; argininosuccinate lyase [EC:4.3.2.1]	3.85	NA	3.47	NA
K01756	ko00250	Amino acid metabolism	Alanine, aspartate and glutamate metabolism	purB, ADSL; adenylosuccinate lyase [EC:4.3.2.2]	4.48	NA	3.58	NA
K01929	ko00300	Amino acid metabolism	Lysine biosynthesis	murF; UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase [EC:6.3.2.10]	3.78	NA	3.45	NA
K02502	ko00340	Amino acid metabolism	Histidine metabolism	hisZ; ATP phosphoribosyltransferase regulatory subunit	3.42	NA	3.40	NA
K10217	ko00380	Amino acid metabolism	Tryptophan metabolism	dmpC, xylG; aminomuconate-semialdehyde/2-hydroxymuconate-6-semialdehyde dehydrogenase [EC:1.2.1.32 1.2.1.85]	NA	NA	NA	NA
K14260	ko00220	Amino acid metabolism	Arginine biosynthesis	alaA; alanine-synthesizing transaminase [EC:2.6.1.66 2.6.1.2]	3.98	NA	3.36	NA
K14260	ko00250	Amino acid metabolism	Alanine, aspartate and glutamate metabolism	alaA; alanine-synthesizing transaminase [EC:2.6.1.66 2.6.1.2]	3.98	NA	3.36	NA
K14260	ko00290	Amino acid metabolism	Valine, leucine and isoleucine biosynthesis	alaA; alanine-synthesizing transaminase [EC:2.6.1.66 2.6.1.2]	3.98	NA	3.36	NA
K15371	ko00220	Amino acid metabolism	Arginine biosynthesis	GDH2; glutamate dehydrogenase [EC:1.4.1.2]	1.80	NA	2.33	NA
K15371	ko00250	Amino acid metabolism	Alanine, aspartate and glutamate metabolism	GDH2; glutamate dehydrogenase [EC:1.4.1.2]	1.80	NA	2.33	NA
K15633	ko00260	Amino acid metabolism	Glycine, serine and threonine metabolism	gpmI; 2,3-bisphosphoglycerate-independent phosphoglycerate mutase [EC:5.4.2.12]	4.56	NA	3.39	NA
K17103	ko00260	Amino acid metabolism	Glycine, serine and threonine metabolism	CHO1, pssA; CDP-diacylglycerol---serine O-phosphatidyltransferase [EC:2.7.8.8]	3.29	NA	3.19	NA
K00029	ko00620	Carbohydrate metabolism	Pyruvate metabolism	E1.1.1.40, maeB; malate dehydrogenase (oxaloacetate-decarboxylating)(NADP+) [EC:1.1.1.40]	2.00	NA	1.64	NA
K00036	ko00030	Carbohydrate metabolism	Pentose phosphate pathway	G6PD, zwf; glucose-6-phosphate 1-dehydrogenase [EC:1.1.1.49 1.1.1.363]	3.27	NA	2.07	NA
K00127	ko00630	Carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism	fdoI; formate dehydrogenase subunit gamma	3.96	NA	3.08	NA

Brackish specialists

Specific response to marine environment

ko	KEGG ortholog	sub-functional category	pathway	gene description	Otu000009_BM	Otu000021_BM	Otu000009_OM	Otu000021_OM
K00140	ko00562	Carbohydrate metabolism	Inositol phosphate metabolism	mmsA, iolA, ALDH6A1; malonate-semialdehyde dehydrogenase (acetylating) / methylmalonate-semialdehyde dehydrogenase [EC:1.2.1.18 1.2.1.27]	NA	4.20	-2.04	NA
K00140	ko00640	Carbohydrate metabolism	Propanoate metabolism	mmsA, iolA, ALDH6A1; malonate-semialdehyde dehydrogenase (acetylating) / methylmalonate-semialdehyde dehydrogenase [EC:1.2.1.18 1.2.1.27]	NA	4.20	-2.04	NA
K00164	ko00020	Carbohydrate metabolism	Citrate cycle (TCA cycle)	OGDH, sucA; 2-oxoglutarate dehydrogenase E1 component [EC:1.2.4.2]	1.29	NA	NA	NA
K00241	ko00020	Carbohydrate metabolism	Citrate cycle (TCA cycle)	sdhC, frdC; succinate dehydrogenase / fumarate reductase, cytochrome b subunit	4.70	NA	3.11	NA
K00241	ko00650	Carbohydrate metabolism	Butanoate metabolism	sdhC, frdC; succinate dehydrogenase / fumarate reductase, cytochrome b subunit	4.70	NA	3.11	NA
K00281	ko00630	Carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism	GLDC, gcvP; glycine dehydrogenase [EC:1.4.4.2]	2.65	NA	1.87	NA
K00615	ko00030	Carbohydrate metabolism	Pentose phosphate pathway	E2.2.1.1, tktA, tktB; transketolase [EC:2.2.1.1]	2.18	NA	1.83	NA
K00658	ko00020	Carbohydrate metabolism	Citrate cycle (TCA cycle)	DLST, sucB; 2-oxoglutarate dehydrogenase E2 component (dihydrolipoamide succinyltransferase) [EC:2.3.1.61]	2.30	NA	2.16	NA
K00703	ko00500	Carbohydrate metabolism	Starch and sucrose metabolism	E2.4.1.21, glgA; starch synthase [EC:2.4.1.21]	2.18	NA	1.49	NA
K00873	ko00010	Carbohydrate metabolism	Glycolysis / Gluconeogenesis	PK, pyk; pyruvate kinase [EC:2.7.1.40]	1.91	4.24	1.87	NA
K00873	ko00620	Carbohydrate metabolism	Pyruvate metabolism	PK, pyk; pyruvate kinase [EC:2.7.1.40]	1.91	4.24	1.87	NA
K01007	ko00620	Carbohydrate metabolism	Pyruvate metabolism	pps, ppsA; pyruvate, water dikinase [EC:2.7.9.2]	3.56	NA	3.06	NA
K01028	ko00650	Carbohydrate metabolism	Butanoate metabolism	E2.8.3.5A, scoA; 3-oxoacid CoA-transferase subunit A [EC:2.8.3.5]	-3.64	NA	-2.31	NA
K01091	ko00630	Carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism	gph; phosphoglycolate phosphatase [EC:3.1.3.18]	3.81	NA	2.84	NA
K01187	ko00052	Carbohydrate metabolism	Galactose metabolism	malZ; alpha-glucosidase [EC:3.2.1.20]	3.96	NA	2.89	NA
K01187	ko00500	Carbohydrate metabolism	Starch and sucrose metabolism	malZ; alpha-glucosidase [EC:3.2.1.20]	3.96	NA	2.89	NA
K01433	ko00630	Carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism	purU; formyltetrahydrofolate deformylase [EC:3.5.1.10]	NA	4.22	NA	4.65
K01595	ko00620	Carbohydrate metabolism	Pyruvate metabolism	ppc; phosphoenolpyruvate carboxylase [EC:4.1.1.31]	3.85	NA	3.66	NA
K01637	ko00630	Carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism	E4.1.3.1, aceA; isocitrate lyase [EC:4.1.3.1]	NA	3.09	NA	1.74
K01652	ko00650	Carbohydrate metabolism	Butanoate metabolism	E2.2.1.6L, ilvB, ilvG, ilvI; acetolactate synthase I/II/III large subunit [EC:2.2.1.6]	1.83	NA	NA	NA
K01652	ko00660	Carbohydrate metabolism	C5-Branched dibasic acid metabolism	E2.2.1.6L, ilvB, ilvG, ilvI; acetolactate synthase I/II/III large subunit [EC:2.2.1.6]	1.83	NA	NA	NA
K01676	ko00020	Carbohydrate metabolism	Citrate cycle (TCA cycle)	E4.2.1.2A, fumA, fumB; fumarate hydratase, class I [EC:4.2.1.2]	2.59	4.16	2.49	NA
K01676	ko00620	Carbohydrate metabolism	Pyruvate metabolism	E4.2.1.2A, fumA, fumB; fumarate hydratase, class I [EC:4.2.1.2]	2.59	4.16	2.49	NA
K01681	ko00020	Carbohydrate metabolism	Citrate cycle (TCA cycle)	ACO, acnA; aconitate hydratase [EC:4.2.1.3]	0.96	1.39	NA	NA
K01681	ko00630	Carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism	ACO, acnA; aconitate hydratase [EC:4.2.1.3]	0.96	1.39	NA	NA
K01682	ko00020	Carbohydrate metabolism	Citrate cycle (TCA cycle)	acnB; aconitate hydratase 2 / 2-methylisocitrate dehydratase [EC:4.2.1.3 4.2.1.99]	-1.43	NA	-2.12	NA

Brackish specialists

Specific response to marine environment

ko	KEGG ortholog	sub-functional category	pathway	gene description	Otu000009_BM	Otu000021_BM	Otu000009_OM	Otu000021_OM
K01682	ko00630	Carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism	acnB; aconitate hydratase 2 / 2-methylisocitrate dehydratase [EC:4.2.1.3 4.2.1.99]	-1.43	NA	-2.12	NA
K01682	ko00640	Carbohydrate metabolism	Propanoate metabolism	acnB; aconitate hydratase 2 / 2-methylisocitrate dehydratase [EC:4.2.1.3 4.2.1.99]	-1.43	NA	-2.12	NA
K01704	ko00660	Carbohydrate metabolism	C5-Branched dibasic acid metabolism	leuD; 3-isopropylmalate/(R)-2-methylmalate dehydratase small subunit [EC:4.2.1.33 4.2.1.35]	3.50	NA	2.91	NA
K01711	ko00051	Carbohydrate metabolism	Fructose and mannose metabolism	gmd, GMDS; GDPmannose 4,6-dehydratase [EC:4.2.1.47]	1.63	NA	2.01	NA
K01711	ko00520	Carbohydrate metabolism	Amino sugar and nucleotide sugar metabolism	gmd, GMDS; GDPmannose 4,6-dehydratase [EC:4.2.1.47]	1.63	NA	2.01	NA
K01759	ko00620	Carbohydrate metabolism	Pyruvate metabolism	GLO1, gloA; lactoylglutathione lyase [EC:4.4.1.5]	3.26	4.48	2.97	NA
K01803	ko00010	Carbohydrate metabolism	Glycolysis / Gluconeogenesis	TPI, tpiA; triosephosphate isomerase (TIM) [EC:5.3.1.1]	2.40	NA	1.70	NA
K01803	ko00051	Carbohydrate metabolism	Fructose and mannose metabolism	TPI, tpiA; triosephosphate isomerase (TIM) [EC:5.3.1.1]	2.40	NA	1.70	NA
K01803	ko00562	Carbohydrate metabolism	Inositol phosphate metabolism	TPI, tpiA; triosephosphate isomerase (TIM) [EC:5.3.1.1]	2.40	NA	1.70	NA
K01854	ko00052	Carbohydrate metabolism	Galactose metabolism	glf; UDP-galactopyranose mutase [EC:5.4.99.9]	2.67	NA	1.66	NA
K01854	ko00520	Carbohydrate metabolism	Amino sugar and nucleotide sugar metabolism	glf; UDP-galactopyranose mutase [EC:5.4.99.9]	2.67	NA	1.66	NA
K01895	ko00010	Carbohydrate metabolism	Glycolysis / Gluconeogenesis	ACSS, acs; acetyl-CoA synthetase [EC:6.2.1.1]	4.33	NA	3.52	NA
K01895	ko00620	Carbohydrate metabolism	Pyruvate metabolism	ACSS, acs; acetyl-CoA synthetase [EC:6.2.1.1]	4.33	NA	3.52	NA
K01895	ko00640	Carbohydrate metabolism	Propanoate metabolism	ACSS, acs; acetyl-CoA synthetase [EC:6.2.1.1]	4.33	NA	3.52	NA
K01961	ko00620	Carbohydrate metabolism	Pyruvate metabolism	accC; acetyl-CoA carboxylase, biotin carboxylase subunit [EC:6.4.1.2 6.3.4.14]	2.15	-6.93	NA	-6.42
K01961	ko00640	Carbohydrate metabolism	Propanoate metabolism	accC; acetyl-CoA carboxylase, biotin carboxylase subunit [EC:6.4.1.2 6.3.4.14]	2.15	-6.93	NA	-6.42
K01962	ko00620	Carbohydrate metabolism	Pyruvate metabolism	accA; acetyl-CoA carboxylase carboxyl transferase subunit alpha [EC:6.4.1.2]	2.06	NA	1.86	NA
K01962	ko00640	Carbohydrate metabolism	Propanoate metabolism	accA; acetyl-CoA carboxylase carboxyl transferase subunit alpha [EC:6.4.1.2]	2.06	NA	1.86	NA
K12452	ko00520	Carbohydrate metabolism	Amino sugar and nucleotide sugar metabolism	rfbH; CDP-6-deoxy-D-xylo-4-hexulose-3-dehydrase	-2.82	NA	-3.60	NA
K15633	ko00010	Carbohydrate metabolism	Glycolysis / Gluconeogenesis	gpmI; 2,3-bisphosphoglycerate-independent phosphoglycerate mutase [EC:5.4.2.12]	4.56	NA	3.39	NA
K15778	ko00010	Carbohydrate metabolism	Glycolysis / Gluconeogenesis	pmm-pgm; phosphomannomutase / phosphoglucomutase [EC:5.4.2.8 5.4.2.2]	2.01	NA	1.65	NA
K15778	ko00030	Carbohydrate metabolism	Pentose phosphate pathway	pmm-pgm; phosphomannomutase / phosphoglucomutase [EC:5.4.2.8 5.4.2.2]	2.01	NA	1.65	NA
K15778	ko00051	Carbohydrate metabolism	Fructose and mannose metabolism	pmm-pgm; phosphomannomutase / phosphoglucomutase [EC:5.4.2.8 5.4.2.2]	2.01	NA	1.65	NA
K15778	ko00052	Carbohydrate metabolism	Galactose metabolism	pmm-pgm; phosphomannomutase / phosphoglucomutase [EC:5.4.2.8 5.4.2.2]	2.01	NA	1.65	NA
K15778	ko00500	Carbohydrate metabolism	Starch and sucrose metabolism	pmm-pgm; phosphomannomutase / phosphoglucomutase [EC:5.4.2.8 5.4.2.2]	2.01	NA	1.65	NA
K15778	ko00520	Carbohydrate metabolism	Amino sugar and nucleotide sugar metabolism	pmm-pgm; phosphomannomutase / phosphoglucomutase [EC:5.4.2.8 5.4.2.2]	2.01	NA	1.65	NA

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ko	KEGG ortholog	sub-functional category	pathway	gene description	Otu000009_BM	Otu000021_BM	Otu000009_OM	Otu000021_OM
K01358	ko04112	Cell growth and death	Cell cycle - Caulobacter	clpP, CLPP; ATP-dependent Clp protease, protease subunit [EC:3.4.21.92]	5.10	NA	4.23	NA
K08738	ko04115	Cell growth and death	p53 signaling pathway	CYC; cytochrome c	4.36	3.87	3.15	NA
K08738	ko04210	Cell growth and death	Apoptosis	CYC; cytochrome c	4.36	3.87	3.15	NA
K00029	ko00710	Energy metabolism	Carbon fixation in photosynthetic organisms	E1.1.1.40, maeB; malate dehydrogenase (oxaloacetate-decarboxylating)(NADP+) [EC:1.1.1.40]	2.00	NA	1.64	NA
K00127	ko00680	Energy metabolism	Methane metabolism	fdoI; formate dehydrogenase subunit gamma	3.96	NA	3.08	NA
K00241	ko00190	Energy metabolism	Oxidative phosphorylation	sdhC, frdC; succinate dehydrogenase / fumarate reductase, cytochrome b subunit	4.70	NA	3.11	NA
K00241	ko00720	Energy metabolism	Carbon fixation pathways in prokaryotes	sdhC, frdC; succinate dehydrogenase / fumarate reductase, cytochrome b subunit	4.70	NA	3.11	NA
K00615	ko00710	Energy metabolism	Carbon fixation in photosynthetic organisms	E2.2.1.1, tktA, tktB; transketolase [EC:2.2.1.1]	2.18	NA	1.83	NA
K00831	ko00680	Energy metabolism	Methane metabolism	serC, PSAT1; phosphoserine aminotransferase [EC:2.6.1.52]	4.14	NA	3.27	NA
K00937	ko00190	Energy metabolism	Oxidative phosphorylation	ppk; polyphosphate kinase [EC:2.7.4.1]	2.68	3.46	1.89	NA
K01007	ko00680	Energy metabolism	Methane metabolism	pps, ppsA; pyruvate, water dikinase [EC:2.7.9.2]	3.56	NA	3.06	NA
K01007	ko00720	Energy metabolism	Carbon fixation pathways in prokaryotes	pps, ppsA; pyruvate, water dikinase [EC:2.7.9.2]	3.56	NA	3.06	NA
K01595	ko00680	Energy metabolism	Methane metabolism	ppc; phosphoenolpyruvate carboxylase [EC:4.1.1.31]	3.85	NA	3.66	NA
K01595	ko00710	Energy metabolism	Carbon fixation in photosynthetic organisms	ppc; phosphoenolpyruvate carboxylase [EC:4.1.1.31]	3.85	NA	3.66	NA
K01595	ko00720	Energy metabolism	Carbon fixation pathways in prokaryotes	ppc; phosphoenolpyruvate carboxylase [EC:4.1.1.31]	3.85	NA	3.66	NA
K01676	ko00720	Energy metabolism	Carbon fixation pathways in prokaryotes	E4.2.1.2A, fumA, fumB; fumarate hydratase, class I [EC:4.2.1.2]	2.59	4.16	2.49	NA
K01681	ko00720	Energy metabolism	Carbon fixation pathways in prokaryotes	ACO, acnA; aconitate hydratase [EC:4.2.1.3]	0.96	1.39	NA	NA
K01682	ko00720	Energy metabolism	Carbon fixation pathways in prokaryotes	acnB; aconitate hydratase 2 / 2-methylisocitrate dehydratase [EC:4.2.1.3 4.2.1.99]	-1.43	NA	-2.12	NA
K01803	ko00710	Energy metabolism	Carbon fixation in photosynthetic organisms	TPI, tpiA; triosephosphate isomerase (TIM) [EC:5.3.1.1]	2.40	NA	1.70	NA
K01895	ko00680	Energy metabolism	Methane metabolism	ACSS, acs; acetyl-CoA synthetase [EC:6.2.1.1]	4.33	NA	3.52	NA
K01895	ko00720	Energy metabolism	Carbon fixation pathways in prokaryotes	ACSS, acs; acetyl-CoA synthetase [EC:6.2.1.1]	4.33	NA	3.52	NA
K01961	ko00720	Energy metabolism	Carbon fixation pathways in prokaryotes	accC; acetyl-CoA carboxylase, biotin carboxylase subunit [EC:6.4.1.2 6.3.4.14]	2.15	-6.93	NA	-6.42
K01962	ko00720	Energy metabolism	Carbon fixation pathways in prokaryotes	accA; acetyl-CoA carboxylase carboxyl transferase subunit alpha [EC:6.4.1.2]	2.06	NA	1.86	NA
K02110	ko00190	Energy metabolism	Oxidative phosphorylation	ATPF0C, atpE; F-type H+-transporting ATPase subunit c	-1.53	NA	-2.78	NA
K02110	ko00195	Energy metabolism	Photosynthesis	ATPF0C, atpE; F-type H+-transporting ATPase subunit c	-1.53	NA	-2.78	NA
K02112	ko00190	Energy metabolism	Oxidative phosphorylation	ATPF1B, atpD; F-type H+-transporting ATPase subunit beta [EC:3.6.3.14]	-1.68	-1.83	-2.24	NA
K02112	ko00195	Energy metabolism	Photosynthesis	ATPF1B, atpD; F-type H+-transporting ATPase subunit beta [EC:3.6.3.14]	-1.68	-1.83	-2.24	NA
K08738	ko00920	Energy metabolism	Sulfur metabolism	CYC; cytochrome c	4.36	3.87	3.15	NA

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Specific response to marine environment

ko	KEGG ortholog	sub-functional category	pathway	gene description	Otu000009_BM	Otu000021_BM	Otu000009_OM	Otu000021_OM
K15371	ko00910	Energy metabolism	Nitrogen metabolism	GDH2; glutamate dehydrogenase [EC:1.4.1.2]	1.80	NA	2.33	NA
K15633	ko00680	Energy metabolism	Methane metabolism	gpmI; 2,3-bisphosphoglycerate-independent phosphoglycerate mutase [EC:5.4.2.12]	4.56	NA	3.39	NA
K00208	ko00061	Lipid metabolism	Fatty acid biosynthesis	fabI; enoyl-[acyl-carrier protein] reductase I [EC:1.3.1.9 1.3.1.10]	3.67	4.62	2.86	NA
K01028	ko00072	Lipid metabolism	Synthesis and degradation of ketone bodies	E2.8.3.5A, scoA; 3-oxoacid CoA-transferase subunit A [EC:2.8.3.5]	-3.64	NA	-2.31	NA
K01961	ko00061	Lipid metabolism	Fatty acid biosynthesis	accC; acetyl-CoA carboxylase, biotin carboxylase subunit [EC:6.4.1.2 6.3.4.14]	2.15	-6.93	NA	-6.42
K01962	ko00061	Lipid metabolism	Fatty acid biosynthesis	accA; acetyl-CoA carboxylase carboxyl transferase subunit alpha [EC:6.4.1.2]	2.06	NA	1.86	NA
K05297	ko00071	Lipid metabolism	Fatty acid degradation	E1.18.1.1; rubredoxin-NAD+ reductase [EC:1.18.1.1]	2.80	NA	2.79	NA
K17103	ko00564	Lipid metabolism	Glycerophospholipid metabolism	CHO1, pssA; CDP-diacylglycerol---serine O-phosphatidyltransferase [EC:2.7.8.8]	3.29	NA	3.19	NA
K02040	ko02010	Membrane transport	ABC transporters	pstS; phosphate transport system substrate-binding protein	NA	-1.30	0.81	-1.50
K02065	ko02010	Membrane transport	ABC transporters	mlaF, linL, mkl; phospholipid/cholesterol/gamma-HCH transport system ATP-binding protein	3.11	NA	2.92	NA
K03075	ko03070	Membrane transport	Bacterial secretion system	secG; preprotein translocase subunit SecG	4.32	NA	3.05	NA
K03106	ko03070	Membrane transport	Bacterial secretion system	SRP54, ffh; signal recognition particle subunit SRP54	3.40	NA	2.40	NA
K08484	ko02060	Membrane transport	Phosphotransferase system (PTS)	PTS-EI.PTSP, ptsP; phosphotransferase system, enzyme I, PtsP [EC:2.7.3.9]	3.53	3.25	3.21	NA
K11085	ko02010	Membrane transport	ABC transporters	msbA; ATP-binding cassette, subfamily B, bacterial MsbA [EC:3.6.3.-]	3.88	NA	2.93	NA
K11720	ko02010	Membrane transport	ABC transporters	lptG; lipopolysaccharide export system permease protein	-2.22	NA	-2.67	NA
K02338	ko03030	Replication and repair	DNA replication	DPO3B, dnaN; DNA polymerase III subunit beta [EC:2.7.7.7]	4.34	NA	3.52	NA
K02338	ko03430	Replication and repair	Mismatch repair	DPO3B, dnaN; DNA polymerase III subunit beta [EC:2.7.7.7]	4.34	NA	3.52	NA
K02338	ko03440	Replication and repair	Homologous recombination	DPO3B, dnaN; DNA polymerase III subunit beta [EC:2.7.7.7]	4.34	NA	3.52	NA
K03553	ko03440	Replication and repair	Homologous recombination	recA; recombination protein RecA	-0.97	NA	-1.38	NA
K01759	ko04011	Signal transduction	MAPK signaling pathway - yeast	GLO1, gloA; lactoylglutathione lyase [EC:4.4.1.5]	3.26	4.48	2.97	NA
K02040	ko02020	Signal transduction	Two-component system	pstS; phosphate transport system substrate-binding protein	NA	-1.30	0.81	-1.50
K02556	ko02020	Signal transduction	Two-component system	motA; chemotaxis protein MotA	NA	4.40	NA	4.69
K04771	ko02020	Signal transduction	Two-component system	degP, htrA; serine protease Do [EC:3.4.21.107]	4.27	NA	3.09	NA
K07665	ko02020	Signal transduction	Two-component system	cusR, copR, silR; two-component system, OmpR family, copper resistance phosphate regulon response regulator CusR	4.09	NA	3.79	NA
K08738	ko02020	Signal transduction	Two-component system	CYC; cytochrome c	4.36	3.87	3.15	NA
K18093	ko02020	Signal transduction	Two-component system	oprD; imipenem/basic amino acid-specific outer membrane pore [EC:3.4.21.-]	1.49	NA	1.26	NA
K01886	ko00970	Translation	Aminoacyl-tRNA biosynthesis	QARS, glnS; glutaminyl-tRNA synthetase [EC:6.1.1.18]	-1.18	NA	-1.40	NA
K02871	ko03010	Translation	Ribosome	RP-L13, MRPL13, rplM; large subunit ribosomal protein L13	4.17	NA	3.90	NA
K02876	ko03010	Translation	Ribosome	RP-L15, MRPL15, rplO; large subunit ribosomal protein L15	3.68	NA	3.23	NA
K02881	ko03010	Translation	Ribosome	RP-L18, MRPL18, rplR; large subunit ribosomal protein L18	4.92	-3.03	3.98	NA
K02884	ko03010	Translation	Ribosome	RP-L19, MRPL19, rplS; large subunit ribosomal protein L19	4.52	NA	4.10	NA

Brackish specialists

Specific response to marine environment

ko	KEGG ortholog	sub-functional category	pathway	gene description	Otu000009_BM	Otu000021_BM	Otu000009_OM	Otu000021_OM
K02887	ko03010	Translation	Ribosome	RP-L20, MRPL20, rplT; large subunit ribosomal protein L20	4.21	NA	4.09	NA
K02888	ko03010	Translation	Ribosome	RP-L21, MRPL21, rplU; large subunit ribosomal protein L21	NA	2.46	-1.28	NA
K02895	ko03010	Translation	Ribosome	RP-L24, MRPL24, rplX; large subunit ribosomal protein L24	3.16	NA	1.96	NA
K02899	ko03010	Translation	Ribosome	RP-L27, MRPL27, rpmA; large subunit ribosomal protein L27	2.89	NA	3.74	NA
K02902	ko03010	Translation	Ribosome	RP-L28, MRPL28, rpmB; large subunit ribosomal protein L28	3.06	NA	1.86	NA
K02906	ko03010	Translation	Ribosome	RP-L3, MRPL3, rplC; large subunit ribosomal protein L3	NA	2.78	NA	2.81
K02909	ko03010	Translation	Ribosome	RP-L31, rpmE; large subunit ribosomal protein L31	2.41	NA	3.35	NA
K02914	ko03010	Translation	Ribosome	RP-L34, MRPL34, rpmH; large subunit ribosomal protein L34	4.72	NA	3.80	NA
K02926	ko03010	Translation	Ribosome	RP-L4, MRPL4, rplD; large subunit ribosomal protein L4	3.59	NA	3.01	NA
K02935	ko03010	Translation	Ribosome	RP-L7, MRPL12, rplL; large subunit ribosomal protein L7/L12	1.92	-1.91	2.86	NA
K02939	ko03010	Translation	Ribosome	RP-L9, MRPL9, rplI; large subunit ribosomal protein L9	3.77	NA	3.69	NA
K02952	ko03010	Translation	Ribosome	RP-S13, rpsM; small subunit ribosomal protein S13	3.31	NA	2.49	NA
K02954	ko03010	Translation	Ribosome	RP-S14, MRPS14, rpsN; small subunit ribosomal protein S14	2.15	NA	2.19	NA
K02961	ko03010	Translation	Ribosome	RP-S17, MRPS17, rpsQ; small subunit ribosomal protein S17	4.45	NA	4.42	NA
K02965	ko03010	Translation	Ribosome	RP-S19, rpsS; small subunit ribosomal protein S19	-2.57	NA	-1.55	NA
K02990	ko03010	Translation	Ribosome	RP-S6, MRPS6, rpsF; small subunit ribosomal protein S6	2.60	NA	2.56	NA
K02992	ko03010	Translation	Ribosome	RP-S7, MRPS7, rpsG; small subunit ribosomal protein S7	1.88	NA	1.66	NA

Table S8 The specific response of the marine specialists to each of the three incubation environments by illustrating the numbers of genes that were shared between the transcriptome dataset pairs. The capital letters following the OTU ID indicate the two environments between which the gene expressions were compared, and the first capital letter indicates that the environment to which the given gene was significantly regulated as a response. The data were presented in fold change of the gene (relative) abundance for the comparisons and the positive and negative fold change indicate the up- or down-regulation of the given genes. Only the genes belonging to the 7 functional clusters are listed.

Specific response to marine environment			Marine specialists			
ko	KEGG ortholog	sub-functional category	pathway	gene description	Otu000033_BM	Otu000033_OM
K00265	ko00250	Amino acid metabolism	Alanine, aspartate and glutamate metabolism	gltB; glutamate synthase (NADPH/NADH) large chain [EC:1.4.1.13 1.4.1.14]	-5.07	-6.07
K00605	ko00260	Amino acid metabolism	Glycine, serine and threonine metabolism	gcvT, AMT; aminomethyltransferase [EC:2.1.2.10]	-5.76	-6.75
K01738	ko00270	Amino acid metabolism	Cysteine and methionine metabolism	cysK; cysteine synthase A [EC:2.5.1.47]	-5.20	-4.93
K00605	ko00630	Carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism	gcvT, AMT; aminomethyltransferase [EC:2.1.2.10]	-5.76	-6.75
K00265	ko00910	Energy metabolism	Nitrogen metabolism	gltB; glutamate synthase (NADPH/NADH) large chain [EC:1.4.1.13 1.4.1.14]	-5.07	-6.07
K01738	ko00920	Energy metabolism	Sulfur metabolism	cysK; cysteine synthase A [EC:2.5.1.47]	-5.20	-4.93
K05813	ko02010	Membrane transport	ABC transporters	ugpB; sn-glycerol 3-phosphate transport system substrate-binding protein	-6.35	-5.83
K15580	ko02010	Membrane transport	ABC transporters	oppA, mppA; oligopeptide transport system substrate-binding protein	-6.13	-7.16

Table S9 Full gene names for the top 10 significantly expressed genes shown in Table 4

Gene abbreviation	Full description
aapQ, bztB	general L-amino acid transport system permease protein
aapM, bztC	general L-amino acid transport system permease protein
aapJ, bztA	general L-amino acid transport system substrate-binding protein
aarC	succinyl-CoA:acetate CoA-transferase;
ABC-2.CPSE.P	capsular polysaccharide transport system permease protein
accC	acetyl-CoA carboxylase, biotin carboxylase subunit
aceB	malate synthase; alaA, alanine-synthesizing transaminase
atzF	allophanate hydrolase
arcA	arginine deiminase;
atpE	F-type H+-transporting ATPase subunit c
atpF	F-type H+-transporting ATPase subunit b
atpH	F-type H+-transporting ATPase subunit delta
aptA	F-type H+-transporting ATPase subunit alpha
aroF, aroG, aroH	3-deoxy-7-phosphoheptulonate synthase
aor	aldehyde:ferredoxin oxidoreductase
argG	argininosuccinate synthase
adiA	arginine decarboxylase
bkdA1	2-oxoisovalerate dehydrogenase E1 component alpha subunit
bdh	3-hydroxybutyrate dehydrogenase
bshA	L-malate glycosyltransferase
carA	carbamoyl-phosphate synthase small subunit
cysK	cysteine synthase A
codA	cytosine deaminase
ccoN	cytochrome c oxidase cbb3-type subunit I
clpP	ATP-dependent Clp protease, protease subunit
COX11	cytochrome c oxidase assembly protein subunit 11
ccoO	cytochrome c oxidase cbb3-type subunit II
ccoP	cytochrome c oxidase cbb3-type subunit III
CYC	cytochrome c
dapC	N-succinyldiaminopimelate aminotransferase
DPO3E, dnaQ	DNA polymerase III subunit epsilon
dnaG	DNA primase
SCD, desC	stearoyl-CoA desaturase (Delta-9 desaturase);
dppA	dipeptide transport system substrate-binding protein
FAD6, desA,	acyl-lipid omega-6 desaturase (Delta-12 desaturase);

Gene abbreviation	Full description
cusS, copS, silS	two-component system, OmpR family, heavy metal sensor histidine kinase CusS
degP, htrA	serine protease Do
eno	enolase
eutB	ethanolamine ammonia-lyase large subunit
eutC	ethanolamine ammonia-lyase small subunit
exaA	alcohol dehydrogenase (cytochrome c)
E1.1.1.3	homoserine dehydrogenase
E3.5.2.10	creatinine amidohydrolase
E6.3.4.6	urea carboxylase
fadD	long-chain acyl-CoA synthetase
fabI	enoyl-[acyl-carrier protein] reductase I
fabF	3-oxoacyl-[acyl-carrier-protein] synthase II
fdoG, fdhF	formate dehydrogenase major subunit
fadJ	3-hydroxyacyl-CoA dehydrogenase / enoyl-CoA hydratase / 3-hydroxybutyryl-CoA epimerase
fadE	acyl-CoA dehydrogenase
fadZ	3-hydroxyacyl-[acyl-carrier-protein] dehydratase
fdoG, fdhF	formate dehydrogenase major subunit
fliA	RNA polymerase sigma factor for flagellar operon FliA
ftsQ	cell division protein FtsQ
ftsZ	cell division protein FtsZ
glcB	malate synthase
gcvT, AMT	aminomethyltransferase
gltB	glutamate synthase (NADPH/NADH) large chain
gdhA	glutamate dehydrogenase (NAD(P)+)
glgP	glycogen phosphorylase
gloA	lactoylglutathione lyase
glpK	glycerol kinase
glgC	glucose-1-phosphate adenylyltransferase
htpG	molecular chaperone HtpG
ivd	isovaleryl-CoA dehydrogenase
icd	isocitrate dehydrogenase
katE, CAT, catB, srpA	catalase
lacZ	beta-galactosidase
iucD	lysine N6-hydroxylase
leuA	2-isopropylmalate synthase

Table S9 Continued

Gene abbreviation	Full description
ligC	2-hydroxy-4-carboxymuconate semialdehyde hemiacetal dehydrogenase
MGD	1,2-diacylglycerol 3-beta-galactosyltransferase
motA	chemotaxis protein MotA
metE	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase
manC, cpsB,	mannose-1-phosphate guanylyltransferase
malQ	4-alpha-glucanotransferase
murA	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
mgsA	methylglyoxal synthase
mcp	methyl-accepting chemotaxis protein
nrtA, nasF, cynA	nitrate/nitrite transport system substrate-binding protein
nuoA	NADH-quinone oxidoreductase subunit A
nuoL	NADH-quinone oxidoreductase subunit L
nuoM	NADH-quinone oxidoreductase subunit M
nuoG	NADH-quinone oxidoreductase subunit G
nrtB	nitrate/nitrite transport system permease protein
nrtC, nasD	nitrate/nitrite transport system ATP-binding protein
ndh	NADH dehydrogenase
oppA, mppA	oligopeptide transport system substrate-binding protein
pyrB	aspartate carbamoyltransferase catalytic subunit
phbC, phaC,	polyhydroxyalkanoate synthase
pheS	phenylalanyl-tRNA synthetase alpha chain
potF	putrescine transport system substrate-binding protein
pstS	phosphate transport system substrate-binding protein
phnE	phosphonate transport system permease protein
plsX	glycerol-3-phosphate acyltransferase PlsX
petC	ubiquinol-cytochrome c reductase cytochrome c1 subunit
phaZ	poly(3-hydroxybutyrate) depolymerase
phbB	acetoacetyl-CoA reductase
phoA, phoB	alkaline phosphatase
phoD	alkaline phosphatase D
phoR	phosphate regulon sensor histidine kinase PhoR
ppk	polyphosphate kinase
psd	phosphatidylserine decarboxylase
stS	phosphate transport system substrate-binding protein

Gene abbreviation	Full description
pucA	light-harvesting protein B-800-850 alpha chain
pucB	light-harvesting protein B-800-850 beta chain
pufB	light-harvesting complex 1 beta chain
puhA	photosynthetic reaction center H subunit
rplJ	large subunit ribosomal protein L10
rpmE	large subunit ribosomal protein
rplM	large subunit ribosomal protein L13
rplC	large subunit ribosomal protein L3
rpsN	small subunit ribosomal protein S14
rplX	large subunit ribosomal protein L24
rplQ	large subunit ribosomal protein L17
rplL	large subunit ribosomal protein L7/L12
rpsP	small subunit ribosomal protein S16
rplR	large subunit ribosomal protein L18
rpmH	large subunit ribosomal protein L34
rplS	large subunit ribosomal protein L19
rplO	large subunit ribosomal protein L15
rpsC	small subunit ribosomal protein S3
rpoZ	DNA-directed RNA polymerase subunit omega
rplW	large subunit ribosomal protein L23
rplP	large subunit ribosomal protein L16
sdhC, frdC	succinate dehydrogenase / fumarate reductase, cytochrome b subunit
secE,	preprotein translocase subunit SecE
speC, speF	ornithine decarboxylase
spuC	putrescine---pyruvate transaminase
tatA	sec-independent protein translocase protein TatA
tolC	aromatic-amino-acid transaminase
tyrB	aromatic-amino-acid transaminase
ugpB	sn-glycerol 3-phosphate transport system substrate-binding protein
ugpC	sn-glycerol 3-phosphate transport system ATP-binding protein
urtA	urea transport system substrate-binding protein
urtB	urea transport system permease protein
urtE	urea transport system ATP-binding protein
valS	valyl-tRNA synthetase
yidC, OXA1	YidC/Oxa1 family membrane protein insertase

Table S10 Nutrient content, microbial cell abundances, number of sequence reads (after removal of the singletons) and bacterial community diversity metrics in each biological replicate of the initial inocula (day 0) and in the microcosms at the end of the expeirment (day 5). Capital letters B and M refer to the incubation environment, while lower case letters b and m refer to the source (origin) of the initial microbial inoculum. ND and DT indicate non-dispersal and dispersal treatments, respectively; and the terminal number represents the biological replicate. Abbreviations: psu: practical salinity unit; DOC: dissolved organic carbon. "NA" indicates that no data were available for the measurement. The detection limit for colorimetric nutrient measurements is: NO_3^-, 0.05 µM; NO_2^-, 0.01 µM; PO_4^{3-}, 0.03 µM; NH_4^+, 0.1 µM after Strickland and Parsons (1972).

Sample ID	Salinity (psu)	NO_3^- (µM)	NO_2^- (µM)	PO_4^{3-} (µM)	NH_4^+ (µM)	SiO_2^- (µM)	DOC (µM)	Bacteria x 10^6 (cells ml^{-1})	Protists x 10^3 (cells ml^{-1})	No. reads	Alpha diversity	Richness	Evenness
Initial_b1	24.29	11.71	0.19	0.87	0.1	16.91	157.6	0.87	1.85	22544	4.52	590.24	0.64
Initial_b2	NA	NA	NA	NA	NA	NA	NA	0.98	1.62	23646	4.59	612.04	0.65
Initial_b3	NA	NA	NA	NA	NA	NA	NA	1.04	1.48	29380	4.63	630.03	0.65
Initial_m1	30.35	1.09	0.07	0.03	0.1	1.84	107	0.87	2.86	23552	3.57	323.25	0.55
Initial_m2	NA	NA	NA	NA	NA	NA	NA	0.87	3.76	28499	3.49	313.35	0.54
Initial_m3	NA	NA	NA	NA	NA	NA	NA	0.9	3.68	22369	3.47	320.52	0.54
Bb_ND1	24.59	1.33	0.09	0.3	0.1	13.21	154.05	1.91	12.08	36177	3.76	502.2	0.54
Bb_ND2	25	0.98	0.01	0.26	0.1	12.49	148.05	1.9	7.08	11877	3.56	391	0.53
Bm_ND1	24.59	1.05	0.06	0.39	0.1	12.41	149.2	3.09	1.87	25925	3.24	456.67	0.48
Bm_ND2	25	0.78	0.05	0.2	0.1	12.49	149.9	3.94	1.89	12980	3.19	397.69	0.48
Bm_ND3	24.78	1.12	0.06	0.35	0.1	12.79	149.25	3.53	1.66	19957	3.31	548.31	0.47
Bb_DT1	25.28	2.78	0.01	0.28	0.1	12.62	135.3	1.83	14.6	22494	3.85	582.87	0.55
Bb_DT2	25.2	1.56	0.01	0.24	0.1	12.56	131.2	2.21	21.22	28511	3.71	571.91	0.53
Bb_DT3	25.2	2.68	0.01	0.33	0.1	12.84	132.8	1.51	18.82	32284	3.95	686.88	0.55
Bm_DT1	25.28	2.09	0.07	0.67	0.1	15.47	144.3	3.11	3.33	13594	3.88	612.56	0.55
Bm_DT2	25.2	2.07	0.09	0.44	0.1	13.37	136.3	3.24	2.49	37647	3.91	576.24	0.55
Bm_DT3	25.2	1.71	0.06	0.5	0.1	12.7	132.5	2.71	4.84	26026	3.78	739.64	0.52
Mb_ND1	29.32	0.68	0.01	0.03	0.1	3.28	117.85	0.4	5.48	15761	4.16	744.92	0.57
Mb_ND2	29.07	0.62	0.01	0.03	0.1	3.26	113.7	0.4	6.45	14950	4.01	644.43	0.56
Mb_ND3	29.27	0.69	0.01	0.11	0.1	3.32	115.85	0.48	5.45	41150	3.89	723.31	0.53
Mm_ND1	29.34	0.64	0.01	0.11	0.1	3.32	115.7	2.52	1.46	11074	3.68	445.6	0.54
Mm_ND2	29.27	0.64	0.01	0.03	0.1	3.1	117.35	2.19	1	39650	3.55	412.91	0.53
Mm_ND3	29.27	0.6	0.01	0.03	0.1	3.13	121.45	2.31	1.28	16192	3.4	316.18	0.53

Sample ID	Salinity (psu)	NO_3^- (μM)	NO_2^- (μM)	PO_4^{3-} (μM)	NH_4^+ (μM)	SiO_2^- (μM)	DOC (μM)	Bacteria x 10^6 (cells ml^{-1})	Protists x 10^3 (cells ml^{-1})	No. reads	Alpha diversity	Richness	Evenness
Mb_DT1	29.45	1.01	0.01	0.03	0.1	3.58	109.33	0.59	5.14	19803	4.15	641.48	0.58
Mb_DT2	29.78	0.96	0.01	0.03	0.1	3.46	108.55	0.75	5.98	19990	4.04	719.85	0.56
Mb_DT3	29.78	1.18	0.01	0.03	0.1	3.36	105.05	0.61	4.96	21006	4.04	690.55	0.56
Mm_DT1	30.1	1.08	0.1	0.13	0.1	27.06	118	2.05	2.46	18370	3.66	537.54	0.52
Mm_DT2	29.75	1.16	0.01	0.11	0.1	5.96	104.95	2.58	2.14	29062	3.4	451.97	0.5
Mm_DT3	30.1	1.01	0.01	0.03	0.1	3.75	113.95	2.31	1.81	21095	3.52	393.69	0.53

Table S11 The results of three-way ANOVAs testing the effect of dispersal, incubation environment, inoculum source and their interactions on microbial abundance and the bacterial community diversity.

	Bacterial abundance		Protist abundance		Alpha diversity		Richness		Evenness	
Factors	F	*P*-value	F	*P*-value	F	*P*-value	F	*P*-value	F	*P*-value
Dispersal	0.93	ns	40.88	***	20.42	***	10.80	**	8.38	*
Incubation environment (IncE)	128.78	***	45.71	***	10.35	**	0.21	ns	13.91	**
Inoculum source (InoS)	224.57	***	293.32	***	59.25	***	21.79	***	34.01	***
Dispersal x IncE	3.14	.	6.85	*	16.53	**	5.39	*	11.68	**
Dispersal x InoS	2.90	ns	3.04	ns	3.77	.	1.41	ns	2.21	ns
IncE x InoS	3.51	.	6.55	*	12.33	**	26.05	***	0.21	ns
Dispersal x IncE x InoS	0.21	ns	3.64	.	6.98	*	0.52	ns	15.22	**

Abbreviation: IncE, incubation environment; InoS, inoculum source; ns, not significant.

Significance codes : '***' $P < 0.001$; '**' $P < 0.01$; '*' $P < 0.05$; '.' $P < 0.1$.

Table S12 PERMANOVA tests showing variance (R^2) explained by dispersal, incubation environment, inoculum source and their interactions in the community composition among all microcosms (**A**) and among the microcosms with the brackish and marine inoculum sources separately (**B**).

A.

	PERMANOVA test	
	Variance (R^2) explained	*P*-value
Dispersal	3.50%	*
Inocubation Environment (IncE)	9.32%	**
Inoculum Source (InoS)	52.23%	***
Dispesal x IncE	2.92%	.
Dispersal x InoS	5.59%	*
IncE x InoS	6.62%	**
Dispesal x IncE x InoS	1.25%	ns

B.

	PERMANOVA test			
	Brackish inoculum source		Marine inoculum source	
	Variance (R^2) explained	*P*-value	Variance (R^2) explained	*P*-value
Dispersal	17.66%	*	18.52%	**
Incubation Environment (IncE)	37.09%	**	31.48%	***
Dispesal x IncE	3.82%	ns	14.35%	*

Abbreviation: IncE, incubation environment; InoS, inoculum source; ns, not significant.

Significance codes : '***' $P < 0.001$; '**' $P < 0.01$; '*' $P < 0.05$; '.' $P < 0.1$.
Number of permutations: 999

Table S13 Explanatory values of environmental variables to differences in community structure along NMDS axes for all microcosms. Factors significant at $P < 0.05$ are in bold.

	NMDS1	NMDS2	R^2	*P*-value	
Salinity	**0.130**	**0.992**	**0.727**	**0.001**	***
NO_3^-	**0.432**	**-0.902**	**0.447**	**0.003**	**
NO_2^-	-0.277	-0.961	0.155	0.18	ns
PO_4^{3-}	**-0.052**	**-0.999**	**0.496**	**0.002**	***
NH_4^+	0.000	0.000	0.000	1	ns
SiO_2^-	**0.039**	**-0.999**	**0.358**	**0.015**	*
DOC	**-0.277**	**-0.961**	**0.772**	**0.001**	***
PA	**-0.791**	**-0.611**	**0.564**	**0.001**	***

Significance codes : '***' $P < 0.001$; '**' $P < 0.01$; '*' $P < 0.05$; '.' $P < 0.1$.

Number of permutations: 999

Table S14 Taxonomic affiliation and maximal relative abundance of the abundant OTUs (mean relative abundance > 1% in any microcosm). The numbers in the brackets indicate the proportion of the sequences for an OTU that was classified as being members of the given taxonomy. "Microcosm ID" refers to the microcosm in which the maximal relative abundance was detected.

OTU ID	Phylum	Class	Order	Family	Genus	Max.abun %	Microcosm ID
Otu000001	Proteobacteria(100)	Gammaproteobacteria(100)	Vibrionales(100)	Vibrionaceae(100)	Vibrio(95)	18.34	Bm_ND
Otu000002	Proteobacteria(100)	Gammaproteobacteria(100)	Vibrionales(100)	Vibrionaceae(100)	unclassified(82)	14.67	Bm_ND
Otu000003	Proteobacteria(100)	Epsilonproteobacteria(100)	Campylobacterales(100)	Campylobacteraceae(100)	Arcobacter(100)	19.85	Bb_ND
Otu000004	Proteobacteria(100)	Gammaproteobacteria(100)	unclassified(100)	unclassified(100)	unclassified(100)	11.41	Bb_DT
Otu000005	Proteobacteria(100)	Gammaproteobacteria(100)	Oceanospirillales(100)	Oceanospirillaceae(100)	Marinomonas(100)	7.64	Bm_ND
Otu000006	Proteobacteria(100)	Gammaproteobacteria(100)	Oceanospirillales(100)	Oceanospirillaceae(100)	Pseudospirillum(100)	5.17	Mm_ND
Otu000007	Proteobacteria(100)	Gammaproteobacteria(100)	Cellvibrionales(100)	Cellvibrionaceae(100)	Simiduia(100)	8.57	Bb_DT
Otu000008	Proteobacteria(100)	Alphaproteobacteria(100)	Rhodobacterales(100)	Rhodobacteraceae(100)	Ascidiaceihabitans(78)	6.42	Mm_ND
Otu000009	Proteobacteria(100)	Gammaproteobacteria(100)	Alteromonadales(100)	Colwelliaceae(100)	Colwellia(100)	4.87	Bm_ND
Otu000010	Proteobacteria(100)	Deltaproteobacteria(100)	Bdellovibrionales(100)	Bdellovibrionaceae(100)	OM27_clade(100)	6.56	Mb_ND
Otu000012	Proteobacteria(100)	Alphaproteobacteria(100)	Rhodobacterales(100)	Rhodobacteraceae(100)	Planktomarina(97)	2.47	Mm_DT
Otu000013	Proteobacteria(100)	Gammaproteobacteria(100)	Alteromonadales(100)	Pseudoalteromonadaceae(100)	Algicola(100)	3.31	Bm_DT
Otu000014	Bacteroidetes(100)	Cytophagia(100)	Cytophagales(100)	Flammeovirgaceae(100)	Reichenbachiella(100)	5.85	Mm_DT
Otu000015	Proteobacteria(100)	Alphaproteobacteria(100)	Rickettsiales(100)	SAR116_clade(100)	Candidatus_Puniceispirillum(100)	2.14	Bb_ND
Otu000016	Proteobacteria(100)	Alphaproteobacteria(100)	Rhodobacterales(100)	Rhodobacteraceae(100)	Sulfitobacter(75)	4.43	Mb_ND
Otu000017	Proteobacteria(100)	Alphaproteobacteria(100)	Rhodobacterales(100)	Rhodobacteraceae(100)	Celeribacter(97)	6.81	Mm_ND
Otu000018	Proteobacteria(100)	Alphaproteobacteria(100)	SAR11_clade(100)	Surface_1(100)	Candidatus_Pelagibacter(93)	1.05	Mb_DT
Otu000019	Proteobacteria(100)	Gammaproteobacteria(100)	Alteromonadales(100)	Colwelliaceae(100)	Thalassotalea(100)	4.59	Mb_DT
Otu000020	Proteobacteria(100)	Gammaproteobacteria(100)	Cellvibrionales(100)	Cellvibrionaceae(100)	Simiduia(100)	5.83	Bb_DT
Otu000021	Bacteroidetes(100)	Flavobacteriia(100)	Flavobacteriales(100)	Flavobacteriaceae(100)	NS3a_marine_group(100)	3.19	Mm_ND
Otu000022	Proteobacteria(100)	Gammaproteobacteria(100)	Alteromonadales(100)	Alteromonadaceae(100)	Alteromonas(95)	2.92	Mb_ND
Otu000023	Bacteroidetes(100)	Flavobacteriia(100)	Flavobacteriales(100)	Flavobacteriaceae(100)	Polaribacter(93)	2.46	Bb_DT
Otu000025	Proteobacteria(100)	Gammaproteobacteria(100)	Pseudomonadales(100)	Pseudomonadaceae(100)	Pseudomonas(89)	2.22	Mb_DT
Otu000027	Proteobacteria(100)	Gammaproteobacteria(100)	Cellvibrionales(100)	Cellvibrionaceae(100)	Simiduia(100)	2.7	Mb_ND

OTU ID	Phylum	Class	Order	Family	Genus	Max.abun %	Microcosm ID
Otu000028	Bacteroidetes(100)	Flavobacteriia(100)	Flavobacteriales(100)	Flavobacteriaceae(100)	Dokdonia(94)	2.74	Bb_ND
Otu000029	Proteobacteria(100)	Gammaproteobacteria(100)	Alteromonadales(100)	Pseudoalteromonadaceae(100)	Pseudoalteromonas(100)	2.27	Mb_ND
Otu000031	Proteobacteria(100)	Gammaproteobacteria(100)	Alteromonadales(100)	Colwelliaceae(100)	unclassified(100)	1.52	Bb_ND
Otu000033	Proteobacteria(100)	Deltaproteobacteria(100)	Desulfuromonadales(100)	GR-WP33-58(100)	unclassified(100)	2.23	Bb_ND
Otu000034	Bacteroidetes(100)	Flavobacteriia(100)	Flavobacteriales(100)	Flavobacteriaceae(100)	unclassified(86)	1.17	Mm_ND
Otu000035	Proteobacteria(100)	Deltaproteobacteria(100)	Desulfuromonadales(100)	GR-WP33-58(100)	unclassified(100)	1.53	Mb_DT
Otu000038	Bacteroidetes(100)	Flavobacteriia(100)	Flavobacteriales(100)	Flavobacteriaceae(100)	Leeuwenhoekiella(100)	2.18	Mm_ND
Otu000039	Proteobacteria(100)	Alphaproteobacteria(100)	Rickettsiales(100)	T9d(100)	unclassified(100)	1.38	Mb_ND
Otu000041	Proteobacteria(100)	Deltaproteobacteria(100)	Desulfuromonadales(100)	GR-WP33-58(99)	unclassified(99)	2.05	Bm_DT
Otu000042	Proteobacteria(100)	Gammaproteobacteria(100)	Alteromonadales(100)	Colwelliaceae(100)	Thalassotalea(100)	1.19	Mb_ND
Otu000043	Proteobacteria(100)	Alphaproteobacteria(100)	Rhodobacterales(100)	Rhodobacteraceae(100)	Citreicella(80)	1.45	Mm_ND
Otu000088	Bacteroidetes(100)	Cytophagia(100)	Cytophagales(100)	Flammeovirgaceae(100)	Reichenbachiella(95)	1.75	Mb_DT

Table S15 The results of three-way ANOVAs testing the effect of dispersal, incubation environment, inoculum source and their interactions on abundances of the main bacterial phyla/classes and their subgroups (bacterial orders and / or families).

A. Alphaproteobacteria and subgroups

	Alproteobacteria		**SAR11 clade**		**Rhodobacteraceae**		**SAR116 clade**	
Factors	F	*P*-value	F	*P*-value	F	*P*-value	F	*P*-value
Dispersal	1.43	ns	0.04	ns	0.16	ns	0.10	ns
Incubation Environment (IncE)	46.43	***	12.14	**	33.34	***	0.60	ns
Inoculum Source (InoS)	28.72	***	20.91	***	57.99	***	0.01	ns
Dispersal x IncE	0.17	ns	3.17	.	0.28	ns	0.42	ns
Dispersal x InoS	6.39	*	0.78	ns	10.21	**	0.82	ns
IncE x InoS	7.05	*	0.01	ns	6.35	*	1.55	ns
Dispersal x IncE x InoS	3.06	ns	5.65	*	1.18	ns	0.06	ns

B. Deltaproteobacteria and subgroup

	Deltaproteobacteria		**Bdellovibrionaceae**	
Factors	F	*P*-value	F	*P*-value
Dispersal	61.21	***	54.58	***
Incubation Environment (IncE)	2.88	ns	0.29	ns
Inoculum Source (InoS)	155.70	***	155.43	***
Dispersal x IncE	12.41	**	10.47	**
Dispersal x InoS	44.16	***	28.86	***
IncE x InoS	16.93	***	24.38	***
Dispersal x IncE x InoS	3.13	.	0.00	ns

C. Epsilonproteobacteria and subgroup

	Epsilonproteobacteria		**Campylobacteraceae**	
Factors	F	*P*-value	F	*P*-value
Dispersal	0.07	ns	0.07	ns
Incubation Environment (IncE)	2.24	ns	2.23	ns
Inoculum Source (InoS)	5.44	*	5.46	*
Dispersal x IncE	1.73	ns	1.77	ns
Dispersal x InoS	5.21	*	5.23	*
IncE x InoS	0.03	ns	0.03	ns
Dispersal x IncE x InoS	0.08	ns	0.07	ns

D. Gammaproteobacteria and subgroups

	Gammaproteobacteria		**Cellvibrionaceae**		**Colwelliaceae**		**Oceanospirillaceae**		**Vibrionaceae**	
Factors	F	*P*-value	F	*P*-value	F	*P*-value	F	*P*-value	F	*P*-value
Dispersal	0.43	ns	0.71	ns	0.01	ns	8.78	**	3.32	.
Incubation Environment (IncE)	14.97	**	25.39	***	0.43	ns	1.69	ns	0.07	ns
Inoculum Source (InoS)	8.68	*	62.29	***	0.05	ns	75.44	***	156.75	***
Dispersal x IncE	1.95	ns	0.67	ns	0.05	ns	0.77	ns	8.42	*
Dispersal x InoS	0.72	ns	0.01	ns	0.58	ns	1.72	ns	0.92	ns
IncE x InoS	1.55	ns	6.51	*	2.53	ns	0.45	ns	5.8	*
Dispersal x IncE x InoS	4.7	*	0.28	ns	0	ns	1.07	ns	6.35	*

E. Bacteroidetes and subgroup

	Bacteroidetes		**Flavobacteriaceae**	
Factors	F	*P*-value	F	*P*-value
Dispersal	1.53	ns	0.01	ns
Inocubation Environment (IncE)	6.74	*	3.79	.
Inoculum Source (InoS)	12.68	**	5.06	*
Dispersal x IncE	1.73	ns	6.09	*
Dispersal x InoS	4.67	*	8.66	*
IncE x InoS	23.14	***	30.46	***
Dispersal x IncE x InoS	1.83	ns	1.86	ns

Abbreviation: IncE, incubation environment; InoS, inoculum source; ns, not significant.

Significance codes : '***' $P < 0.001$; '**' $P < 0.01$; '*' $P < 0.05$; '.' $P < 0.1$.

Curriculum Vitae

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Educations

2018. June-Present Postdoctoral research in the Lab of Prof. Klaus Jürgens.

2014. Jan – 2018. May Doctoral thesis: **Micro**bial diversity and ecosystem **fun**ctions along the salinity gradients (MicroFun, funded by DFG), under supervision of Prof. Klaus Jürgens.

2013 Research Assistant under supervision of Dr. Richard Svanbäck, Uppsala University, Sweden

2010-2012 MSc in Aquatic Ecology, Linnaeus University, Sweden

Master thesis "*Temporal dynamics of urea uptake and ureolytic microbial communities in Swedish lakes*" under supervision of Prof. Stefan Bertilsson, Uppsala University, Sweden

2005-2009 BSc in Marine Science, Hebei University, China

Research Overview

Microbial composition varies over space and time. Yet, the question remains whether or not such heterogeneity in microbial composition matters to the ecosystem functions and the magnitude of the effects. Broadly, my research interests focus on the links between microbial biodiversity and ecosystem functions across environmental gradients or under adaptation scenarios. Specific focus is partly related to carbon acquisition by Baltic Sea bacterioplankton (*e.g.* cellulose degradation). In the perspective of microbial adaptation, my aim is to investigate the compositional changes and metabolic traits of indigenous brackish microbes and its freshwater and marine counterparts. To provide mechanistic understanding of microbial diversity, distribution, and their key functional traits,

I will integrate eco-physiological techniques with contemporary molecular tools throughout my PhD study, such as stable isotope probing, MAR-FISH, next generation sequencing, and meta-transcriptomics.

Research Cruises

- *Alkor* 439 (June 2014)

- Sampling along salinity gradient of the Baltic Sea

- Maria S. Merian (*MSM* 46) (August, 2015)

- Sampling along salinity gradient of the St. Lawrence Estuary

Posters and Talks

- **Shen, D.,** Beier, S., Jürgens, K.: Metatranscriptomic analysis of aquatic bacterioplankton in response to marine-oligohaline transitions in transplant microcosms, **Poster presentation** at 17 International Symposium on Microbial Ecology (ISME 17) 2018, Leipzig, Germany.

- **Shen, D.,** Jürgens, K.: Experimental insights into composition, community assembly and functional traits, **Oral presentation** at IOW scientific advisory board 2018, Warnemünde, Germany.

- **Shen, D**., Langenheder, S., Jürgens, K.: Effects of dispersal on diversity and composition of bacterioplankton following a salinity change, **Poster presentation** at 15th Symposium on Aquatic Microbial Ecology (SAME 15) 2017, Zagreb, Croatia; **awarded by FEMS Young Scientist Meeting Grant.**

- **Shen, D**., Beier, S., Jürgens, K.: Differential bacterial dynamics and transcriptional responses promote community robustness to changed salinity, **Poster presentation** at 16th International Symposium on Microbial Ecology (ISME 16) 2016, Montreal, Canada.

- **Shen, D**., Beier, S., Jürgens, K.: Bacterial responses to different salinities in a transplant experiment, **Poster presentation** at IOW Scientific advisory board 2016, Warnemünde, Germany.

- **Shen, D**., Beier, S., Jürgens, K.: Salinity adaptations of freshwater, brackish and marine bacterial communities of the Baltic Sea: results from a transplant experiment, **Oral presentation** at Aquatic Science meeting (ASLO) 2015, Granada, Spain.

- **Shen, D**., Beier, S., Jürgens, K.: Adaptations of freshwater, brackish and marine bacterial communities of the Baltic Sea to changed salinity, **Poster presentation** at IOW Internal seminar 2014, Warnemünde, Germany.

- **Shen, D**., Beier, S., Jürgens, K.: Salinity adaptations of freshwater, brackish and marine bacterial communities of the Baltic Sea, **Poster presentation** at CEMEB workshop - Marine Evolution under Climate Change 2014, Gothenburg, Sweden.

Publications and manuscripts

I. Sara Beier, **Dandan Shen**, Thomas Schott, and Klaus Jürgens. (2017). **Metatranscriptomic data reveal the effect of different community properties on multifunctional redundancy**. *Molecular Ecology* **26**: 6813-6826.

II. **Dandan Shen**, Klaus Jürgens, and Sara Beier. (2018). **Experimental insights into the importance of ecologically dissimilar bacteria to community assembly along a salinity gradient**. *Environmental Microbiology* 20: 1170-1184.

III. **Dandan Shen**, Silke Langenheder, and Klaus Jürgens. **Dispersal modifies the diversity and composition of active bacterial communities in response to a salinity disturbance**. (In press) *Frontiers in Microbiology,* doi: 10.3389/fmicb.2018.02188.

IV. **Dandan Shen,** Sara Beier, and Klaus Jürgens. **Metatranscriptomic analysis of aquatic bacterioplankton in response to marine-oligohaline transitions from transplant microcosms**. (*Manuscript*)

V. **Dandan Shen**, Anna-Lena Höger, Daniel Herlemann, and Klaus Jürgens. **Stable abundance-occupancy ranks despite high taxonomic variability between particle-attached and free-living bacterial communities**. (*Manuscript)*

VI. Han Z, **Shen D**, Mu SM, Guo MS, Kang XJ. (2010). 莠去津对雌性日本沼虾的毒性作用 (Toxic effect of Atrazine on female Macrobrachium nipponense). *Journal of Hebei University (Natural Science Edition)* **6**: 701-705.

Teaching Experience

Present- Supervision on master thesis of Hendrik Langeloh (master student in University of Rostock), Thesis "The impact of salinity changes to microbial and planktonic communities in the warnow river"

6/2018 Practical section in the course *Microbial Ecology* at the master level in Leibniz Institute for Baltic Sea Research (IOW) and at University of Rostock

4/2016 – 10/2016 Supervision on master thesis of Anna-Lena Höger (master student in University of Rostock), Thesis "*Examining the ecological coherence of particle-attached and free-living bacteria in the Gulf of St. Lawrence*"

11/2015 Practical sections in the course *Marine Biology* at the master level in Leibniz Institute for Baltic Sea Research (IOW) and at University of Rostock

11/2014 Practical sections in the course *Marine Biology* at the master level in Leibniz Institute for Baltic Sea Research (IOW) and at University of Rostock

Statement of Independence

Selbstständigkeitserklärung

The opportunity for this doctoral project has not been conveyed to me commercially. In particular, I did not engage an organization that, for a fee, seeks supervisions for the preparation of dissertations. Nor did I engage an organization to, in whole or in part, take care of incumbent oligations with regard to the examinations for me.

I hereby declare that I produced the present thesis independently and without any external help. I did not use any auxiliary materials or sources other than the ones indicated and passages used literally or with regards to the contents have been declared as such.

Hiermit versichere ich, dass ich die vorliegende Arbeit selbstständig angefertigt und ohne fremde Hilfe verfasst habe, keine außer den von mir angegebenen Hilfsmitteln und Quellen dazu verwendet habe und die den benutzten Werken inhaltlich und wörtlich entnommenen Stellen als solche kenntlich gemacht habe.

6th. February. 2018…… Rostock……Dandan Shen

Date/Datum Place/Ort Signature/Unterschrift