Bacterial communities associated with Jellyfish

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Wenjin Hao

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1. Gutachter: Prof. Dr. Rudolf Amann

2. Gutachter: Dr. Gunnar Gerdts

3. Prüfer: Prof. Dr. Wilhem Hagen

4. Prüfer: PD Dr. Bernhard M. Fuchs

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

SUMMARY

GENERAL INTRODUCTION 1

RESEARCH AIMS...13

OUTLINE..15

CHAPTER I 17
Bacterial communities associated with Ctenophores at Helgoland Roads

CHAPTER II 43

Bacterial communities associated with Scyphomedusae at Helgoland Roads

CHAPTER III...85

Bacterial communities respond to the excretion of DOM released by live jellyfish

GENERAL DISCUSSION.. 123

REFFERENCES...135

ACKNOWLEDGEMENT 155

DECLARATION...157

List of abbreviations

ARISA	Automated ribosomal intergenic spacer analysis
AMP	Antimicrobial peptide
ASW	Artificial seawater
BCC	Bacterial community composition
BCS	Bacterial community structure
CTAB	Cetyl-trimethyl-ammonium bromide
CARD-FISH	Catalyzed reporter deposition FISH
CFB	Cytophaga-Flavobacter-Bacterioides
DAPI	4´,6-diamidino-2-phenylindole
DNA	Deoxyribonucleic acid
DOM	Dissolved organic matter
DOC	Dissolved organic carbon
DPA	Dissolved primary amines
DFAA	Dissolved free amino acids
FISH	Fluorescent in situ Hybridization
HMW	High molecular weight
ITS	Internal transcribed spacer
LT	Lag time
LMW	Low molecular weight
MPD	Maximum population density
OTU	Operational taxonomic units
PCO	Principal co-ordinate analysis
POM	Particulate organic matter
PERMANOVA	Permutational multivariate analysis of variance
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
SGR	Specific growth rate
T-RFLP	Terminal restriction fragment length polymorphism

SUMMARY

This thesis represents the first investigation to understand the bacterial community associated with jellyfish, with special emphasis on ctenophores and scyphomedusae, at Helgoland Roads in the German Bight (North Sea, Germany).

Bacterial communities associated with the frequently occurring ctenophore species *Mnemiopsis leidyi*, *Beroe* sp., *Bolinopsis infundibulum* and *Pleurobrachia pileus* were investigated. Species-specific differences regarding the different ctenophores were revealed in the present study. The bacterial communities of all ctenophore species were dominated by Proteobacteria as revealed by pyrosequencing. *M. leidyi* and *P. pileus* mainly harbored Gammaproteobacteria, with *Marinomonas* being the dominant phylotype of *M. leidyi*. These results do confirm other studies obtained from the Tampa Bay of Florida and the Gullmar fjord at the west coast of Sweden, suggesting ctenophores from geographically distinct regions shared high similarity in their dominant bacterial communities. *P. pileus* presented a different assemblage with *Pseudoalteromonas* and *Psychrobacter* as the dominating phylotypes of Gammaproteobacteria. *Beroe* sp. was mainly dominated by Alphaproteobacteria, particularly by the genus *Thalassospira*. For *B. infundibulum*, the bacterial community comprised of Alphaproteobacteria and Gammaproteobacteria in equal amounts consisting in particular of the genera *Thalassospira* and *Marinomonas*. Although *Marinomonas* was always predominant, seasonal variation of bacterial community was observed in *M. leidyi* on a small scale.

Regarding the typical metagenetic life cycle of scyphomedusae, the bacterial communities associated with two scyphomedusae species (*Cyanea lamarckii* and *Chrysaora hysoscella*) were firstly investigated at Helgoland Roads. Two aspects were studied: different body parts and different life stages. Concerning the analysis of different body parts (umbrella, gonad, tentacle and mouth arm), significant differences were revealed between umbrella and other body parts (gonad and tentacle) in terms of the associated bacterial community in both species. With regard to the different life stages, bacterial community structure varied from the early stage planula larvae to polyps even to adult medusae with significant differences in both species with completely distinct patterns. Statistical analyses (PCO analyses) revealed that these surfaces obviously represent a passive substrate colonized by a diverse bacterial community as presented by a dispersive structure among three life stages in *C. lamarckii*. In contrast a strong selected

processed of bacterial colonization in each life stage represents a highly separated community structure in *Ch. hysoscella*. Furthermore, the impact of the food source on the associated bacterial community was investigated with respect to polyps. Bacterial communities associated with polyps were significantly distinct from the food in both species. Interestingly, the diversity of bacterial community composition (BCC) associated with polyps was highly correlated with different food sources. However, for the bacterial community composition, significant differences were presented in response to different food source. Polyps might react differently during metabolic processing in response to different food source (*A. salina* and plankton) resulting in a significantly different bacterial community structure. In general, the bacterial communities associated with two scyphomedusae species are species-specific as confirmed in each life stage.

The utilization of DOM released by live jellyfish was firstly investigated in the third part of this thesis. We focused on the compositional succession of bacterioplankton community in response to the DOM released by live scyphomedusae (*Cyanea lamarckii* and *Chrysaora hysoscella*). Bacterial community structure was determined via Automated Ribosomal Intergenic Spacer Analysis (ARISA) fingerprints at the end of the experiments. Catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH) analysis was applied to reveal the bacterial community composition at different time points. Bacterial communities were significantly different regarding to different DOM source including jellyfish treatment (DOM released by live jellyfish), "Kabeltonne" seawater (natural DOM from seawater) and artificial seawater (DOC-free) based on ARISA fingerprints. The bacterial community was significantly stimulated by the DOM released by live jellyfish with different dominant phylotypes regarding to different scyphomedusae species. According to CARD-FISH analysis, Gammaproteobacteria and Bacteroidetes were consistently present in the experiment conducted with *Ch. hysoscella*, while Bacteroidetes decreased at the beginning and recovered at the end of the experiment conducted with *C. lamarckii*. Alphaproteobacteria played a minor role in the present experiments. The significant differences in the bacterial community composition and succession indicate that the DOM released by jellyfish might consist of different compounds which are species specific. Last but not the least, DOM released by live jellyfish strongly impacted the natural bacterioplankton community not only on the bacterial abundance, but also on the community composition.

ZUSAMMENFASSUNG

Die vorliegende Arbeit beschäftigt sich mit marinen Bakteriengemeinschaften assoziiert mit Quallen. Besonderes Augenmerk liegt dabei auf den Bakterien von Ctenophoren und Scyphomedusen, die häufig in der Deutschen Bucht vor Helgoland vorkommen.

Häufig vorkommenden Ctenophoren Arten in der Deutschen Bucht sind *Mnemiopsis leidyi*, *Beroe* sp., *Bolinopsis infundibulum* und *Pleurobrachia pileus.* Zur Analyse ihrer Bakteriengemeinschaften wurden Fingerprints der mit ihnen assoziierten Bakteriengemeinschaften erstellt sowie 454 Pyrosequenzierungen der ribosomalen Gene durchgeführt. In allen untersuchten Ctenophoren Arten wurden die Bakteriengemeinschaften von den Proteobakterien dominiert. Spezifische Unterschiede bezüglich der vorkommenden Bakteriengemeinschaften in diesen Ctenophoren wurden im Detail deutlich. *M. leidyi* und *P. pileus* wiesen überwiegend Gammaproteobakterien auf, wobei die Gattung *Marinomonas* der dominierende Phylotyp in *M. leidyi* war. Dieses Ergebnis bestätigt Untersuchungen der Bakterienzusammensetzung in *Mnemiopsis*, die sowohl in Tampa Bay (Florida) sowie im Gullmar Fjord (Schweden) durchgeführt wurden. *Mnemiopsis* scheint somit in sehr unterschiedlichen geographischen Regionen sehr ähnlich hinsichtlich der dominanten Bakterien zu sein. Obwohl *Marinomonas* die dominierende Bakteriengattung war, konnten kleine saisonale Unterschiede in Bezug auf die vorkommenden Bakteriengruppen in *M. leidyi* beobachtet werden, die allerdings aktuell nicht weiter untersucht werden konnten. *P. pileus,* die ebenfalls Gammaproteobakterien als dominierende Gruppe beinhalten, wies auf einer niedrigeren taxonomischen Ebene eine andere Bakterienzusammensetzung auf. *Pseudoalteromonas* und *Psychrobacter* waren hier die dominierenden Phylotypen. Die Bakteriengemeinschaft in *Beroe* sp. wurde von den Alphaproteobacteria der Gattung *Thalassospira* dominiert. Die Bakteriengemeinschaft in *B. infundibulum* setzte sich gleichermaßen aus Alphaproteobacteria und Gammaproteobacteria zusammen. Sowohl die Gattung *Thalassospira* als auch *Marinomonas* spielen hier eine deutliche Rolle.

Bakteriengemeinschaften der Scyphomedusen wurden erstmalig im Zusammenhang mit deren verschiedenen Lebenscyclen untersucht. Zwei Arten, *Cyanea lamarckii* und *Chrysaora hysoscella* aus Helgoländer Gewässer wurde hinsichtlich verschiedenen Kompartimente der

adulten Tiere (Schirm, Gonaden, Tentakel und Mundregion) sowie hinsichtlich unterschiedlicher Stadien (Planula Larven, Polypen und adulte Tiere) untersucht. Der Vergleich der Bakteriengemeinschaften assoziiert mit unterschiedlichen Gewebekompartimenten beider Scyphomedusen Arten zeigte deutliche Unterschiede insbesondere zwischen dem Schirm und anderen Teilen, wie Gonaden oder Tentakel. Auch die Bakteriengemeinschaften der drei untersuchten Stadien beider Schirmquallen Arten wiesen signifikante Unterschiede auf. Zusätzlich waren die Gemeinschaften beider Arten deutlich unterschiedlich. Interessant dabei ist, dass die Bakteriengemeinschaften offenbar alle drei Stadien von *C. lamarckii* als Oberfläche eher passive bzw. zufällig besiedeln, wohingegen die signifikanten Unterschiede der Besiedlung in *Ch. hysoscella*.auf einen Selektionsdruck hinsichtlich spezifischer Bakterien hindeuten. Auch der Einfluss unterschiedlicher Futterangebote (*Artemia salina* und natürliches Planktonkonzentrat) auf die Diversität und die Zusammensetzung der Bakteriengemeinschaften wurde an den Polypen Stadien beider Quallen Arten untersucht. Interessant ist, dass die bakterielle Diversität in den Polypen, die mit natürlichem Plankton gefüttert wurden höher war, als die Diversität der mit Artemien gefütterten Polypen. Dies spiegelte sich ebenfalls in dem Futter selber wider. Und auch bezüglich der Bakteriengemeinschaften in den Polypen gab es bei beiden Quallen Arten signifikante Unterschiede bei unterschiedlicher Futtergabe. Wie zu erwarten, waren neben der unterschiedlichen Diverstiät die Bakteriengemeinschaften der jeweiligen Futterarten ebenfalls deutlich unterschiedlich. Die Polypen der beiden Quallen Arten scheinen das angebotene Futter unterschiedlich zu verwerten, was wiederum zu einem unterschiedlichen Nahrungsangebot für die Bakterien führen könnte und somit andere Bakterien selektiert werden. Zusammenfassend konnten jedoch eine artspezifisch assoziierte Bakteriengemeinschaften für die verschiedenen Lebens Stadien der Schirmquallen nachgewiesen werden.

Mit dem gelösten organischen Material (DOM), dass von Quallen in das Umgebungswasser ausgeschieden wird, und mit deren Verwertung als Nährstoffquelle von marinen Bakterien beschäftigt sich der dritte Teil dieser Arbeit. Dabei wurde mittels Card-FISH speziell auf die Sukzession sowie die Zusammensetzung des Bakterioplanktons als Antwort auf die Zugabe von "Quallen-DOM" geschaut. Dieses DOM wurde von lebenden Scyphomedusen an das Umgebungswasser abgegeben, zwei Arten wurden untersucht (*Cyanea lamarckii* und *Chrysaora hysoscella*). Als Kontrollen diente einerseits DOM-freies künstliches Seewasser sowie das DOM

in natürlichem Seewasser der Probennahmestation „Kabentonne“ Helgoland Reede. Die Fingerprints der Bakteriengemeinschaften machten deutlich, dass die Bakterien signifikant unterschiedlich auf das DOM verschiedenen Ursprungs reagieren. Im Detail zeigte sich, dass die Bakteriengemeinschaften der drei Treatments signifikant unterschiedlich stimuliert wurde, sowohl über den Versuchszeitraum als auch hinsichtlich der beiden unterschiedlichen Quallen Arten. Gammaproteobacteria und Bacteroidetes waren durchgängig in allen Ansätzen mit *Ch. hysoscella*-DOM deutlich present. Im Gegensatz dazu nahmen im Experiment mit *C. lamarckii*-DOM die Bacteroidetes am Anfang kontinuierlich ab und nahmen erst am Ende der Versuchszeit wieder zu. Diese signifikanten Unterschiede in der Zusammensetzung der Gemeinschaft und deren unterschiedlicher Sukzession macht deutlich, dass das DOM dieser beiden Quallen Arten offenbar spezifisch ist und unterschiedliche chemische Substanzen enthält, die von verschiedenen Bakterien verwertet werden können. Letztendlich hat diese Untersuchung gezeigt, dass das Quallen-DOM tatsächlich einen Einfluss sowohl auf die Anzahl als auch auf die Zusammensetzung der Bakteriengemeinschaften hat.

GENERAL INTRODUCTION

Jellyfish

The term "jellyfish" is used in reference to free-floating gelatinous animals belonging to the phyla Ctenophora and Cnidaria (Richardson *et al.*, 2009). The phylum Ctenophora, also called comb jellies, propel themselves through the sequential beating of rows of cilia (comb rows). Ctenophores are armed with colloblast cells which discharge glue to ensnare prey. Ctenophores are holoplanktonic, remaining in the plankton for their entire life. The phylum Cnidaria contains animals such as anemones and corals but also a range of jellyfish, including four main classes: Scyphozoa, Cubozoa, Hydrozoa and Staurozoa. Jellyfish of phylum Cnidaria range in size from a few millimeters (*Aglaura* and *Obelia* spp.) to 2 m (*Nemopilema nomurai*) in diameter. Generally, they have two alternating generations (swimming medusa and sessile polyp) in their metagenetic life cycles. Cnidarians have stinging cells or cnidocysts (nematocysts) concentrated in the tentacles and mouth appendages. Although both of these two phyla are not closely related, these organisms share many characteristics, including gelatinous and symmetry structure, generally transparent or translucent (composed of > 95% water), no specialized digestive, central nervous, respiratory or circulatory systems (Scolardi *et al.*, 2006).

The ctenophore *Mnemiopsis leidyi*, which originally occurred in South America (GESAMP, 1997), has invaded the Black Sea (Vinogradov *et al.*, 1989), the Mediterranean (Uysal & Mutlu, 1993) and the Caspian Sea (Ivanov *et al.*, 2000) during the last two decades (Purcell *et al.*, 2001). It was first recorded in the North Sea almost at the same time (Faasse & Bayha, 2006; Boersma *et al.*, 2007; Tendal *et al.*, 2007) and was identified as an invasive species at Helgoland Roads (Boersma *et al.*, 2007; Hamer *et al.*, 2011). Other ctenophore species, *Beroe* sp., *Bolinopsis infundibulum* and *Pleurobrachia pileus* are indigenous at Helgoland Roads (Greve, 1970). For scyphozoan, *Aurelia aurita* (Linnaeus, 1758), *Cyanea capillata* (Linnaeus, 1758), *Cyanea lamarckii* (Péron & Lesueur, 1809), *Chrysaora hysoscella* (Linnaeus, 1767) and *Rhizostoma octopus* (Linnaeus, 1788) are common medusa species in the German Bight and usually occur periodically during summer (Möller, 1980; Hay *et al.*, 1990; Barz & Hirche, 2007).

Jellyfish bloom and its impact on the ecosystem

Dramatic spatial increases and temporal shifts of jellyfish (ctenophores and medusae) occurred in many estuarine, coastal, and open-ocean ecosystems worldwide over the past decades (Graham *et al.*, 2001; Brodeur *et al.*, 2002; Brodeur *et al.*, 2008; Hamner & Dawson, 2009; Brotz *et al.*, 2012). The increasing populations of jellyfish have stimulated speculation regarding possible causes including over-fishing, eutrophication, climate change, and invasions (Arai, 1996; Mills, 2001; Hay, 2006; Purcell *et al.*, 2007). Many fish compete for the same zooplankton prey as jellyfish. Some fish are also predators of jellyfish, with benthic and reef fish species ingesting polyps and pelagic fish species feeding on ephyrae and small individuals (Purcell & Arai, 2001). Therefore, over-fishing opens up the ecological space for jellyfish. Coastal eutrophication encourages phytoplankton blooms that can ultimately lead to jellyfish outbreaks (Purcell *et al.*, 2001). In the coastal eutrophic zone, nutrients are rich in nitrogen and phosphorus but poor in silica. Under such conditions, non-siliceous phytoplankton and flagellates (include harmful red-tide species) proliferate and replace diatoms resulting in a reduction in the size of primary and secondary producers (Cushing, 1989; Harashima *et al.*, 2006). It has been hypothesized that such altered food web is more favorable for jellyfish (Parsons & Lalli, 2002). On the other hand, jellyfish and polyps have greater tolerance than fish to low oxygen conditions. This ensures jellyfish can survive and even reproduce under hypoxic conditions (Purcell & Arai, 2001). Global warming might also favor jellyfish. Warmer temperature accelerates jellyfish growth and ephyrae production (Purcell *et al.*, 2007; Holst, 2012 a). Gibbons & Richardson (2009) showed that jellyfish abundance is temperature dependent over 50 years in the North Atlantic, with more jellyfish occurring in warmer years. In addition, the human-assisted movement of species to new marine areas is most commonly caused by the exchange of ballast water between regions and the transport of fouling biota (e.g. polyps) on ship hulls (Graham & Bayha, 2007). As parts of the oceans become increasingly disturbed and overfished, it has been evidenced that energy previously went into production of fishes may be switched over to the production of pelagic Cnidaria or Ctenophora (Mills, 2001).

As the major component of the pelagic system, jellyfish represent a conspicuous element of the zooplankton (Brodeur *et al.*, 2002). The accidental introduction and subsequent expansion of jellyfish have significantly altered food web structure and heavily impacted commercial fisheries

because they are voracious predators on copepods and ichthyoplankton (Parsons & Lalli, 2002; Richardson *et al.*, 2009). Scyphomedusae consume significant quantities of microphytoplankton and microzooplankton including phototrophic and heterotrophic dinoflagellates, ciliates and flagellates, as well as copepods, small ctenophores and fish larvae (Schneider & Behrends, 1998; Purcell & Arai, 2001; Brodeur *et al.*, 2002; Sommer *et al.*, 2002; Purcell, 2003; Hansson *et al.*, 2005) and have a strong impact on zooplankton standing stocks in all parts of the world. It has been shown that *Aurelia aurita* exerts direct predatory pressure on mesozooplankton and microzooplankton populations (Stoecker *et al.*, 1987). Similar impacts have been observed in native habitats, where temporal shifts in *M. leidyi* blooms have occurred and consequently driven copepod populations to summer extinction (Sullivan *et al.*, 2001). Despite obvious predatory impacts on food web structure during their life span, jellyfish are known to release nutrients and bio-available dissolved organic matter (DOM) (Hansson & Norrman, 1995) to the microbial loop via several possible pathways, such as sloppy feeding, excretion of fecal material mucus production (Riemann *et al.*, 2006; Condon *et al.*, 2011) and decaying biomass (Titelman *et al.*, 2006; Tinta *et al.*, 2010). Jellyfish acquire C, N and P by assimilating organic compounds from ingested prey. Inorganic nutrients excreted by jellyfish populations provide a small but significant proportion of the N and P, which are required by phytoplankton for primary production. Organic forms of C and N are recycled to the environment as mucus production and decomposition and it may support microbial production. It is suggested that *A. aurita* is the second most important source for regenerated nutrients in Kiel Bight (Schneider, 1989). In addition, large live medusae accumulatively damage and gradually break down in the water column throughout the season during decomposition (Mills, 2001). The abundant carcasses of jellyfish at the termination of the bloom can represent an important source of labile organic substrates and inorganic nutrients for bacteria due to their rapid turnover rates (Riemann *et al.*, 2006). Hence, decomposition processes of jellyfish bloom may not only influence carbon and nutrient cycling through excretion of DOM, inorganic nitrogen and phosphorus (Schneider, 1989; Nemazie *et al.*, 1993; Billett *et al.*, 2006; West *et al.*, 2009), but also potentially impact the microbial community composition for stimulating the bacterial growth (Martinez, 1996; Titelman *et al.*, 2006; Tinta *et al.*, 2010).

Bacterial community in the pelagic system

It has become obvious that marine prokaryotic microbes are a fundamental part in the marine ecosystems (Decho, 1990; Jensen & Fenical, 1994) and an integral part of the microbial loop (Fenchel, 2008). Generally, bacteria have been recognized primarily as responsible for remineralization of inorganic nutrients and decomposition of organic material (Fuhrman, 1992). The microorganisms affect most biogeochemical processes in the oceans and their activities influence the element cycling on a global scale (Davey & O'toole, 2000). As significant component, planktonic bacteria play a critical role in determining the fate of organic energy and the cycling of carbon, nitrogen, phosphorus, sulfur (Azam *et al.*, 1983; Mary, 2006; Jiao *et al.*, 2010). Bacteria manage to populate all parts of the ocean by capturing nutrients and energy from diverse sources. Photosynthetic bacteria derive energy from the primary production of organic matter through photosynthesis and chemolithotrophy. Heterotrophic bacteria capture dissolved organic molecules from seawater as well as organic particles that they can digest with enzymes. Some bacteria, such as chemoautotrophic, oxidize inorganic chemicals for energy, and the carbon they fix into organic matter serves as basis for food webs in diverse ecosystems.
Alpha- and Gammaproteobacteria and Bacteroidetes generally dominate the bacterioplankton communities (Glöckner *et al.*, 1999; Mary, 2006). Interestingly, the major prokaryotic groups appear to have cosmopolitan distributions. Members of the Alphaproteobacteria are comprised of the Roseobacter clade. They are chemoorganotroph and occur exclusively in the marine environment. Some of these species are able to synthesize bacteriochlorophyll a (Allgaier *et al.*, 2003). Another important group within the Alphaproteobacteria, apart from the Roseobacter clade species belonging to Sphingomonadales, is SAR 11 and SAR 116 which can be highly specialized (Rappé *et al.*, 2000). Members of Gammaproteobacteria are the most cultivable group of the bacterioplankton. These chemoorganotrophic bacteria are often associated with surface of the organisms. Species like *Alteromonas* sp. and *Pseudoalteromonas* sp. belong to this group as well as species like *Oceanospirillum* sp. or *Marinobacter* sp. which form a separate clade. Members of the phylum Bacteroidetes display the ability for gliding motility as well as the ability to degrade biomacromolecules like chitin, agar, cellulose or DNA. They are widespread distributed in the marine environment. Typically, the Bacteroidetes are associated with surfaces

such as algal cells or marine snow but they can also be found free-living (Pinhassi *et al.*, 2004; Hahnke & Harder, 2013; Mann *et al.*, 2013).

Significant seasonal changes in abundance and composition of bacterial communities were observed in several studies (Eilers *et al.*, 2001; Gerdts *et al.*, 2004; Fuhrman, 2006). Especially, phytoplankton dynamics and seasonal cycles in the source of DOM regulated the free-living bacterial communities (Crump *et al.*, 2003). And direct relationship between temperature and bacterial production (Pinhassi & Hagstrom, 2000) as well as seasonal succession of the community structure (Gerdts *et al.*, 2004) was shown for marine bacterioplankton. Sapp *et al.* (2007 b) has shown that the increase in temperature contributed to shifts in the bacterial community which was strongly correlated with the phytoplankton bloom. The massive increase of bacterial abundance during the collapse of phytoplankton blooms consist of the group Bacteroidetes, Alpha- and Gammaproteobacteria suggesting their involvement in the degradation of mucopolysaccharides produced by the phytoplankton (Teeling *et al.*, 2012). On the one hand, these bacteria consume low molecular weight organic compounds such as amino acids, acetate or sugars which can be transported directly across the cell membrane. On the other hand, extracellular hydrolysis or cleavage is needed for the consumption of polysaccharides or proteins. In marine environment, bacteria have a strong affinity to locate and attach on a variety of surfaces. Since marine invertebrates exhibit the greatest phyletic diversity in the biosphere, it is likely that the greatest variety of animal-bacterial interactions occurs within this group (Brusca & Brusca, 1990). Studies characterizing the bacterial communities associated with marine organisms have largely focused on corals (Sunagawa *et al.*, 2009), *Hydra* (Bosch, 2012), sponges (Wichels *et al.*, 2006; Webster & Taylor, 2012), hydrothermal vent worms (Campbell *et al.*, 2003) and copepod (Carman & Dobbs, 1997; Gerdts *et al.*, 2013). The relationship of bacteria with marine organisms ranges from mutualism through commensalisms and competition, to antagonism (Nair, 2004). Although it is difficult to identify general principles of bacterial adhesion because of the diversity of attachment mechanisms and the ability of microorganisms depending on environmental conditions (Fletcher, 1994), these bacterial associations play a vital role in marine ecosystem.

The interactions between bacteria and host could be neutral, where one does not influence the other by any ways like the epiphytic bacteria. In this kind of relationship, neither of the interacting organisms are affected by the association. Certain marine bacteria attach themselves

to the surfaces of organisms and differentiate to form a complex, multicellular structure called biofilm. Though the formation and interaction in biofilms involve neutral processes, they play active roles in the multicellular biofilm development and subsequent dispersal of surviving cells within the marine environment (Nair, 2004). Mai-Prochnow *et al.* (2004) observed that the biofilm formed by newly described green pigmented bacterium *Pseudoalteromonas tunicate* (D2) produces target-specific inhibitory compounds against bacteria, algae, fungi, and invertebrate larvae and is frequently found in association with living surfaces in the marine environment. Hence, it can be understood that these relationships are not stable but are constantly changing and adapting to their environment.

Nevertheless depending on the effect of interaction on the host, the relationship can be positive or negative. The profitable cooperation by different organisms can either be symbiotic or commensal. Symbiosis occurs when two organisms form a relationship, which provides an advantage for both partners at least temporarily. Symbiosis may be divided into two distinct categories, ectosymbiosis and endosymbiosis.

In ectosymbiosis, the symbiont lives on the body surface of the host including internal surfaces such as lining of the digestive tube and ducts of glands (Nair, 2004). Bivalve mollusc has established symbiosis with chemosynthetic bacteria that use inorganic reduced compounds as an electron source, in regions where sulfide and oxygen are present in the water perfusing the sediments (Dubilier *et al.*, 2008; Vrijenhoek, 2010). The sulfur-oxidizing epibacteria found on nematodes living in marine sands, at the oxic-anoxic interface; the nematodes appear to meet their carbon requirements directly by feeding on their symbionts (Polz *et al.*, 1994; Nussbaumer *et al.*, 2004).

In endosymbiosis, the symbiont lives within the cells or intracellular space of the host. Such a symbiont is called an endosymbiont. Occurrence of endosymbiosis is well documented in the hydrothermal vent systems (Cary & Giovannoni, 1993; Moyer *et al.*, 1995; Hurtado *et al.*, 2003). The deep-sea hydrothermal vent sites are characterized by high concentration of reduced sulfur compounds. There life is supported by the growth of chemolithoautotrophic bacteria, capable of oxidizing hydrogen sulfide to generate energy that is used to fuel carbon dioxide fixation into macromolecules (Compère *et al.*, 2002; López-García *et al.*, 2003). An extraordinary animal in these ecosystems is the giant tubeworm *Riftia pachyptila*. The adult worms do not have mouth, gut, or anus, and obtain all their energy and food from endosymbiotic chemolithoautotrophic

bacteria which are harbored in a specialized organ called trophosome. These worms have unique hemoglobin in their blood that binds oxygen and sulfide thereby transporting both from the surrounding seawater to the bacteria (Cavanaugh, 1994; Grzymski *et al.*, 2008; Robidart *et al.*, 2008). In addition, many of the luminous animals show monospecific associations with bioluminescent bacteria and these are associated in specialized light organs. The selecting process of the single bacterial species, *Vibrio fischeri*, by the bobtail squid (*Euprymna scolopes*) is an example of environmental harvesting to take advantage of this bacteria's bioluminescent capability for predator avoidance (Nyholm & McFall-Ngai, 2004).

In addition, these associated bacteria also produce certain bioactive secondary metabolites. Rinehart (2008) reported the occurrence of such metabolites in sponges, molluscs, bryozoans and ascidians. Waddell & Pawlik (2000) showed the anti-fouling or anti-infective nature of these secondary metabolites which conferred anti-predatory protection (Pisut & Pawlik, 2002). In return, the host supplies adequate nutrition for proliferation of its endosymbionts. Graf & Ruby (1998) reported that *Euprymna scolopes* produce free amino acids as well as peptides to support the growth of its endosymbiont, *Vibrio fischeri*.

In commensalism, one-organism benefits and the other is neither harmed nor helped. Commensalism represents a simple type of positive interaction and perhaps is the first step towards the development of beneficial relations (Reinheimer, 1986). Bacteria occur as ecto and endocommensals. In this relationship the bacteria do not extract nutrients from the host, but they do utilize the habitat provided by the host.

Bacteria communities associated with coelenterate

Coelenterates contain two phyla, the Ctenophora (comb jellies) and the Cnidaria, e.g. including corals, true jellyfish and sea anemones. Here, the associated bacterial community will be specifically addressed with corals, hydra and jellyfish (ctenophores and scyphozoan).

Bacteria communities associated with coral

Coral reefs are the most diverse biological marine ecosystems and harbor an enormous diversity of marine organisms (Frias-Lopez *et al.*, 2004; Rosenberg *et al.*, 2007). Bacteria are known to be abundant and active around corals (Rohwer *et al.*, 2002; Ritchie, 2006; Raina *et al.*, 2009). Coral provides three habitats for bacterial community, including the coral surface mucus layer (Lampert *et al.*, 2006; Ritchie, 2006), the coral tissue (the gastrodermal cavity) (Frias-Lopez *et al.*, 2002) and the calcium carbonate skeleton within coral tissue itself (Kushmaro *et al.*, 1996; Banin *et al.*, 2000). The associations between bacteria and corals are suspected to play important roles, including nitrogen fixers (Williams *et al.*, 1987; Shashar *et al.*, 1994), sulfur compound metabolization (Raina *et al.*, 2009), chitin decomposers (Ducklow & Mitchel, 1979) and provide organic compounds to the coral tissue (via photosynthesis produced by Cyanobacteria in the skeleton) (Fine & Loya, 2002).

The abundance of bacteria associated with coral tissue is similar to those found in the mucus layer and both of them are 100-1000 fold higher than that in the surrounding seawater (Ducklow & Mitchel, 1979; Koren & Rosenberg, 2006). Moreover, these abundant bacterial groups seem to be specialized since the dominant bacterial communities associated with coral tissue are different from that colonized the surface mucus layer (Bourne & Munn, 2005; Koren & Rosenberg, 2006). In addition, the composition of the coral bacterial community differs from that of the surrounding seawater suggesting the association between the coral and its microbiota is specific (Frias-Lopez *et al.*, 2002; Bourne & Munn, 2005; Sunagawa *et al.*, 2009). Similar bacterial communities, in terms of composition, are associated with the same coral species (even if geographically separated), whereas different communities are found on different coral species indicating the species-specific associations with coral (Frias-Lopez *et al.*, 2002; Bourne & Munn, 2005; Lampert *et al.*, 2006; Wegley *et al.*, 2007).

The most common bacterial communities of coral associations are Gammaproteobacteria, Alphaproteobacteria, Firmicutes, Bacillus/Clostridium (BC), Cytophaga-Flavobacter/Flexibacter-Bacterioides (CFB) and Cyanobacteria (Ducklow & Mitchel, 1979; Rohwer *et al.*, 2002; Frias-Lopez *et al.*, 2004; Bourne & Munn, 2005; Rosenberg *et al.*, 2007). None of the coral-associated bacterial 16S rDNAs belonged to the ubiquitous pelagic marine groups SAR11 and SAR116 (Rohwer *et al.*, 2002). The most abundant bacterial groups

identified by culture-independent sequencing are Cyanobacteria and Alphaproteobacteria. In contrast, the cultured bacteria from the same sample are closely related to Gammaproteobacteria (Rohwer *et al.*, 2001).

Bacteria communities and *Hydra*

The hydrozoan species "*Hydra*" is a model system in developmental biology since the 1980s (Fraune & Bosch, 2010). Epithelia of all organisms are colonized by complex and dynamic communities of microbes (Dale & Moran, 2006). The *Hydra* body represents a simplified structure. It consists of only two cell layers and few number of cell types. All cell types in *Hydra* are derived from only three distinct stem cell lineages, the ectodermal and endodermal epithelial stem cells and the interstitial stem cell lineage (Bosch, 2009).
Bacteria have profound effects on tissue proliferation in *Hydra* (Rahat & Dimentman, 1982). The sterilized *Hydra* cannot proliferate asexually by budding (Rahat & Dimentman, 1982). Interestingly, this effect could be rescued by inoculating the bacteria from standard culture of *Hydra* (Rahat & Dimentman, 1982). Therefore, resident microbiota strongly affects development in *Hydra*. While this probiotic effect in *Hydra* remains mostly unexplained, in several invertebrates and vertebrates there is accumulating evidence that beneficial microbes have a crucial role in metabolism, immunity and development (Fraune *et al.*, 2010). Bosch and his colleagues (Fraune *et al.*, 2010) investigated the bacterial community associated with the different stages of *Hydra* from embryo to polyp. They found fewer and different bacterial community associated with early embryos than with later developmental stages. They further demonstrated that early embryonic stages of *Hydra* are capable of developing specific antimicrobial activity against certain bacterial to control bacterial colonization by using maternal antimicrobial peptides (Fraune *et al.*, 2010).
Similarly, a complex and dynamic community of bacterial colonizers in *Hydra* epithelium greatly differed from different species (Fraune & Bosch, 2007). They even found that individuals living in the wild were colonized by a similar bacterial community as compared with polyps grown in the lab over long period of time (Fraune & Bosch, 2007). These findings strongly indicate that distinct selective pressures are imposed on and within the *Hydra* epithelium. The active selection of the specific bacterial community associated with *Hydra* is genetically encoded

in the epithelium (Fraune & Bosch, 2007). Furthermore, bacterial community was significantly modulated in response to changes of the epithelia cell composition (Fraune *et al.*, 2009). In particular, the dominant bacterial phylotype belonging to the Betaproteobacteria was decreased in polyps lacking the interstitial stem cell lineage (Fraune *et al.*, 2009). These changes in the bacterial composition depending on the loss of the interstitial cell imply a direct interaction between cellular tissue composition and microbiota (Fraune *et al.*, 2009).

Bacteria and Jellyfish

Bacterial communities associated with the lobate ctenophore *Mnemiopsis leidyi* and its natural predator *Beroe ovata* were firstly investigated in Tampa Bay, Florida, USA (Daniels & Breitbart, 2012). They found that both ctenophore species contained fewer bacterial operational taxonomic units (OTUs) by terminal restriction fragment length polymorphism (T-RFLP) and lower diversity communities by 16S rRNA gene sequencing than the surrounding water column. Each ctenophore genus contained a unique microbiota (Daniels & Breitbart, 2012). Overall, Alphaproteobacteria, Gammaproteobacteria, and Bacteroidetes were the most abundant groups detected in ctenophores (Daniels & Breitbart, 2012). The *B. ovata* sample was dominated by an uncultured Alphaproteobacteria from the family Rhodospirillaceae. An uncultured *Marinomonas* sp. (Gammaproteobacteria belonging to the order Oceanospirillales) dominated the *M. leidyi* community, but was not detected in the water column or the *B. ovata* specimen. In addition, a bacterium of the genus *Tenacibaculum* accounted for the second highest number of clones in the *B. ovata* sample and was exclusive to this ctenophore (Daniels & Breitbart, 2012). Interestingly, Dinasquet *et al*. (2012) also reported that the dominant community associated with the empty gut of *M. leidyi* was related to Flavobacteriaceae (Bacteroidetes). In addition, temporal variation of bacterial community associated with ctenophore was revealed by Daniels & Breitbart (2012).
In Cnidarian, pelagic larvae need undergo metamorphosis to develop into sessile polyp (Woollacott & Hadfield, 1996; Leitz, 1997; Holst & Jarms, 2010). Generally, an external cue is necessary for metamorphosis process. In marine environments almost all substrates are covered by biofilms. Within the biofilm certain bacteria are suggested to deliver the metamorphosis-inducing stimulus (Clare *et al.*, 1998). Bacteria and/or their products are also involved in the induction of settlement and metamorphosis in some scyphozoan (Müller & Leitz, 2002). Under

aseptic conditions, no settlement of planulae of the jellyfish *Aurelia aurita* (Schmahl, 1985), *Cassiopea andromeda* (Hofmann & Brand, 1987) and the hydrozoan *Hydractinia echinata* (Leitz & Wagner, 1993) occurred. They were induced by incubation with *Vibrio* sp. isolated from aquarium material found to induce metamorphosis of *Cassiopea* (Hofmann *et al.*, 1978). The density of microorganisms on artificial substrata positively correlated with settlement responses in *Cyanea capillata* (Brewer, 1976). The pedal stolons of *Aurelia aurita* were induced to settle by a bacterial species of the Family Micrococcaceae (Schmahl, 1985).

Bacteria thrive in the dissolved organic carbon (DOC) released by live jellyfish which have been described both in the laboratory (Hansson & Norrman, 1995) and the field (Riemann *et al.*, 2006). Titelman *et al.*(2006) confirmed that jellyfish could be a suitable substrate for specific bacteria community. Tinta and colleagues (Tinta *et al.*, 2010; Tinta *et al.*, 2012) investigated the decomposition process of jellyfish. The addition of jellyfish homogenate resulted in increased bacterial abundance and production, coupled with NH_4^+ accumulation and oxygen consumption and dramatic changes in bacterial community composition. They observed a rapid shift in community composition from unculturable Alphaproteobacteria to culturable species of Gammaproteobacteria and Flavobacteria (Tinta *et al.*, 2012). Jelly-DOM favored the rapid growth and dominance of specific bacterial phylogenetic groups (primarily Gammaproteobacteria) that were rare in ambient waters, implying that jelly-DOM was channeled through a small component of the *in situ* microbial assemblage and thus induced large changes in bacterial community composition (Condon *et al.*, 2011). Condon *et al.* (2011) demonstrated that the effect of gelatinous organisms could alter food web dynamics by promoting microbial respiration, further fueling the microbial loop rather than being incorporated into biomass.

RESEARCH AIMS

The aim of this thesis is to investigate the bacterial communities associated with jellyfish at Helgoland Roads (North Sea, Germany) from a wide range of angles. In contrast to the well-described coral and *Hydra* microbiota, few previous studies have looked at the bacterial communities associates with jellyfish. Therefore, many questions regarding bacterial community associated with jellyfish, their abundance, composition, diversity and response to changing environmental parameters are unresolved. The current study aimed to answer some of the questions with special emphasis on the ctenophore and scyphomedusae.

Bacterial communities associated with Ctenophore

Although several studies on the bacterial community composition (BCC) of coelenterata were published in the past, it must be stated that the majority of these studies focused on corals. Compared to other marine invertebrates (e.g. sponges), the knowledge on ctenophore is still scarce here. The current study investigated the bacterial communities associated with the frequently occurring ctenophore species *Mnemiopsis leidyi*, *Beroe* sp., *Bolinopsis infundibulum* and *Pleurobrachia pileus* at Helgoland Roads in the German Bight (North Sea). The aim was to find out whether different ctenophore species harbor distinct bacterial community. The bacterial community structure was determined via automated ribosomal intergenic spacer analysis (ARISA) fingerprinting and community composition was analyzed by ribosomal amplicon pyrosequencing. Small temporal scale comparison was carried out in *Mnemiopsis leidyi*. The experiment is presented in Chapter I.

Bacterial communities associated with Scyphomedusa

The metagenetic life cycle of scyphozoans consist of the sexual reproductive medusa generation and the asexual reproductive polyp generation. Although numerous studies have focused upon the general life history distribution, reproductive behavior, factors leading to strobilation, growth rates and impact of predation rate of medusae, bacteria associated with different life stages of Scyphozoa have received little attention. Therefore, in the current study, bacterial communities associated with different body parts and different life stages of two scyphomedusae *Cyanea*

lamarckii and *Chrysaora hysoscella*, which are common species in the German Bight, North Sea, were analyzed. This study aimed at investigating the following questions: (1) Are different body parts of scyphomedusae associated with different bacterial communities? (2) Does the bacterial community composition (BCC) vary in different life cycle stages of scyphomedusae? (3) Does the food source influence the bacterial community associated with polyps? The experiment is presented in Chapter II.

The influence of DOM on bacterial community

Previous studies focused on the impact of decomposition of jellyfish biomass on the microbial community. Their biomass is highly bio-available to "jellyfish – associated" and/or "free – living" heterotrophic bacteria. Jellyfish are also known to release large amounts of organic matter through excretion, feeding and mucus production. However, it remains unclear if jellyfish release specific carbon compounds during their metabolize processing and whether particular bacteria preferentially utilize such compounds. The aim was to identify how the planktonic bacterial communities respond to the dissolved organic matter released by live jellyfish. This was determined by a combination of ARISA fingerprinting and catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH) analysis. The impacts on bacterial abundance were investigated by flow cytometry. The study is presented in Chapter III.

OUTLINE

The present thesis consists of a general introduction, three chapters and a general discussion.

Chapter I (submitted to *FEMS Microbial Ecology*)

Hao WJ, Gerdts G, Peplies J and Wichels A.

Bacterial community associated with Ctenophore at Helgoland Roads, German Bight

The laboratory investigations were carried out by Wenjin Hao. The planning, evaluation and manuscript writing was carried out by Wenjin Hao under the guidance of Antje Wichels and Gunnar Gerdts. Jörg Peplies was in charge of the analysis of 16S ribosomal amplicon pyrosequencing.

Chapter II

Hao WJ, Wichels A, Holst S and Gerdts G.

Bacterial community associated with Scyphomedusae at Helgoland Roads, German Bight

The planning, evaluation and manuscript writing was carried out by Wenjin Hao under the guidance of Antje Wichels and Gunnar Gerdts. Sabine Holst provided the technology for polyp culturing and fruitful discussions.

Chapter III

Hao WJ, Wichels A , Fuchs B and Gerdts G.

Bacterial communities respond to the excretion of DOM released by live jellyfish

The planning, evaluation and manuscript writing was carried out by Wenjin Hao under the guidance of Antje Wichels and Gunnar Gerdts. Jutta Niggemann assisted with the preparation of artificial seawater and will provide the data analysis of DOC and DOM. Bernhard Fuchs provided access to CARD-FISH methodology and Jörg Wolf assisted with the analysis of CARD-FISH samples.

Chapter I

Bacterial communities associated with four Ctenophores in the German Bight (North Sea)

Wenjin Hao[1], Gunnar Gerdts[1], Jörg Peplies[2], Antje Wichels[1]

[1] Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Biologische Anstalt Helgoland, 27498 Helgoland, Germany

[2] Ribocon GmbH Bremen Germany

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Abstract

Intense research has been carried out on jellyfish and ctenophores in recent years because they are increasingly recognized as marine ecosystem key elements, critical indicators and drivers of ecosystem performance and change. Contrary, the bacterial community associated with ctenophores is still poorly investigated. Based on Automated Ribosomal Intergenic Spacer Analysis (ARISA) fingerprinting and ribosomal amplicon pyrosequencing, we investigated the bacterial community associated with the frequently occurring ctenophore species *Mnemiopsis leidyi*, *Beroe* sp., *Bolinopsis infundibulum* and *Pleurobrachia pileus* at Helgoland Roads in the German Bight (North Sea). We observed significant differences between the associated bacterial communities of the different ctenophore species based on ARISA patterns. The bacterial communities of all ctenophore species were dominated by Proteobacteria as revealed by pyrosequencing. *M. leidyi* and *P. pileus* mainly harbored Gammaproteobacteria, with *Marinomonas* being the dominant phylotype of *M. leidyi*. In contrast, in *P. pileus*, *Pseudoalteromonas* and *Psychrobacter* were the dominating phylotypes of Gammaproteobacteria. *Beroe* sp. was mainly dominated by Alphaproteobacteria, particularly by the genus *Thalassospira*. For *B. infundibulum*, the bacterial community is comprised of Alphaproteobacteria and Gammaproteobacteria in equal amounts consisting in particular of the genera *Thalassospira* and *Marinomonas*. Our results indicated that the bacterial community associated with ctenophores might be highly species-specific.

Introduction

Ctenophores (comb jellies) represent a distinct phylum of gelatinous invertebrates that are ubiquitous in all marine environments. Blooms and invasions of ctenophore have been documented in many estuarine, coastal, and open-ocean ecosystems worldwide over the past a few decades (Purcell & Arai, 2001; Pitt *et al.*, 2009; Richardson *et al.*, 2009). It was hypothesized that ctenophores benefit from multiple changes in the ocean which are attribute to anthropogenic impacts, including eutrophication, overfishing and global warming. The numbers of blooms continue to increase globally and some predictions suggest that these gelatinous animals may be ultimately dominant in the oceans in the future, instead of fish-dominated systems (Purcell *et al.*, 2007; Richardson *et al.*, 2009).

Ctenophores and scyphomedusae play an important role in the marine ecosystems by substantially affecting the structure of the planktonic food web structure (Schneider & Behrends 1998; Brodeur *et al.*, 2002; Sommer *et al.*, 2002; Purcell, 2003). On the other hand, they release large amount of nutrients and dissolved organic matter (Nemazie *et al.*, 1993; Schneider & Behrends, 1998) through their metabolic activities, presumably directly stimulating bacterial growth and potentially influencing bacterial community composition (Hansson & Norrman, 1995; Titelman *et al.*, 2006). Condon *et al.* (2011) found that the microbial loop was promoted by the jelly-like DOM produced by ctenophores which was readily available for heterotrophic bacteria. Furthermore, the bacterial community of water column shifted from Alphaproteobacteria to Gammaproteobacteria in response to the addition of the jelly-DOM. All these findings directly indicate the ability of ctenophores to affect carbon cycling and the structure of free-living bacterial communities.

Studies of bacterial communities associated with marine animals have largely focused on sponges, corals, bryozoans and crustaceans (Frias-Lopez *et al.*, 2004; Wang *et al.*, 2004; Kittelmann & Harder, 2005; Ritchie, 2006; Webster *et al.*, 2010). It has been demonstrated that bacterial communities associated with marine invertebrates differ from those in the water column and display host-specificity (Rohwer *et al.*, 2002; Thakur *et al.*, 2004; Webster *et al.*, 2010), while being sometimes variable for species, habitats or seasons (Friedrich *et al.*, 2001; Wichels *et al.*, 2006; Sharp *et al.*, 2007). The outer surface of marine organisms represents the major physiological interface with the environment. Bacterial colonization of a “living” surface may be

influenced by several factors including the age of the colonized organism and the release of organic metabolites or extracellular polymers which has been exemplified for various marine invertebrates such as ascidians (Wahl *et al.*, 1994), corals (Neulinger *et al.*, 2008), sponges (Thakur *et al.*, 2003) and bryozoans (Kittelmann & Harder, 2005). These epibiotic bacteria have been reported not only to pose direct positive or negative effects on the colonized organism, such as interferences with gas and nutrient exchange (Wahl *et al.*, 2011) or a susceptibility to diseases (Ritchie, 2006), but also to involve in the development and evolution of the organism (McFall-Ngai & Ruby, 1991; Nyholm & McFall-Ngai, 2004).

Unfortunately, bacterial communities associated with ctenophores so far have not been fully characterized and the functions and mechanisms of associated bacterial communities have not been determined. Until now, only two studies have been published concerning this subject. Daniels and Breitbart (2012) found that specimens of two ctenophore species (*Mnemiopsis leidyi* and *Beroe ovata*) from Tampa Bay contained fewer bacterial OTUs by T-RFLP and a low diversity as revealed by 16S rRNA clone library analysis, when compared with the water column. Interestingly, Dinasquet (2012) who applied 454 pyrosequencing of 16S rRNA genes found a similar bacterial community composition (BCC) associated with *M.leidyi* in the Gullmar fjord at the west coast of Sweden.

The aim of this study was to investigate the bacterial community associated with different ctenophore species in the German Bight (North Sea). Specimens were collected from the natural environment as they occurred in the water column. *Mnemiopsis leidyi*, which originally occurred in South America (GESAMP, 1997), has invaded the Black, Azov, Marmara and Aegean Seas during the last two decades (Purcell *et al.*, 2001) and was recently identified as an invasive species at Helgoland Roads (Boersma *et al.*, 2007; Hamer *et al.*, 2011). Conversely, *Beroe* sp., *Bolinopsis infundibulum* and *Pleurobrachia pileus* are indigenous ctenophores at Helgoland Roads (Greve, 1970).

Materials and methods

Sample collection and preparation

Individual specimen of ctenophores *Mnemiopsis leidyi*, *Beroe* sp., *Bolinopsis infundibulum* and *Pleurobrachia pileus* were collected at Helgoland Roads in the Germany Bight (54˚11.3′N, 7˚54.0′E) from Nov. 2009 to Oct. 2010 three times per week using a 500 μm mesh trawl towed by the research vessel ‘‘Aade’’. The samples were transferred to the laboratory within 2 h and observed under a dissecting microscope for morphological identification. The identification was determined based on Greve (1975), Faasse & Bayha (2006) and Byern *et al*. (2010). Maximum 10 intact individuals of each species were collected at each sampling day. Each individual was carefully rinsed 5 times with sterile seawater to remove transient and loosely associated microorganisms from the surfaces of ctenophores. All specimens were checked to make sure no visible gut content and stored at -20˚C and freeze-dried prior to DNA extraction.

DNA extraction

Total genomic DNA was extracted from freeze-dried tissue using CTAB (cetyl-trimethyl-ammonium bromide) according to the modified protocol of Gawel (1991). Ctenophore samples were homogenized by using a sterile mortar and pestle. Aliquots from ground samples (1 mg) were transferred to 2 mL pre-heated (60°C) CTAB buffer (3% CTAB, 1.4M NaCl, 20mM EDTA, 100mM Tris-HCl, 0.2% mercaptoethanol) and incubated at 65°C for 30 min. 1 mL STE buffer (6.7% saccharose, 50mM Tris, 1mM EDTA, pH 8), lysozyme (10mg/mL) and proteinase K (10mg/mL) were added to samples and incubated at 50°C for further 30min. DNA extraction was performed twice using phenol–chloroform–isoamylalcohol (25 : 24 : 1) , followed by DNA precipitation with isopropanol overnight at -20°C. DNA was washed with 75% ethanol and finally dried in sterile bench. All DNA extracts were dissolved 30-50µl sterile water and served as template DNA for the PCR. The quantity and quality of extracted DNA were determined by microphotometry using Tecan Infinite 200 NanoQuant.

Automated Ribosomal Intergenic Spacer Analysis (ARISA)

For the PCR reaction, the internal transcribed spacer region was amplified with the forward primers L-D-Bact-132-a A-18 (5'-CCG GGT TTC CCC ATT CGG-3') and the fluorescently labeled reverse primer S-D-Bact-1522-b-S-20 (5'-TGC GGC TGG ATC CCC TCC TT-3') (Ranjard *et al.*, 2000). PCR reaction and cycling conditions were performed as described previously (Krause *et al.*, 2012). In particular, 50 ng of genomic DNA template was applied in each reaction. Based on the intensities of PCR products on agarose gels, original or diluted PCR products were mixed with equal volume of stop mix and separated on 5.5% polyacrylamide gels prepared according to the manufacturer's protocol (LI-COR Biosciences, Lincoln, NE, USA), as well as the sample preparation and running condition. We used a 50-1500bp standard as a size reference (all materials: LI-COR Biosciences, Lincoln, NE, USA).

16S ribosomal amplicon pyrosequencing

Based on significant differences in bacterial community structure as revealed by ARISA fingerprints, 96 samples from *M. leidyi*, 44 from *Beroe* sp. 44 from *B. infundibulum* and 18 samples from *P. pileus* were chosen and pooled for each ctenophore species. The sequencing approach was performed with these 4 pooled samples by LGC Genomics (Berlin, Germany). The V1-V6 region of the 16S RNA gene was amplified using the following primer set: forward GM3 5'-AGAGTTTGATCMTGGC-3' and reverse 907R 5'-CCGTCAATTCMTTTGAGTTT-3'. Sequencing was performed on a 454 Roche Genome Sequencer FLX + Titanium.

Statistical analysis

Analysis of ARISA data

ARISA gel images were analyzed by using BioNumerics 6.6 software (Applied Maths, Sint-Martens-Latem, Belgium). Normalization of band patterns was conducted automatically referencing by the size standard and the presence or absence of each band was determined based on the normalized minimum threshold density (5%). Binning to band classes was performed according to Kovacs *et al*. (2010) and Brown (2005). Bands smaller than 300bp were neglected for multivariate analyses.

Multivariate analyses

The permutational multivariate ANOVA (PERMANOVA) with fixed factor was applied to investigate the differences of four species regarding their associated bacterial community structure (BCS) based on Jaccard coefficient. Principal co-ordinate analysis (PCO) was performed to visualize patterns of the bacterial community influence by species. For all multivariate analyses, we used Primer 6 with the add-on package PERMANOVA+ (PRIMER-E Ltd, Plymouth, UK).

Processing of pyrosequencing data

Sequence reads from PCR amplicon pyrosequencing were processed by bioinformatics pipeline of the SILVA rRNA gene database project (Pruesse, 2007) which has been described by Teeling *et al*. (2012) and Ionescu *et al*. (2012). Dereplication (identification of identical reads ignoring overhangs), clustering (OTU definition based on a non-redundant subset of reads) and classification were independently performed with sufficient quality sequences. Finally, the quantitative information of individual reads representing a taxonomic path was obtained. The relative abundances of the identified taxonomic groups were calculated as percentages. However it should be mentioned here, that the numbers of the sequence reads do only reflect the rDNA abundances in the amplicon pool and are therefore an approximation of the natural abundance.

Results

BCC of different ctenophores species

Four ctenophore species, including *Mnemiopsis leidyi*, *Beroe* sp., *Bolinopsis infundibulum* and *Pleurobrachia pileus*, were occurred at Helgoland Roads from Nov. 2009 to Oct. 2010. From January to March in 2010 no animals could be observed. Furthermore, the freeze-dried biomass of juvenile (diameter small than 10mm) *Pleurobrachia pileus* collected in April 2010 was not sufficient for BCC analyses. Hence, no data of ctenophores were obtained from January to April in 2010. In total, 496 ctenophore specimens were collected and analyzed (Tab.1). *M. leidyi* was present and abundant throughout the whole sampling period except for May 2010 and 354 individuals were collected. In contrast, *Beroe* sp. and *P. pileus* occurred generally in low numbers. 44 individuals of *Beroe* sp. were collected except in August 2010 and 42 individuals of *P. pileus* were collected in May, June and July 2010. *B. infundibulum* only appeared in June 2010 where 56 individuals were collected. For all samples DNA was successfully extracted and amplified by the modified protocols of Gawel (1991) and Ranjard (2000).

Based on ARISA fingerprints, the PCO plot depicted the BCC of all specimens of the four ctenophore species (*M. leidyi*, *Beroe* sp., *B. infundibulum* and *P. pileus*) (Fig.1). The bacterial communities of different ctenophore species were well separated in PCO ordination which was confirmed by PERMANOVA main test (Tab. 2). Pair-wise comparisons further illustrated that the four ctenophore genera contained distinct different bacterial community (P=0.001) (Tab. 3). The highest amount of variation occurred between *M.leidyi* and all other three species (*t* statistic). The bacterial richness (alpha diversity) (supplementary Fig.1), as estimated by ARISA-OTUs (operational taxonomic units) numbers, was variable among all four species. The highest OTU richness was recorded in *Beroe* sp. (S = 36), followed by *B. infundibulum* (S = 22) and *M.leidyi* (S = 21), while the lowest richness was observed in *P.pileus* (S = 5).

Temporal variation of BCC in *M. leidyi*

Mnemiopsis leidyi was the only species which was collected almost throughout the whole sampling period except for May (no occurrence) and June 2010 (one specimen). Hence, the seasonal trend in the BCC was only analyzed for this species. In the PCO plot (Fig. 2), BCCs of

summer samples (July and August) clearly clustered together and were separated from those of autumn and winter (Sep., Oct., Nov. and Dec.). Consistent with the PCO ordination, PERMANOVA main test and pair-wise comparisons of BCCs revealed significant differences regarding factor “month” (P=0.001) (Tab. 4 and Tab. 5). This indicated a significant seasonal variation in the bacterial community of *M. leidyi*.

Classification of DNA sequence reads

Based on the findings of the ARISA analyses, randomly chosen samples from each ctenophore species were pooled and subjected to 16S ribosomal amplicon pyrosequencing. Concerning this subset of samples, PCO ordination and PERMANOVA analyses again displayed significant difference between the BCCs of the ctenophore species (Fig. 3, Tab.6 and Tab.7). Similar variation of both selected (Tab. 6) and total samples (Tab. 2) explained by Sq.root indicated that the sample subsets represented the bacterial community associated with each ctenophore species.
In total 244885 raw pyrosequencing reads of the 16S rDNA spanning the hypervariable regions V1-V6 were obtained from the four ctenophore species *M. leidyi*, *Beroe* sp., *B. infundibulum* and *P. pileus*. After removing insufficient quality sequences and sequences that could not be adequately sub-classified, 128244 reads were used for subsequent analysis including 21721 of *Beroe* sp., 21118 of *B. infundibulum*, 28318 of *M. leidyi* and 57087 of *P. pileus*. Taxonomic classification of bacterial reads from the four ctenophore species at different levels were determined by SILVA classifier based on 98% similarity. Singletons (n=1) and rare reads (<1%) were omitted in the results presented in Figure 4. The percentages of the different phylogenetic groups were taken to describe the relative abundances of these groups within the samples. However it should be mentioned here, that the numbers of the sequence reads are only an approximation of the natural abundance and do only reflect the rDNA abundances in the amplicon pool.
Altogether four bacterial phyla were recovered from the four ctenophore species (Fig. 4A). Proteobacteria was the dominating phylum in all four ctenophore species, with the highest ratio in *P.pileus* (98%), followed by *Beroe* sp. (93%), *B. infundibulum* (89%) and *M. leidyi* (88%). Actinobacteria were present in four ctenophores species with different ratio, the highest in *B. infundibulum* (9%), followed by *M. leidyi* (6%), *Beroe* sp. (2%) and *P. pileus* (1%). Reads

matching to Tenericutes (1-5%) and Firmicutes (0-1%) were found to be the minor groups (Fig.4A). Tenericutes were mainly present in *Beroe* sp. and *M.leidyi* with similar ratios (~ 5%).
Within Proteobacteria (Fig.4B), Gammaproteobacteria were dominant both in *M. leidyi* (99%) and *P.pileus* (98%). In contrast in *Beroe* sp., Alphaproteobacteria (94%) were the major group of Proteobacteria, followed by Gammaproteobacteria (4%) and Betaproteobacteria (2%). For *B.infundibulum*, Proteobacteria consisted of 47% Alphaproteobacteria and 53% Gammaproteobacteria.
Within the Gammaproteobacteria at the family level (Fig.4C), particularly Oceanospirillaceae were the dominant bacterial group in *M. leidyi* (94%) and *B. infundibulum* (62%), and interestingly also in *Beroe* sp. (59%) with only a small proportion of Gammaproteobacteria (4%). Contrary, Pseudoalteromonadaceae (55%) and Moraxellaceae (43%) constituted a significant portion of reads from *P. pileus*. In addition, the bacterial community of *Beroe* sp. and *M. leidyi* consisted of 19% and 4% Vibrionaceae, respectively. At the genus level of Gammaproteobacteria (Fig. 4D), *Marinomonas* dominated the bacterial community in *M. leidyi* (94%), *B. infundibulum* (63%) and *Beroe* sp. (60%). For *P. pileus*, there were two dominant genera: *Psychrobacter* (43%) and *Pseudoalteromonas* (56%). Additionally, reads affiliated to *Pseudoalteromonas* (20%) were also detected in *B. infundibulum*.
Within Alphaproteobacteria at the family level (Fig.4E), Rhodospirillaceae were the dominant bacterial family in *Beroe* sp. (100%), *B. infundibulum* (93%) and *P. pileus* (87%). In contrast regarding *M. leidyi*, the Alphaproteobacteria representing only 1% at class level were dominated by Rhodobacteraceae family (56%). At the genus level (Fig.4F), *Thalassospira* dominated the bacterial community of *Beroe* sp. (100%), *B. infundibulum* (94%) and *P. pileus* (89%). The Alphaproteobactria in *M. leidyi* consisted of 18 different genera, each with very low numbers and uncultured Rhodobacteraceae (20%) as the main group.

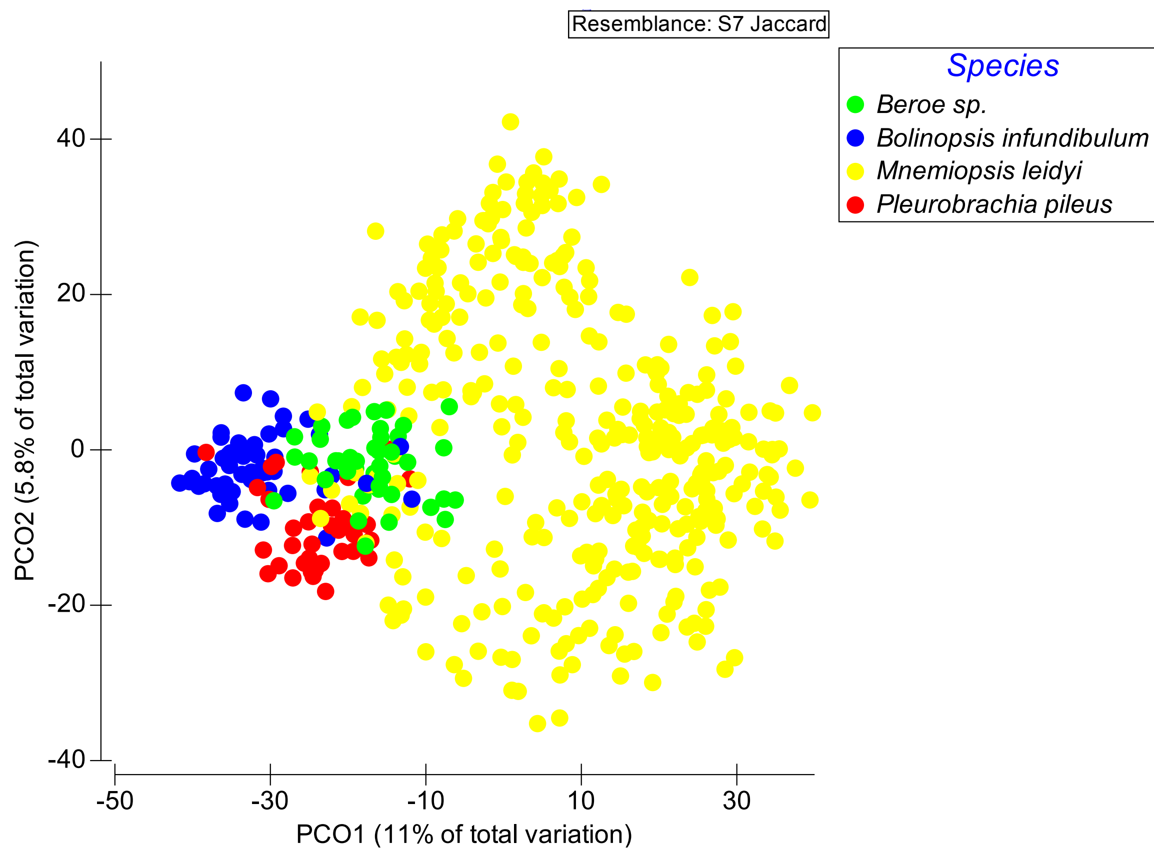


Figure 1. Principal coordinate (PCO) analysis presenting the bacterial communities associated with four ctenophore species based on Jaccard coefficient from ARISA profiles.

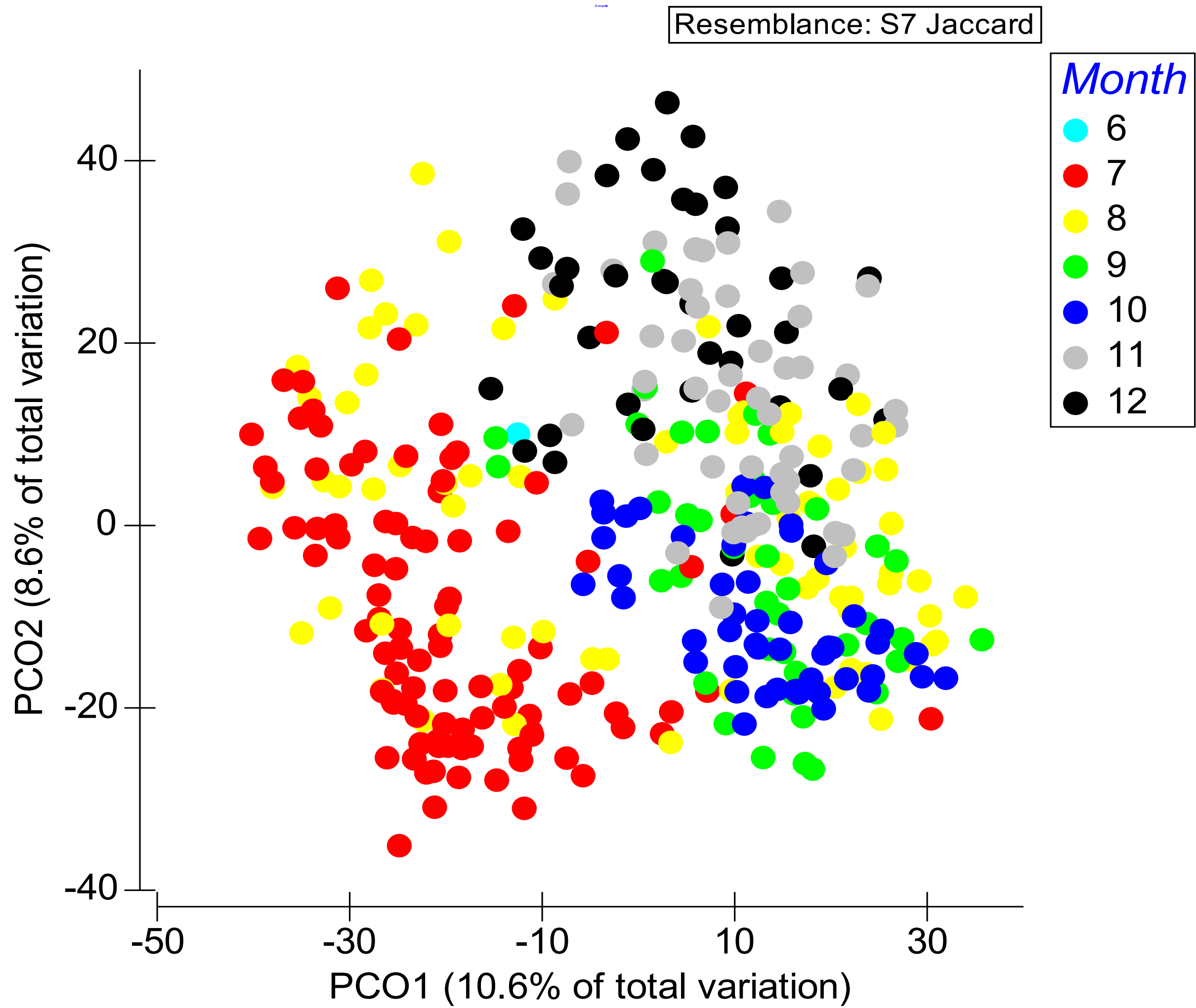


Figure 2. Principal coordinate (PCO) analysis presenting the differences of bacterial communities associated with *Mnemiopsis leidyi* based on Jaccard coefficient from ARISA profiles

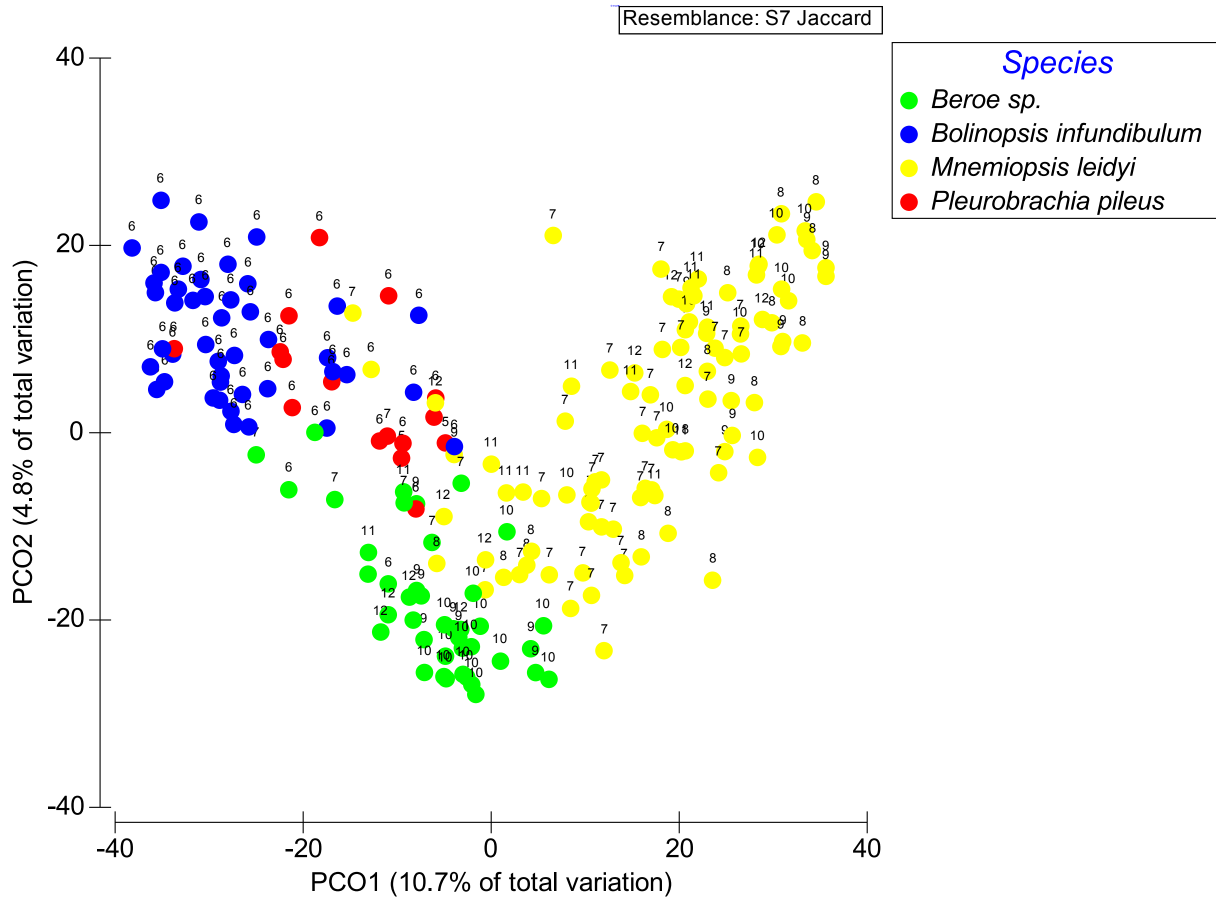


Figure 3. Principal coordinate (PCO) analysis presenting the bacterial communities associated with four ctenophores species which applied for 16s ribosomal amplicon pyrosequencing based on Jaccard coefficient. (Number of month are shown beside the species symbols)

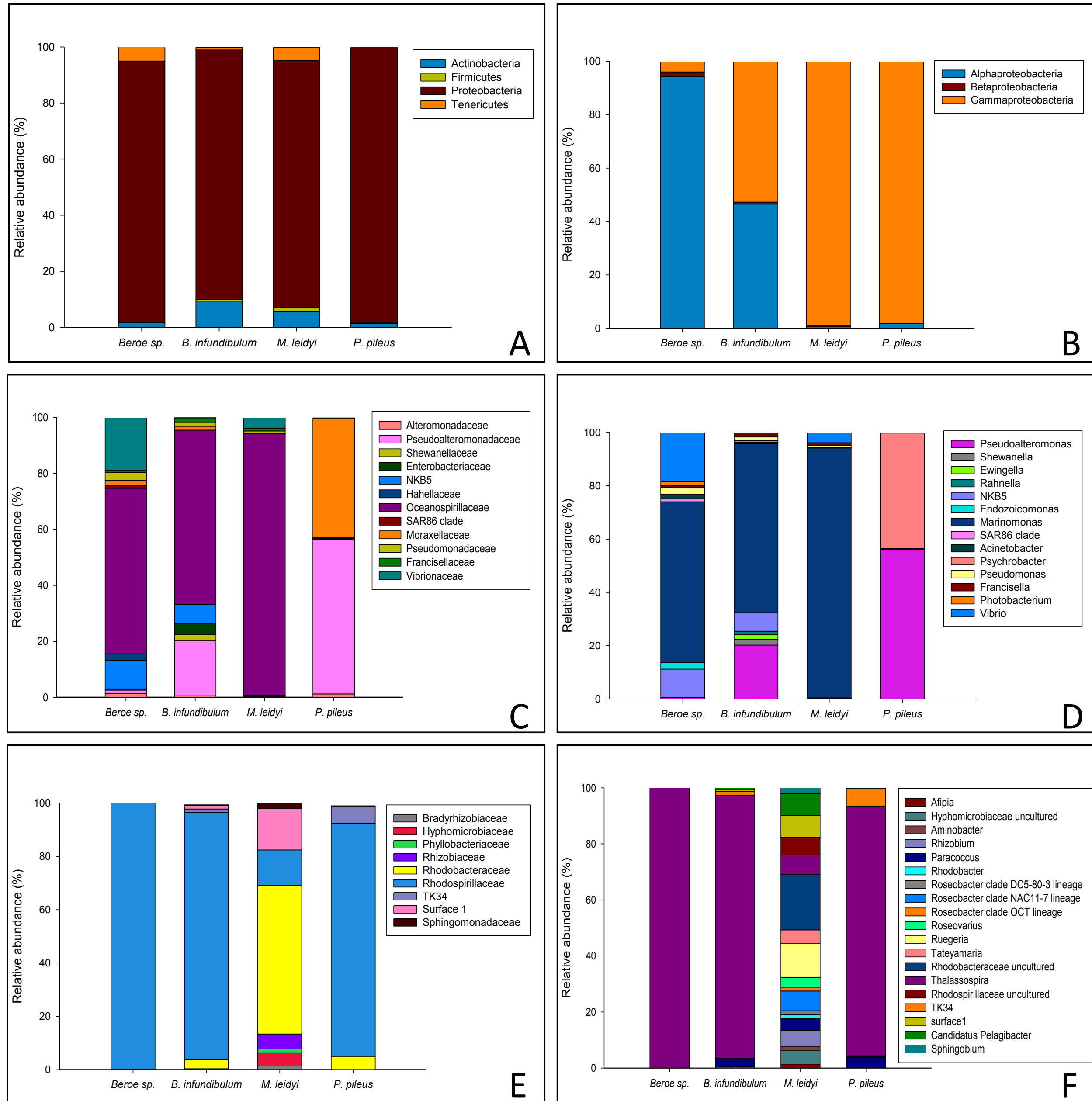


Figure 4. Taxonomic classification of bacterial reads from different ctenophore species at different levels using SILVA classifier based on 98% similarity omitting singletons (n=1) and rare reads (<1%). (A. Phylum, B. Classes of Proteobacteria, C. Family of Gammaproteobacteria, D. Genera of Gammaproteobacteria, E. Family of Alphaproteobacteria, F. Genera of Alphaproteobacteria.)

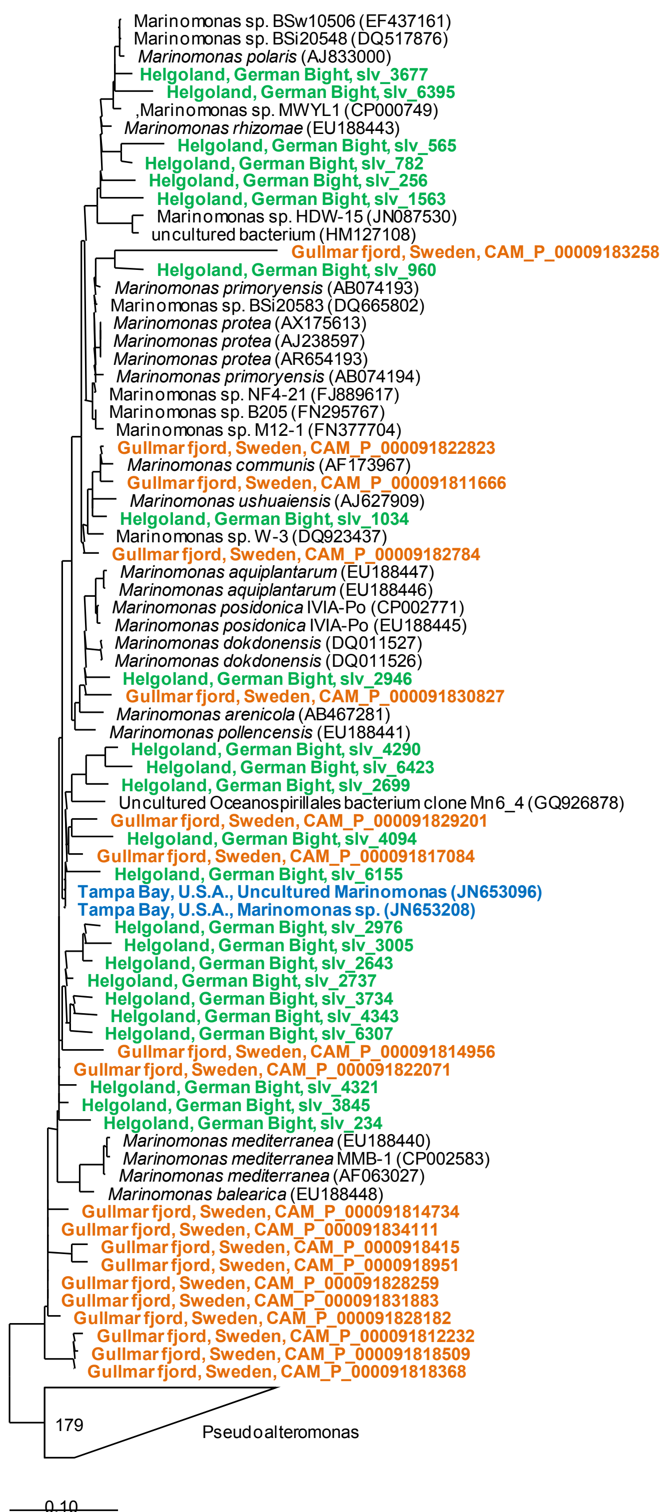


Figure 5. Phylogenetic tree based of partial 16S ribosomal gene of the genus *Marinomonas* spp. illustrating the occurrence of closely related members of this genus in the ctenophore species Mnemiopsis leidyi from different geographical origin (green: this study, orange: Dinasquet *et al*., 2012, blue: Daniels & Breitbart, 2012)

Table 1. Amount of ctenophores collected at Helgoland Roads during the sampling period from November 2009 to October 2010. (The numbers shown here are summed up by weeks of each month)

Month. Year / Species	11.09	12.09	05.10	06.10	07.10	08.10	09.10	10.10
Mnemiopsis leidyi	52	38	0	1	98	74	42	49
Beroe sp.	2	5	0	3	6	0	9	19
Bolinopsis infundibulum	0	0	10	28	3	0	0	0
Pleurobrachia pileus	0	0	0	57	0	0	0	0

Table 2. PERMANOVA main tests of bacterial community composition associated with four ctenophore species based on Jaccard dissimilarities of ARISA profiles.

Significant results (p (perm)<0.05) are highlighted in bold. Displayed are tests for the factor ‘species’ and the partitioning of multivariate variation. p-values were obtained using type III sums of squares.

d.f.: degrees of freedom, SS: sums of squares, Sq. root: square root of the component of variation attributable to that factor in the model, in units of Jaccard dissimilarities.

Source of variation	d.f.	SS	*pseudo F*	*p (perm)*	Perms	Sq. root
Species	3	223000	21.718	**0.001**	994	30.739
Residuals	489	1680000				58.551
Total	492	1900000				

Table 3. PERMANOVA pair-wise comparisons of bacterial community composition associated with four ctenophore specie based on Jaccard dissimilarities of ARISA profiles.

Significant results (p (perm)<0.05) are highlighted in bold. Displayed are pair-wise *a posteriori* comparisons of the factor “species”.

Comparison	*t (perm)*	*p (perm)*	Perms
Beroe sp. vs. *B.infundibulum*	3.4608	**0.001**	998
Beroe sp. vs. *M. leidyi*	4.0271	**0.001**	999
Beroe sp. vs. *P. pileus*	3.1212	**0.001**	997
B. infundibulum vs. *M. leidyi*	5.7974	**0.001**	999
B. infundibulum vs. *P. pileus*	3.6843	**0.001**	998
M. leidyi vs. *P.pileus*	4.7464	**0.001**	996

Table 4. PERMANOVA main tests of bacterial community composition associated with *Mnemiopsis leidyi* based on Jaccard dissimilarities of ARISA profiles.

Significant results (p (perm) <0.05) are highlighted in bold. Displayed are tests for the factor ‘Month’ and the partitioning of multivariate variation. p-values were obtained using type III sums of squares.

d.f.: degrees of freedom, SS: sums of squares, Sq. root: square root of the component of variation attributable to that factor in the model, in units of Jaccard dissimilarities.

Source of variation	d.f.	SS	*pseudo F*	*p (perm)*	Perms	Sq. root
Month	6	211000	12.424	**0.001**	997	25.98
Residuals	347	984000				53.246
Total	353	120000				

Table 5. PERMANOVA pair-wise comparisons of bacterial community composition associated with *Mnemiopsis leidyi* based on Jaccard dissimilarities of ARISA profiles.

Significant results (p (perm) <0.05) are highlighted in bold. Displayed are pair-wise *a posteriori* comparisons of the factor “Month”.

Comparison	t (perm)	*p (perm)*	Perms
7, 8	3.3283	**0.001**	997
7, 12	4.0879	**0.001**	998
7, 11	4.7004	**0.001**	998
7, 9	3.976	**0.001**	998
7, 10	4.6726	**0.001**	999
8, 12	3.1392	**0.001**	999
8, 11	3.7009	**0.001**	997
8, 9	2.6566	**0.001**	998
8, 10	3.3845	**0.001**	999
12, 11	2.5652	**0.001**	999
12, 9	3.4674	**0.001**	998
12, 10	4.3174	**0.001**	999
11, 9	3.7679	**0.001**	997
11, 10	4.5288	**0.001**	997
9, 10	3.8645	**0.001**	999

Table 6. PERMANOVA main tests of bacterial community composition associated with four ctenophores species which applied for 16s ribosomal amplicon pyrosequencing based on Jaccard dissimilarities of ARISA profiles.

Significant results (p (perm)<0.05) are highlighted in bold. Displayed are tests for the factor 'species' and the partitioning of multivariate variation. p-values were obtained using type III sums of squares.

d.f.: degrees of freedom, SS: sums of squares, Sq. root: square root of the component of variation attributable to that factor in the model, in units of Jaccard dissimilarities.

Source of variation	d.f.	SS	*pseudo F*	*p (perm)*	Perms	Sq. root
Species	3	115000	11.092	**0.001**	995	28.24
Residuals	195	674000				58.804
Total	198	789000				

Table 7. PERMANOVA pair-wise comparisons of bacterial community composition associated with four ctenophores species which applied for 16s ribosomal amplicon pyrosequencing based on Jaccard dissimilarities of ARISA profiles.

Significant results (p (perm)<0.05) are highlighted in bold. Displayed are pair-wise *a posteriori* comparisons of the factor "species".

Comparison	t (perm)	*p (perm)*	Perms
Beroe sp. vs. *B.infundibulum*	3.1676	**0.001**	997
Beroe sp. vs. *M. leidyi*	3.2441	**0.001**	999
Beroe sp. vs. *P. pileus*	2.3305	**0.001**	996
B. infundibulum vs. *M. leidyi*	4.4218	**0.001**	998
B. infundibulum vs. *P. pileus*	2.4871	**0.001**	996
M. leidyi vs. *P.pileus*	2.8418	**0.001**	999

Discussion

Although several studies on the bacterial community composition (BCC) of coelenterata were published in the past, it must be stated that the majority of these studies focused on corals. Compared to other marine invertebrates (e.g. sponges), the knowledge on ctenophore is still scarce here (studies from below). This is even more the fact concerning ctenophora with only two currently published studies (Daniels & Breitbart, 2012; Dinasquet *et al.*, 2012). In this context, present study is the first investigation on the BCC of frequently occurring ctenophore species at Helgoland Roads: *Mnemiopsis leidyi*, *Beroe* sp., *Pleurobrachia pileus* and *Bolinopsis infundibulum*.

Gammaproteobacteria were highly dominant in the bacterial community associated with *M. leidyi* and *P. pileus*. However, the composition of the Gammaproteobacteria was completely different in *M. leidyi* and *P. pileus*. In the present study, three major groups Oceanospirillaceae, Pseudoalteromonadaceae and Moraxellaceae were detected in these ctenophore species, in particular, *Marinomonas*, *Pseudoalteromonas* and *Psychrobacter*. *Marinomonas* highly dominated the community of *M. leidyi*, *Beroe* sp. and *B. infundibulum* in our study. Bacteria of the genus *Marinomonas* have been previously reported in bacterial communities of corals, sponges and bryozoans (Li *et al.*, 2007; Heindl *et al.*, 2010; Chimetto *et al.*, 2011). Interestingly in two recent studies on the BCC of ctenophore (Daniels & Breitbart, 2012; Dinasquet *et al.*, 2012) performed in the Tampa Bay, Gulf of Mexico and the Gulmar Fjord, *Marinomonas* was also identified as the prominent genus. Figure 5 exemplarily shows a phylogenetic tree of *Marinomonas* spp. related DNA sequences retrieved from those two studies as well as from our study. This occurrence of closely related *Marinomonas* species from different geographical origins gives probably evidence to suggest that *Marinomonas* is a common member of the bacterial community associated with ctenophora on a more global scale, but this need to be proven in further studies. If this is the case, the functional role of this genus in this consortium has to be elucidated (symbiosis, commensalism and pathogen). It is known, that *Marinomonas* contain multifunctional polyphenol oxidases which are able to oxidize a wide range of substrates (Solano & Sanchez-Amat, 1999) and are involved in a series of secondary metabolism and biodegradative processes (Sanchez-Amat *et al.*, 2001). Lucas-Elio (2005) found that *M. mediterranea* synthesizes an antibacterial protein with activity against both gram-positive and

gram-negative bacteria. Hence, it may be speculated that these bacteria have the metabolic capacity to colonize the surface of ctenophore species as well as to degrade gelatinous tissue.

Bacteria of the genus *Pseudoalteromonas* usually do not account for more than 1% of the total counts in the free-living bacterial community in North Sea. Interestingly, they are frequently detected in the attached fraction of the marine bacterial community (Eilers *et al.*, 2000). Moreover, they were also frequently found in association with living surface of eukaryotes such as sponges, mussels, pufferfish and a range of algae (Holmström *et al.*, 2002). Our pyrosequencing results showed that *Pseudoalteromonas* occurred in *B. infundibulum* and *P. pileus*. Bacteria of this genus are known to produce a variety of highly bioactive compounds, including extracellular enzymes, exopolysaccharides and a range of different molecular weight compounds with antimicrobial, anti-fouling, algicidal and various pharmaceutically relevant activities (Holmström & Kjelleberg, 1999; Bowman, 2007). These capabilities successfully enable them to compete for nutrients and colonize surfaces (Holmström *et al.*, 2002). It is possible that such antibacterial activities might prevent the colonization of other bacterial groups resulting in the lower diversity in the community of *B. infundibulum* and *P. pileus* compared with other ctenophores (*M. leidyi* and *Beroe* sp.) (Fig. 4 and Supplemental Fig.1). Tinta *et al.* (2012) observed a rapid shift in the community composition from Alphaproteobacteria to Gammaproteobacteria, which was highly dominated by *Pseudoalteromonas*, in response to the addition of jellyfish substrate. The majority of *Pseudoalteromonas* species are characterized by specifically hydrolytic activities (Ivanova *et al.*, 1998). Hence, it is not surprising that jellyfish biomass is faster decomposed compared to non-gelatinous zooplankton (West *et al.*, 2009; Frost *et al.*, 2012) whose biochemical composition is comprised of protein rich organic matter (Hoeger, 1983; Clarke *et al.*, 1992; Finenko, 2001). This indicates that jellyfish represent high quality particulate organic matter (POM) for bacteria (Titelman *et al.*, 2006; Pitt *et al.*, 2009; Tinta *et al.*, 2010).

Bacteria of the genus *Psychrobacter*, which comprise of strictly aerobic chemo-organotrophic, mainly psychro-tolerant and halo-tolerant, nonmotile gram-negative cocci (Shcherbakova *et al.*, 2009), were observed only in *P. pileus*. These bacteria have previously been isolated from a variety of low temperature marine environments (Romanenko *et al.*, 2004), Antarctic sea ice (Heuchert *et al.*, 2004; Shivaji *et al.*, 2005), deep seawater (Maruyama *et al.*, 2000) and the stomach contents of the Antarctic krill (Denner *et al.*, 2001). So far little is known specifically

concerning their role and function in marine ecosystems. Recent investigations indicate that the distribution of this genus is even more cosmopolitan than originally anticipated (Pukall *et al.*, 2001).

Alphaproteobacteria, another important group in the associated bacterial community of ctenophores, dominated the communities of *Beroe* sp. (~94%) and *B. infundibulum* (~47%). Most prominent group is the genus *Thalassospira* (Rhodospirillaceae) which is known to be chemotactic toward inorganic phosphate (Hütz *et al.*, 2011). *Thalassospira* can utilize hydrocarbons, carbohydrates, organic acids or amino acids as sole carbon sources for growth and degrade polycyclic aromatic hydrocarbons (PAHs) in oil-contaminated seawater (Kodama *et al.*, 2008). Interestingly the bacterial community of *B. ovate* from Tampa Bay was also dominated by members of this genus which is in accordance with our findings. In their study, the bacterial clones affiliate to *Thalassospira* in both *M. leidyi* and *B. ovate* (Daniels & Breitbart, 2012). In contrast, Dinasquet *et al*. (2012) found *Thalassospira* neither in tissue nor in gut samples. Bacteria of the Rhodobacteraceae dominated the Alphaproteobacteria associated with *M. leidyi* in the Gullmar fjord (Dinasquet *et al.*, 2012) which is in agreement with our findings that Rhodobacteraceae was the major group in Alphaproteobacteria associated with *M. leidyi* at Helgoland Roads even though they only account for 1% of the whole community (Fig. 4B and 4E).

Except for the predominant Proteobacteria, other phyla, such as Actinobacteria, Tenericutes and Firmicutes, were detected in small numbers in our study. Additionally the taxonomic composition of these phyla in each ctenophore species was different among the four ctenophores species with no common trend. Actinobacteria mainly occurred in the community of *B. infundibulum* and *M. leidyi*. Notably Dinasquet *et al*. (2012) found Actinobacteria especially in the gut community of *M. leidyi*. Probably the presence of Actionbacteria, although we checked the animals for empty guts, is the remnants of the gut community which were still present in some of our samples. *Mycoplasma* and *Spiroplasma*, from the phylum Tenericutes, occurred occasionally in four ctenophores (data not shown). *Mycoplasma* occurred in *M.leidyi* and *B. infundibulum* with varying ratios of 4% and 1%, respectively. *Spiroplasma* was present in *M.leidyi* and *Beroe* sp. with 1% and 5%, respectively. Both bacterial groups were reported in *B. ovate* and *M.leidyi* from the Gulf of Mexico also in low numbers (Daniels & Breitbart, 2012).

The presence of Tenericutes in ctenophores from geographically disparate regions might again hint to a specialization of certain bacterial groups associated with ctenophores.

Bacteria of the Bacteroidetes phylum usually occur in coastal and marine environments throughout the year, partly dominating the bacterioplankton in the North Sea especially during or after phytoplankton blooms (Teeling *et al.*, 2012). Interestingly, the Bacteroidetes seem to play a minor role with < 1% occurrence in all ctenophore species in present study. However, members of this phylum were present in *M. leidyi* (10%) and *B. ovata* (25%) at Tampa Bay (Daniels & Breitbart, 2012). Investigation of different body parts of *M. leidyi,* Dinasquet *et al*. (2012) reported that specifically the gut community of starved *M. leidyi* was dominated by the genus *Tenacibaculum,* a Flavobacterium related to the fish pathogen *Tenacibaculum maritimum* with strong proteolytic activity (Bernardet & Nakagawa, 2006; Ferguson *et al.*, 2010; Delannoy *et al.*, 2011; Mitchell *et al.*, 2013). Recent research showed that the physical damage in the gill disease of *Salmo salar* (farmed salmon) was caused by nematocysts which was accompanied by the introduction of pathogen bacteria, such as *Tenacibaculum maritimum* (Ferguson *et al.*, 2010). *T. maritimum* was isolated from various species of jellyfish, including the hydromedusae *Solmaris corona* (Baxter *et al.*, 2011), the scyphomedusae *Pelagia noctiluca* (Doyle *et al.*, 2008) and *Aurelia aurita* (Mitchell *et al.*, 2013). It has recently been implicated that certain jellyfish species may act as vectors of bacterial disease for fishes (Mitchell & Rodger, 2011). However, according to our findings, the absence of Bacteroidetes in our study can be taken as evidence for successful defecation and cleaning of the ctenophores before further processing ctenophore biomass since Bacteroidetes are commonly found to be associated with small plankton organisms, potential food of ctenophores at Helgoland Roads.

In the present study, the bacterial communities associated with ctenophores are clearly dominated by Proteobacteria of the Alpha- and Gamma class and some minor phyla with variable composition in respect to the different ctenophore species. Comparison between the BCC associated with ctenophore and the bacterioplankton community at Helgoland Roads reveals strong differences both in diversity and composition. Teeling *et al*. (2012) stated that distinct populations of Bacteroidetes (Flavobacteria 89-98%), Gammaproteobacteria (*Reinekea* spp. and SAR92) and Alphaproteobacteria (2/3 SAR11 and 1/3 Rosebacter) were predominant in the bacterioplankton at Helgoland Roads. These phyla also dominated the community of various planktonic organisms such as diatoms and dinoflagellates (Kan *et al.*, 2007; Sapp *et al.*, 2007 a;

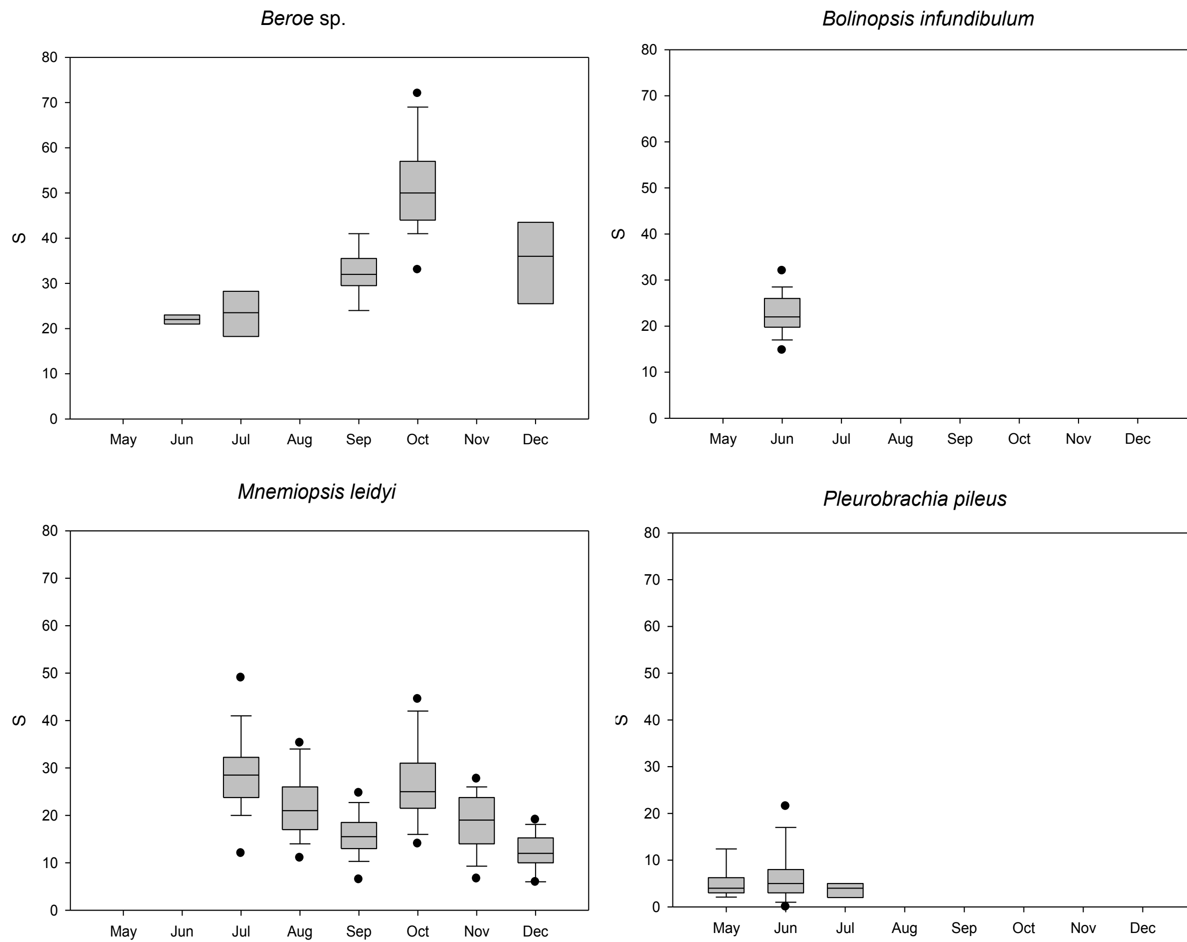
Sapp *et al.*, 2007 b; Sapp *et al.*, 2007 c; Gilbert *et al.*, 2012). The dominant groups of ctenophores within Gammaproteobacteria, such as *Pseudoalteromonas*, and *Psychrobacter* are detected frequently in seawater but with relative low percentages. In contrast *Marinomonas* so far was identified only occasionally in seawater (Eilers *et al.*, 2000). Another prominent class in ctenophores, the Alphaproteobacteria was composed of clearly different members as compared with planktonic bacteria. *Thalassospira*, as the most abundant group associated with *Beroe sp*., *B. infundibulum* and *P. pileus*, has not been found in the waters of Helgoland Roads so far (Eilers *et al.*, 2000; Eilers *et al.*, 2000; Eilers *et al.*, 2001; Gerdts *et al.*, 2004; Teeling *et al.*, 2012). Obviously, the ctenophores share similar bacterial groups on a large geographical scale in waters of the German Bight, the Gulmar Fjord and Tampa Bay (e.g. *Marinomonas* spp., Fig 5). In addition the BCC of the surrounding seawater is different in all cases. From these clear compositional differences in the bacterial communities of ctenophores and bacterioplankton, we assume that the observed bacterial community in ctenophores is specifically associated.

Bacterial communities associated with other marine invertebrates have been investigated at Helgoland Roads in the past. Schuett *et al.* (2007) found that intra-tentacular bacteria of the sea anemone *Metridium senile* were affiliated to Gamma- and Betaproteobacteria, including the families Hahellaceae, Burkholderiaceae and Comamonadaceae (Specifically, *Endozoicimonas elysicola, Pseudomonas saccharophilia* and *Ralstonia pickettii*). These three genera were also detected with very low ratios (less than 1%) in the bacterial community associated with *Beroe* sp. and *M.leidyi* in our study. Both of *R. pickettii* and *P. saccharophilia* are common endobiotic bacteria and were already identified in the ascidian tunic matrix of *Diplosoma migrans* collected from Helgoland (Schuett *et al.*, 2005). Pukall *et al*. (2001) investigated the culturable bacteria associated with the North Sea bryozoans *Flustra foliacea*. Gammaproteobacteria identified as *Shewanella frigidimarina*, *Pseudoalteromonas* sp. and *Psychrobacter* sp. were predominant in bryozoan samples from Helgoland Roads and the genus *Pseudoalteromonas* was also found in the tentacle of *Cyanea capillata* (Schuett & Doepke, 2010). Schuett & Doepke (2010) proposed this genus as a common partner of Cnidarian species.

In conclusion, the associated bacterial communities of different ctenophores at Helgoland Roads illustrate on the one hand differences to the surrounding seawater both in diversity and composition and on the other hand species-specific difference regarding the different ctenophores. The genera *Marinomonas*, *Thalassospira*, *Pseudoalteromonas* and *Psychrobacter*

were identified as predominant groups associated with ctenophores. The results obtained from the Tampa Bay of Florida and the Gullmar fjord at the west coast of Sweden were confirmed by our study, suggesting ctenophores from geographically distinct regions shared high similarity in their dominant bacterial communities. For further studies it is worthwhile to localize the associated bacteria in or on the animals in order to reveal the possible origin (lateral transfer, epibiosis, gut or food related). Based on the observed seasonal influenced patterns of the bacterial community of *M. leidyi*, the seasonal variation of bacterial community composition needs to be analyzed in more details. The biogeographic distribution of these bacterial associations needs to be processed further which might be correlated with nutrients, phytoplankton bloom and so on. A comparative analysis of the bacterial community present at different life stages of ctenophores would give possible evidence for the mechanisms of acquisition and maintenance of these associates. Moreover, bacteria of the genus *Tenacibaculum* occurred in other ctenophore studies (Daniels & Breitbart, 2012; Dinasquet *et al*., 2012) serving as a vector for pathogenic bacteria (Ferguson *et al.*, 2010; Delannoy *et al.*, 2011; Daniels & Breitbart, 2012). Although *Tenacibaculum* were not identified in our study, bacteria of the genus *Vibrio* were found in *Beroe* sp. (19%) and *M. leidyi* (4%) and probably serve as potentially pathogenic bacteria in present study (Fig. 4D). Additional information concerning the interaction between pathogen and ctenophore need to be established in future.

Supplementary



Supplementary Figure 1. Box plot of bacterial OTU number (alpha diversity) obtained by ARISA for four ctenophore species grouped by month. The box presents the 25%-75% variation. The middle line in each box depicts the median of the respective data set. Whiskers and outliers (black dots) indicate the distribution of remaining data points, representing the overall variation.

Chapter II

Bacterial community associated with scyphomedusae at Helgoland Roads

Wenjin Hao[1]*, Antje Wichels[1], Sabine Holst[2], Gunnar Gerdts[1]

[1] Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Biologische Anstalt Helgoland, 27498 Helgoland, Germany

[2] Senckenberg am Meer, German Center for Marine Biodiversity Research, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany

Key words: Bacterial community structure, scyphomedusae, life stages, polyps, ARISA

Abstract

Different modes of asexual and sexual reproduction are typical for the life-history of metagenetic scyphozoa. Numerous studies have focused upon the general life history, reproductive behavior, factors leading to strobilation, growth rates and impact of predation rate of medusa. However, bacteria associated with different life stages of scyphozoa have received less attention. In the present study, bacterial communities associated with different body parts and different life stages of two common scyphomedusae species in the German Bight of the North Sea, *Cyanea lamarckii* and *Chrysaora hysoscella*, were analyzed by automated ribosomal intergenic spacer analysis (ARISA). Regarding the analysis of different body parts (umbrella, gonad, tentacle and mouth arm), significant differences were revealed between umbrella and other body parts (gonad and tentacle) in terms of the associated bacterial community in both species. With regard to the different life stages, bacterial community structure varied from the early stage larvae to polyps even to adult medusae with significant differences in both species. Furthermore, the bacterial communities associated with polyps were significantly distinct from that with food in both species. Polyps might react differently in response to different food source (*A. salina* and plankton) resulting in a significantly different bacterial community structure. In general, the bacterial communities associated with two scyphomedusae species were species-specific confirmed in each life stage.

Introduction

As a major component of the pelagic ecosystem, jellyfish represent a conspicuous element of the zooplankton (Brodeur *et al.*, 2002). Scyphomedusae utilize a wide spectrum of zooplankton prey, including fish larvae and eggs, copepods, small ctenophores, and can have a strong impact on zooplankton standing stocks in all parts of the world (Brodeur, 1998; Barz & Hirche, 2007; Decker *et al.*, 2007). The composition of phyto- and zooplankton communities changes considerably accompanied by increasing frequency and intensity of scyphomedusae blooms around the world (Brodeur *et al.*, 1999; Brodeur *et al.*, 2002; Beaugrand *et al.*, 2002; Beaugrand, 2004; Hay, 2006; Barz & Hirche, 2007). The abundance of scyphomedusae showed interannual fluctuations and large variability between regions in the North Sea (Hay *et al.*, 1990).

The metagenetic life cycle of most scyphozoans consists of the sexual reproductive medusa generation and the asexual reproductive polyp generation. They go through five life stages: egg, planula, polyp, ephyra and medusa. Conspicuous mature medusae sexually reproduce eggs that develop into millions of ciliated larvae (planulae) after fertilization. Planulae settle on suitable substrates and metamorphose into inconspicuous polyps (scyphistomae). The scyphistomae are able to asexually produce offspring polyps by successive stolon formation, vegetative budding, longitudinal fission, or cyst formation (Lucas, 2001). Eventually the upper part of the scyphistoma undergoes a series of transverse constrictions and comes to resemble a stack of saucers. By a process called strobilation, the "saucers" develop to star-shaped bodies which successively detach. In this way a sequence of newly released free-swimming juvenile individuals (ephyrae) are produced asexually. The timing of the strobilation process is temperature dependant and species specific (Purcell *et al.*, 2007; Holst, 2012 a). In the North Sea medusae typically are most abundant from late spring to autumn (Möller, 1980; Hay *et al.*, 1990; Barz & Hirche, 2007), whereas medusa numbers reduce in late autumn, and they are nearly absent from the waters in winter. In contrast the benthic polyp stage (scyphistomae) can survive in the winter as a dormant cyst which excysts in the spring to strobilate again (Decker *et al.*, 2007). Strobilation can be monodisc (only one ephyra produced by each polyp at a certain time) or polydisc (more than one ephyra produced by each polyp at a certain time) (Arai, 1996). All scyphozoa from the German Bight undergo polydisc strobilation (Holst, 2012 b) which

contributes to the development of jellyfish blooms because one single polyp can produce many ephyrae (Boero *et al.*, 2008).

Epi-microbia communities have been found to be important in the larval settlement processes of most marine invertebrates (Wieczorek & Todd, 1998), such as sponges (Woollacott & Hadfield, 1996), cnidarians (Bosch, 2013), ascidians (Wahl *et al.*, 1994; Schuett *et al.*, 2005) and bryozoans (Pukall *et al.*, 2001; Kittelmann & Harder, 2005) . Under aseptic conditions, no settlement of planulae of the jellyfish *Aurelia aurita* (Schmahl, 1985), *Cassiopea andromeda* (Hofmann & Brand, 1987) and the hydrozoan *Hydractinia echinata* (Leitz & Wagner, 1993) occurred. Interestingly, bacteria and/or their products induced the settlement and metamorphosis of each of these organisms (Müller & Leitz, 2002). Intensive studies of scyphomedusae focused upon the general life history strategies, including the detailed morphology description of planula, scyphistoma and young ephyra, process of strobilation, development of ephyra to young medusa (Svane & Dolmer, 1995; Holst *et al.*, 2007; Lucas *et al.*, 2012), reproductive behavior, different changing factors leading to strobilation, growth rate and impact of predation rate of ephyrae (Hofmann *et al.*, 1978; Båmstedt, 1990; Olesen *et al.*, 1996; Barz & Hirche, 2007; Holst *et al.*, 2007; Holst, 2012 a and b)**.** Whereas the presence and activity of bacteria associated with of different life stages of scyphomedusae have received less attention. A few studies characterizing the bacterial community associated with early life stages were carried out on fish larva (Bergh, 1995; Hansen & Olafsen, 1999; Romero & Navarrete, 2006), scallop (Torkildsen *et al.*, 2005) focusing on the colonization by pathogenic bacteria during early stage of commercial fish. Fraune *et al.*(2010) described the bacterial colonization in the early development of embryo in *Hydra*. However, little is known about the early step of bacterial colonization in scyphomedusae larvae, the establishment of microbiota and its stability, especially after strobilation. This knowledge is prerequisite for understanding the aspect of microbial ecology of jellyfish regarding to the controlling of reproduction mechanisms. The knowledge on the interrelations of bacteria communities and different life stages in scyphozoa could help to provide profound insights into understanding a microbe-dependent life style and its evolutionary consequences.

Cyanea lamarckii and *Chrysaora hysoscella* (Russell, 1970), investigated in the present study, are common medusa species at Helgoland Roads in the German Bight and usually occur during summer (Möller, 1980; Hay *et al.*, 1990; Barz & Hirche, 2007). Adult medusae were collected from the field and the larvae, which are released directly by medusae, were collected. In the

laboratory, the planulae of these two species were hatched. Polyps were grown from larvae until scyphistoma stage. The bacterial communities associated with planula, polyps and medusae were determined using automated ribosomal intergenic spacer analysis (ARISA). Specifically, we aimed to answer following questions: (1) Are different body parts of the scyphomedusae associated with different bacterial communities? (2) Do the (bacterial community composition) BCCs vary in different life stages scyphomedusae? (3) Does the food influence the bacterial community associated with polyps? (4) Do different scyphomedusae species harbor different bacterial communities?

Materials and methods

Sample collection and preparation

Medusae of the scyphozoan species *Cyanea lamarckii* and *Chrysaora hysoscella* were collected in the vicinity of Helgoland Roads in the Germany Bight (54˚11.3′N, 7˚54.0′E) from May to July 2011 two times per week using a 500 µm mesh trawl towed by the research vessel ‘‘Aade’’. Additional specimens were collected from land around the island of Helgoland with a bucket. The samples were transferred to the laboratory within 1 h after collection. Identification on the genus and species level was determined based on morphological traits according to Russell (1970) and Holst (2012 b). Five intact individuals of each species were collected at each sampling day to analyze the bacterial community of different body parts. Medusae were dissected under the stereomicroscope using sterile forceps and scissors. Separated body parts (umbrella, gonad, tentacle and mouth arm) were rinsed five times with sterile seawater to eliminate transient and loosely attached microorganisms from the surface. All samples were frozen at -20˚C and lyophilized prior to molecular analysis. In addition, at least one mature medusa carrying planula larvae was chosen at each sampling day and transferred to a 30L aquarium for planula collection. Released planulae were collected with a pipette from the bottom and rinsed five times with sterile seawater. Individual planula (one) was collected with sterile capillary pipette respectively for subsequent molecular analysis. Remaining rinsed planula was used for larval settlement experiments.

Planula settlement

Larval settlement was achieved according to the modified protocol of Holst & Jarms (2010). Three replicates were set up for each specimen. 20 ml of concentrated planula suspension was pipetted into 100-ml plastic beaker, which was filled up with 15-20ml sterile seawater in advance. The lid of one polystyrene Petri dish (47 mm diameter) was add to each jar, placed on the water surface to provide a swimming settlement substrate which is preferred by planulae (Brewer, 1976; Brewer, 1984; Holst & Jarms, 2007). Settlement experiments were conducted under in *situ* temperature (15 ± 2°C). The water of each beaker was carefully replaced with fresh sterile seawater after 48h when planula settled on the underside of the floating Petri dish lids. The unsettled planulae were carefully removed by pipette.

Feeding of polyps

Polyps of each species were fed by two different food sources, brine shrimp (*Artemia salina*) nauplii reared in sterile seawater from eggs in the laboratory and natural plankton collected at Helgoland Roads by a plankton net (75µm mesh size) at the day of feeding. Feeding with both food types was conducted at the same time and in the same manner for both scyphozoan species. The food organisms were mashed before feeding until the polyps developed four tentacles (usually after 5-6 d). In the eight tentacles stage, they were fed with intact living food organisms. Feeding was carried out twice a week for 1-2h. The jars were cleaned and the water was replaced with sterile seawater every time after feeding to keep a debris-free environment for polyps. During the strobilation stage, only half of the water was changed carefully, and remaining food was removed from the beaker with a pipette to avoid disturbing the strobilation process.

Only well-developed polyps with extended tentacles were collected for the analyses of the associated bacterial community. Polyps were carefully removed from the underside of the Petri dish with a needle under the stereomicroscope. The collection of polyps was conducted one or two days after feeding and lasted for 5 months. Collected polyps were rinsed and frozen in the same manner as described for larvae.

In order to estimate the impact of food resource on the bacterial community of polyps during feeding process, 1ml of seawater was collected from *A. salina* cultures and plankton samples, respectively, to analyze the community of free-living bacteria associated with each food sources.

In addition, 500ml of the hatching water of *A. salina* and plankton sample seawater was filtered through 0.2 μm membrane filters (GTTP, Millipore, Schwalbach, Germany) to analyze the community of attached bacteria with each food sources.

DNA extraction

Total genomic DNA of medusae (different body parts) was extracted from freeze-dried tissue using CTAB (cetyl-trimethyl-ammoniumbromide) according to the modified protocol of Gawel (1991) described in details in Chapter I. Briefly, Aliquots from ground samples (1 mg) were incubated in 2 mL pre-heated (60°C) CTAB buffer for 30 min at 65°C. Afterwards, 1 mL STE buffer, lysozyme (10mg/mL) and proteinase K (10mg/mL) were added to samples and incubated at 50°C for further 30min. DNA was extracted twice by phenol–chloroform–isoamylalcohol (25 : 24 : 1) and DNA was precipitated with isopropanol overnight at -20°C.

For larvae and polyps, DNA extraction was performed as previously described (Sapp *et al.*, 2007 b) with some modifications. Briefly, the tube containing larva or polyp was centrifuged for 1 min at 2504 g to remove supernatant water, Biomass of larva or polyp was resuspended in STE buffer (6.7% saccharose, 50mM Tris, 1mM EDTA, pH 8) and homogenized with sterile pestle formatted in 1.5ml microtube, then lysozyme (10mg/mL) and proteinase K (10mg/mL) were added for 30min at 37°C. Cell lyses was performed by adding Tris-EDTA (50mM Tris, 250mM EDTA, pH 8) and SDS-Tris-EDTA (20% sodium dodecyl sulfate, 50mM Tris, 20mM EDTA, pH 8) for 60min at 50°C with slow agitation. DNA was extracted using 1/10 volume NaCl (5M) and 1 volume phenol– chloroform–isoamylalcohol (25: 24: 1) and was precipitated with isopropanol.

For the free-living bacterial community in *A. salina* and plankton, DNA was extracted with the same procedure as larva and polyp. For the bacterial community attached with *A. salina* and plankton, DNA was extracted from cut filters (0.2 μm) with the same procedure as larva and polyp without centrifugation. DNA of the all extracts were washed with 75% ethanol and finally dried in sterile bench. All DNA extracts were dissolved 30-50μl sterile water and served as template DNA for the PCR. The quantity and quality of extracted DNA were determined by microphotometry using Tecan Infinite 200 NanoQuant.

Automated Ribosomal Intergenic Spacer Analysis (ARISA)

Characterization of the bacterial communities associated with the different life stages and different body parts of two scyphomedusae species were performed by the ARISA fingerprinting method, as described previously in details in Chapter I. Briefly, the internal transcribed spacer region was amplified with the forward primers L-D-Bact-132-a A-18 and the fluorescently labeled reverse primer S-D-Bact-1522-b-S-20 (Ranjard *et al.*, 2000) in the PCR reaction. PCR reaction and cycling conditions were performed as described previously (Krause *et al.*, 2012). In particular, 50 ng of genomic DNA template was applied in each reaction. Based on the intensities of PCR products on agarose gels, original or diluted PCR products were mixed with equal volume of stop mix and separated on 5.5% polyacrylamide gels prepared according to the manufacturer's protocol (LI-COR Biosciences, Lincoln, NE, USA), as well as the sample preparation and running condition. We used a 50-1500bp standard as a size reference (all materials: LI-COR Biosciences, Lincoln, NE, USA).

Statistical analysis

Analysis of ARISA data

ARISA fingerprints were analyzed by using BioNumerics 6.6 software (Applied Maths, Sint-Martens-Latem, Belgium). Normalization of band patterns was conducted automatically referencing by the size standard and the presence or absence of each band was determined based on the normalized minimum threshold density (5%). Depending on the length of the detected fragment, bins of 3bp were used for fragments up to 700 bp in length, bins of 5bp for fragments between 700 and 1000bp and bins of 10bp for fragments larger than 1000bp. Binning to band classes was performed according to Kovacs *et al*. (2010) and Brown (2005). Bands smaller than 300bp were neglected for multivariate analyses. The alpha diversity (OTU richness) (operational taxonomic units) of each sample which obtained from ARISA fingerprints was estimated by summing up the total number of band classes present in the respective samples. ARISA-OTUs were analyzed based on a constructed binary table. Differences between the groups were tested by one-way analysis of variance (ANOVA) with the software package Statistica (Version 9).

Multivariate analyses

For multivariate statistical analyses, the software package PRIMER v.6 and the add-on PERMANOVA+ (both PRIMER-E Ltd, Plymouth, UK) was used. The permutational multivariate analysis of variance (PERMANOVA) with fixed factor was applied to investigate the difference of associated bacterial community composition (BCC) of two scyphomedusae species regarding different life stages and different body parts. For the permutation tests, we performed resemblance measure and used 999 permutations on the basis of Jaccard coefficient (S7). Principal co-ordinate analysis (PCO) was performed to visualize patterns of the bacterial community influenced by different life stage and body compartment.

Results

General features of larvae and primary polyps

The planulae of both scyphomedusae species are oval to pear-shaped in yellow-white color, more dominantly milk-white (Fig. 1A). Planulae are completely covered by cilia and highly motile by rotating. Planulae swam for about 1 to 2 days before they settled on the substrate. Planulae of *C. lamarckii* and *Ch. hysoscella* directly developed into young polyps within 2-3 days after settlement (Fig. 1B), and a few of the planulae of *C. lamarckii* encysted (Fig. 1D, arrow). Polyps developed into the four-tentacle stage around 5-6 day and developed a thin stalk with a rounded basal attachment disk (Fig. 1B, arrow). The four tentacles were highly extensile. At the four tentacles stage (Fig. 1C), polyps started to eat the mashed food. Until eight tentacles stage (Fig. 1E), they were easily capable of catching and eating intact brine shrimp (*Artemia salina*) nauplii. After feeding, most polyps were contracted and distinctly orange-colored, while the polyps fed with plankton food were in white-brown color. For the analysis of the bacterial community composition associated with polyps, white-colored and empty gut polyps were collected 1 or 2 days after feeding. In addition, the polyps fed with plankton, didn't grow very well compared with those fed with *A. salina*. Examined under stereomicroscope even after feeding, polyps of both scyphozoan species fed with plankton were inanimate and died off earlier than the one fed with *A. salina*. For all samples DNA was successfully extracted and amplified by the modified protocols of Gawel (1991) and Ranjard (2000).

BCCs associated with body parts of Scyphomedusae

To investigate the bacterial community associated with scyphomedusae, the BCC associated with four different body parts, including umbrella, gonad, mouth arm and tentacle, were analyzed separately. In total, 44 specimen of *Cyanea lamarckii* and 17 specimen of *Chrysaora hysoscella* were applied in the bacterial community analysis. Based on the ARISA fingerprints, the PCO plots (Fig. 2A and B) depict the bacterial communities associated with different body parts (umbrella, gonads, mouth arm and tentacle) of two scyphomedusae species. The first two axes of the PCO of BCC associated with two scyphomedusae species capture 25.4 % and 23.1% of the total variation, respectively. No distinct patterns are present in the BCCs of different body parts in two species (Fig. 2A and B). The PERMANOVA main test indicates significantly different bacterial communities regarding to the body parts in *C. lamarckii* ($p=0.001$, Tab. 1). Contrary, in *Ch. hysoscella*, the bacterial community displays no differences regarding to different body parts ($p=0.119$, Tab. 3). According to the PERMANOVA pair-wise comparisons, the bacterial community associated with umbrella is significantly different from the community associated with gonad, mouth arm and tentacle in *C. lamarckii* ($p=0.001$, Tab. 2). For *Ch. hysoscella*, the community associated with umbrella only differed from the community of gonad and tentacle ($p=0.017$ and 0.015, respectively, Tab. 4).

Bacterial OTU number (alpha diversity) for different body parts of two scyphomedusae species varied from each other. However, the pattern of richness was the same for both scyphomedusae species investigated here (Fig. 3). The highest OTU richness was observed in the community of umbrella in both scyphomedusae species (*C. lamarckii* and *Ch. hysoscella*) with similar numbers 23 and 25, respectively, followed by the community of mouth arm ($S=20$ and 18, respectively) and tentacle ($S=15$), the lowest richness was observed in the community of gonad with the same number for both species ($S=14$). Based on the ANOVA, there is no difference between the different body parts in *C. lamarckii* in respect to the diversity of bacterial community (OTU number) ($F_{3,\,116}=2.4184$, $p=0.06978$). However, the diversity of bacterial community associated with different body parts were significantly different in *Ch. hysoscella* ($F_{3,\,64}=7.2818$, $p=0.00028$). Specifically, the diversity of bacterial community associated with umbrella significantly differed from that of gonad and tentacle ($p<0.001$).

Impact of food on the BCCs of polyps

To investigate the influence of food on the bacterial communities associated with polyps, the individual polyps fed with two kinds of food sources (*A. salina* and plankton) under lab condition were compared. Additionally, 29 samples of *A. salina* and 18 samples of plankton from the attached BCCs with each food source were analyzed respectively. Accordingly, water samples of both *A. salina* hatching water and plankton water were analysed as free-living BCCs. The PCO plots (Fig. 4) represent the BCCs associated with polyps (fed with *A. salina* and plankton) in two scyphozoan species, as well as the BCCs extracted from food including the attached community and free living community from water environment.

For both scyphozoan species, *C. lamarckii* and *Ch. hysoscella*, the first PCO axes explain the majority of the variation among these communities (nearly 26%) and are strongly associated with the separation of assemblages in the polyps (on the left) from those in food (on the right) (Fig. 4 A and B). The bacterial communities associated with polyps of *C. lamarckii* fed with different food are clearly distinguishable from each other (Fig. 4 A). For *Ch. hysoscella*, on this two dimension plot, the communities of polyps fed with *A. salina* are well mixed and not easily distinguishable from the bacterial communities associated with polyps fed with plankton (Fig. 4 B). However, the PERMANOVA main tests of the BCCs of polyps and food sources show significant difference (P=0.001) for both scyphozoan species (Tab. 5 and 7). Consistent with the PERMANOVA pairwise comparison, there is fairly strong evidence to suggest that all of the groups differ from one another (P=0.001 for most comparisons, Tab. 6 and 8) in both species. Overall, the BCCs of polyps fed either with plankton or *A. salina* are significantly different from the BCCs of food including the attached and the free-living community based on the PERMANOVA. Therefore, the bacterial community of food source has no influence on the selection of BCC of polyps, whereas the BCCs of polyps are significantly distinct between the different food sources (*A. salina* and plankton).

The alpha diversity of bacterial community associated with polyps fed with different food of two scyphomedusae, as well as the attached and free-living communities of two kinds of food sources, is depicted as bar plot of median values with 95% confidence of ARISA OTU numbers (Fig. 5). Generally, among the food sources, the highest ARISA OTU number is detected in free-living community of plankton water (S=50), following by the attached community of plankton

(S=31). The attached community of *A. salina* (S=26) has a higher diversity compared with free-living community of *A. salina* (S=20). Among the polyps, the one fed with plankton apparently presents a higher diversity compared with the one fed with *A. salina* in both scyphomedusae species. The highest OTU number is observed in the polyps fed with plankton in *C. lamarckii* (S=36), followed by the one in *Ch. hysoscella* (S=30). The polyps fed with *A. salina* display a similar diversity concerning the BCC in both scyphomedusae species. The ARISA OTU number in polyps fed with *A. salina* is 20 and 23, respectively in *C. lamarckii* and *Ch. hysoscella*. Based on the ANOVA, significant differences are revealed among all groups in respect to the diversity of bacterial community (OTU number) ($F_{7, 558}$=37.749, p=0.0000).

BCCs associated with different life stages of Scyphozoan

Because of the metagenetic nature of scyphozoan, another aspect of the bacterial community associated with scyphozoan in respect to the different life stages were analyzed in the present study. Larvae were collected from medusae adult and polyps were hatched from the larvae stage with two different kinds of food under lab condition. In this part, the four parts of each scyphomedusae species were considered as an integral part presenting the medusae adult. Therefore, the BCCs associated with different body fractions were combined together for the BCC associated with medusa adult for each specimen.

The Principal coordinate analysis (PCO) was applied to visualize the variation of different stages including larvae, polyps (fed with *A. salina* and plankton) and medusae adult in terms of their bacterial community. It is apparent that the bacterial communities associated with all stages of *C. lamarckii* are separated from each other at different degrees as represented by the ARISA fingerprints pattern (Fig. 6 A). In this two-dimensional plot, 22.8% of the total variation is explained by the first two axes. The analysis of PERMANOVA main test and pair-wise comparisons (Tab. 9 and 10) regarding different life stages reveal that BCCs are significantly different (p=0.001). Regarding the bacterial community structure of *Ch. hysoscella*, the PCO plot displays a clear separation among each life stages (Fig. 6 B). The BCCs corresponding to larvae, medusae and polyps form a very tight cluster. The first two axes of the PCO capture 27% of the total variation with an overlapped community of polyps fed with *A. salina* and plankton. The analyses of PERMANOVA main test and pair-wise comparisons reveal significant difference between each stage in *Ch. hysoscella* (P=0.001) (Tab. 11 and 12). The bacterial communities

associated with polyps fed with two kinds of food sources overlap together in the PCO plot (Fig. 6 B). However, a significant difference is observed in these two communities based on PERMANOVA pair-wise comparisons (P=0.001) (Tab. 12).

The alpha diversity of each life stage of the two scyphomedusae species is depicted as bar plot of median values with 95% confidence of ARISA OTU numbers (Fig. 7). Generally, lowest richness in *C. lamarckii* and *Chr. hysoscella* is observed in the polyp stage (S=20). Significantly highest richness is detected in the medusa stage of both scyphomedusae species (S=69). In general, the bacterial community diversity displays a similar pattern in both scyphomedusae species, where the highest richness occurred in medusae and lowest richness occurred in polyps fed with *A. salina*. Based on the ANOVA, bacteria associated with different life stages display significant differences regarding to the diversity of bacterial community (OTU number) in both scyphozoan species ($F_{3,\ 317}$=119.86, p=0.0000 in *C. lamarckii*, $F_{3,\ 294}$=77.911, p=0.0000 in *Ch. hysoscella*)

Comparison between *Cyanea lamarckii* and *Chrysaora hysoscella*

Bacterial communities associated with different life stages are compared between two scyphomedusae species. For the community of larvae (Fig. 8 A), clear separation between *C. lamarckii* and *Ch. hysoscella* is observed in the PCO plot. Regarding to polyps, BCCs of polyps compared between two scyphomedusae species are specified by different food source in Fig. 8 B. The BCCs of polyps not only form a tight cluster on the factor of “species” on the first axis (PCO1), but the communities also clearly separate on the second axis (PCO2) by the factor of “food source” (Fig. 8 B). The community of polyps fed with plankton from *Ch. hysoscella* is slightly dispersed. The first two axes of the PCO capture 45.7% and 33.5 % of the total variation of larvae and polyps, respectively. In accordance with PERMANOVA analysis (Tab. 13), factor “species” significantly influenced the bacterial community structure associated with larvae (p=0.001). For polyps, both experimental factors (species and food source) and their interactions significantly influenced the bacterial community structure (PERMANOVA Tab. 14). The highest amount of variation is explained by “species” (Sq. root, Tab. 14). Furthermore, the community of larvae and polyps display similar variation which is also influenced by species (Sq. root, Tab. 13 and 14). For the community of adult medusae, no distinct difference is observed in the PCO two-dimension plot. The adult medusa stages form a homogeneous structure in both scyphozoan

species (Fig. 8 C). However, the PERMANOVA analysis reveals that regarding two species, the communities of medusae in *C. lamarckii* and *Ch. hysoscella* are different from each other with p=0.001 (Tab.15). Compared with the community of larvae and polyps, medusae display less variation regarding to the bacterial community (Sq. root=10.03, Tab. 15). Overall, the BCCs of all three life stages (larvae, polyps and medusae) significantly differ between *C. lamarckii* and *Ch. hysoscella* indicating a species-specific bacterial association in scyphozoan.

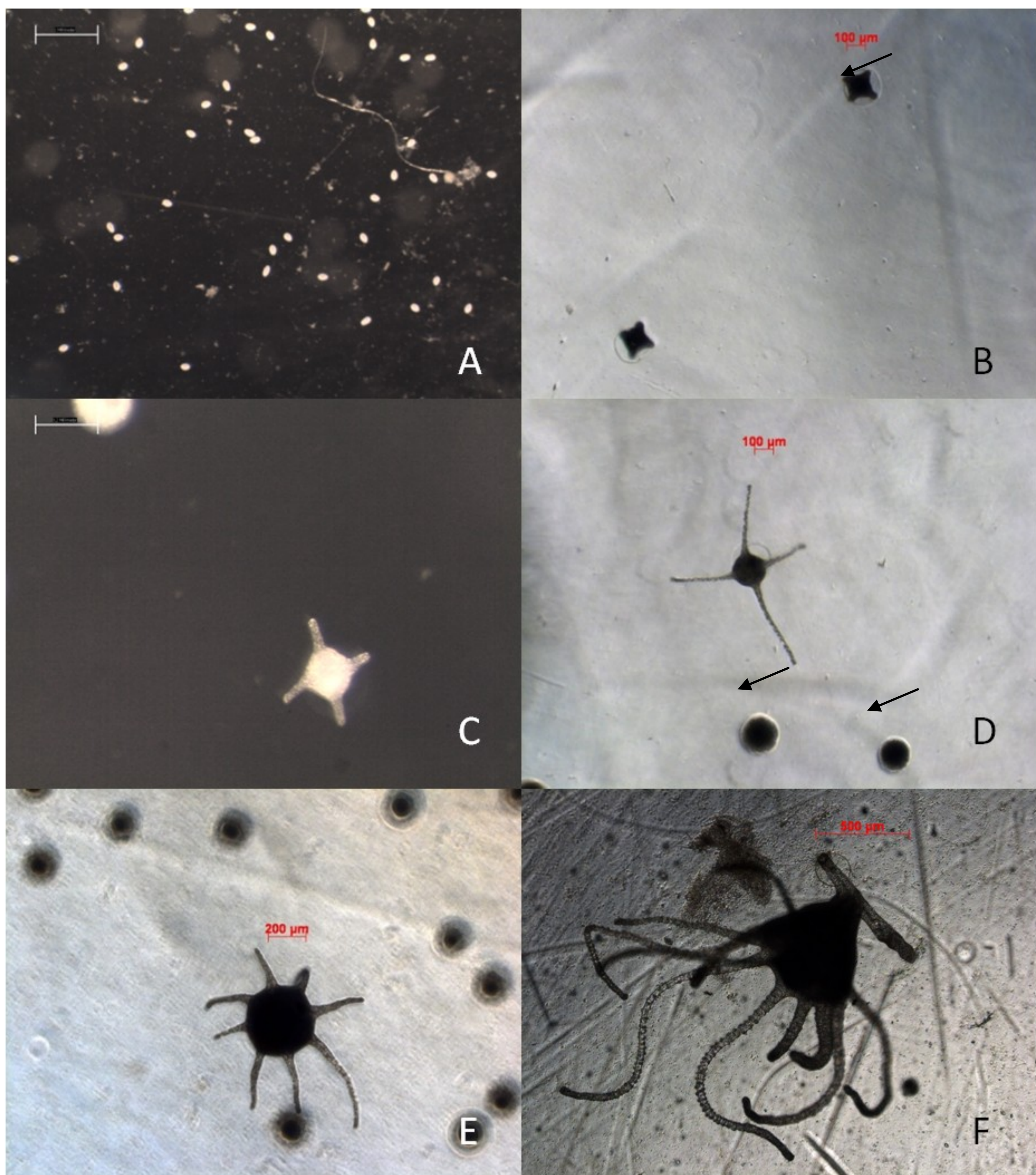
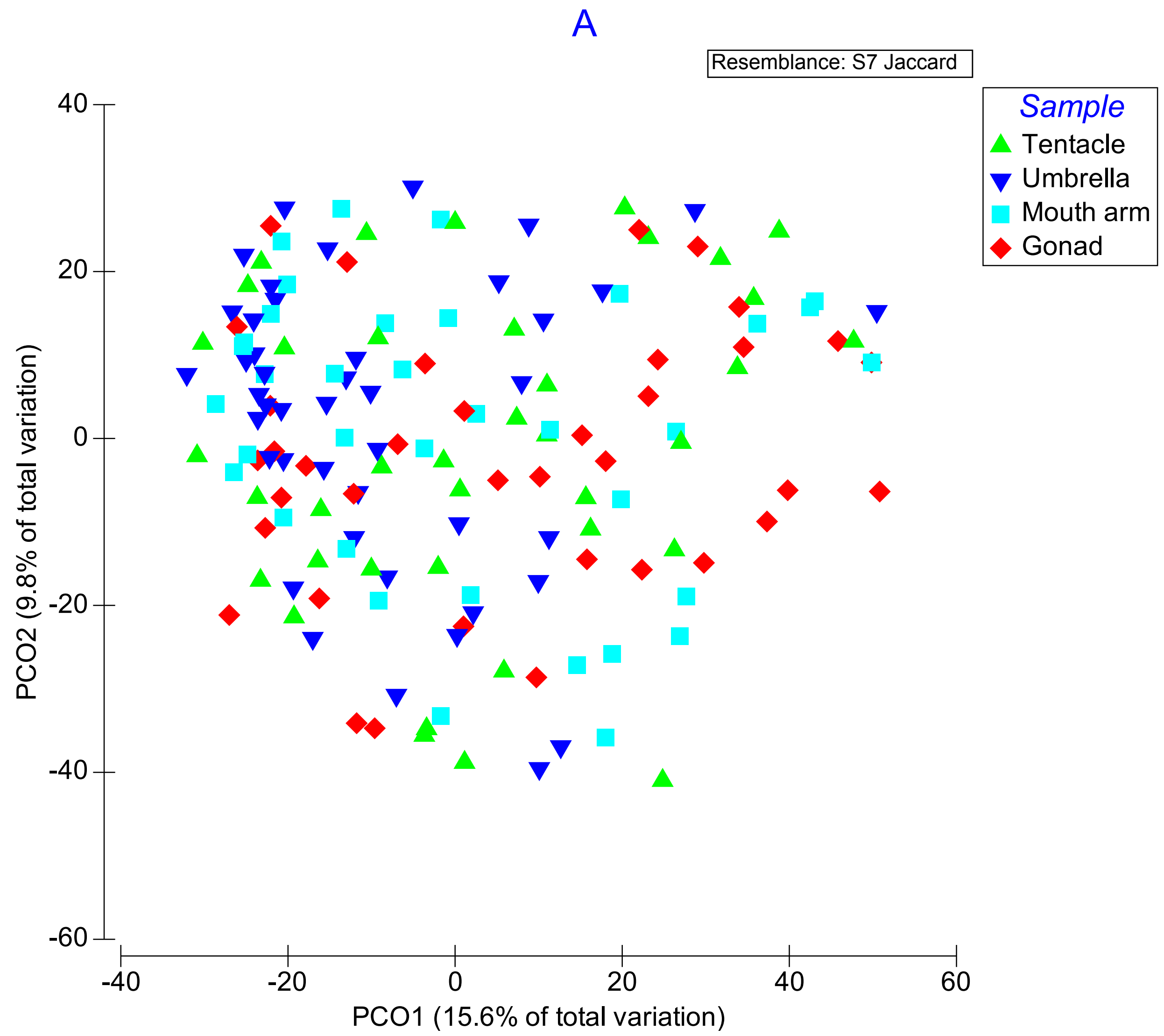


Figure 1. Different development stages of *Cyanea lamarckii*. A, Planula larvae; B, Initial stage of polyp; C and D, Four tentacle stage polyp; E, Eight tentacle stage polyp; F, Full developed polyp. (Scale bar: A. 1 millimeter, B. D. 100 micrometer, C. E. 200 micrometer, F. 500 micrometer. Arrow: B. the rounded basal attachment disk, D. Polyp encystment)


A
Resemblance: S7 Jaccard
Sample
Tentacle
Umbrella
Mouth arm
Gonad
40
20
0
-20
-40
-60
PCO2 (9.8% of total variation)
-40
-20
0
20
40
60
PCO1 (15.6% of total variation)

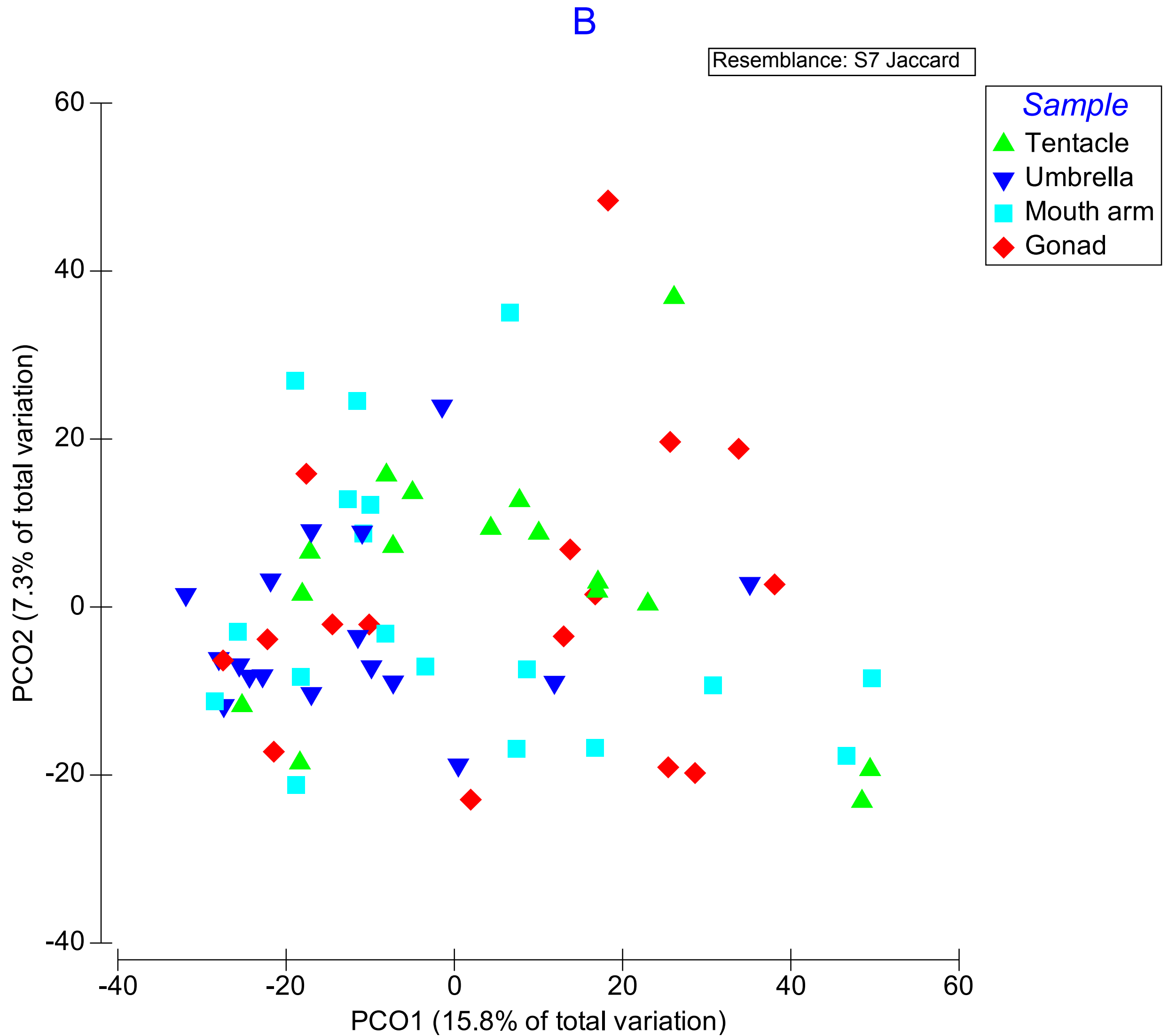


Figure 2. Principal coordinate (PCO) analysis presenting the bacterial communities associated with different body parts of scyphomedusae (A: *Cyanea lamarckii* and B: *Chrysaora hysoscella*) based on Jaccard coefficient from ARISA profiles.

Table 1. PERMANOVA main tests of bacterial community structure of different body parts of *C. lamarckii* based on Jaccard dissimilarities of ARISA profiles.

Significant results (p (perm) <0.05) are highlighted in bold. Displayed are tests for the factor 'sample' and the partitioning of multivariate variation. p-values were obtained using type III sums of squares.

d.f.: degrees of freedom, SS: sums of squares, Sq. root: square root of the component of variation attributable to that factor in the model, in units of Jaccard dissimilarities.

Source of variation	d.f.	SS	*pseudo F*	*p (perm)*	Perms	Sq. root
Sam	3	19091	2.1109	**0.001**	998	9.2795
Res	152	4.5822E5				54.906
Total	155	4.7731E5				

Table 2. PERMANOVA pair-wise comparisons of bacterial community structure of different body parts of *C. lamarckii* based on Jaccard dissimilarities of ARISA profiles.

Significant results (p (perm) <0.05) are highlighted in bold. Displayed are pair-wise comparisons of the factor "sample type".

Comparison	t (perm)	*p (perm)*	Perms
Tentacle vs. Umbrella	1.7168	**0.001**	999
Tentacle vs. Mouth arm	1.0246	0.372	996
Tentacle vs. Gonad	1.0434	0.305	999
Umbrella vs. Mouth arm	1.4854	**0.005**	998
Umbrella vs. Gonad	2.013	**0.001**	999
Mouth arm vs. Gonad	1.1372	0.156	997

Table 3. PERMANOVA main tests of bacterial community structure of different body parts of *Ch. hysoscella* based on Jaccard dissimilarities of ARISA profiles.

Significant results (p (perm) <0.05) are highlighted in bold. Displayed are tests for the factor 'sample' and the partitioning of multivariate variation. p-values were obtained using type III sums of squares.

d.f.: degrees of freedom, SS: sums of squares, Sq. root: square root of the component of variation attributable to that factor in the model, in units of Jaccard dissimilarities.

Source of variation	d.f.	SS	*pseudo F*	*p (perm)*	Perms	Sq. root
Sam	3	11280	1.2061	0.119	993	6.1956
Res	63	1.964E5				55.835
Total	66	2.0768E5				

Table 4. PERMANOVA pair-wise comparisons of bacterial community structure of different body parts of *Ch. hysoscella* based on Jaccard dissimilarities of ARISA profiles.

Significant results (p (perm) <0.05) are highlighted in bold. Displayed are pair-wise comparisons of the factor "sample type".

Comparison	t (perm)	*p (perm)*	Perms
Umbrella vs. Mouth arm	1.1088	0.195	998
Umbrella vs. Gonad	1.3221	**0.017**	999
Umbrella vs. Tentacle	1.3015	**0.015**	997
Mouth arm vs. Gonad	0.9675	0.537	995
Mouth arm vs. Tentacle	1.0171	0.392	998
Gonad vs. Tentacle	0.8578	0.886	998

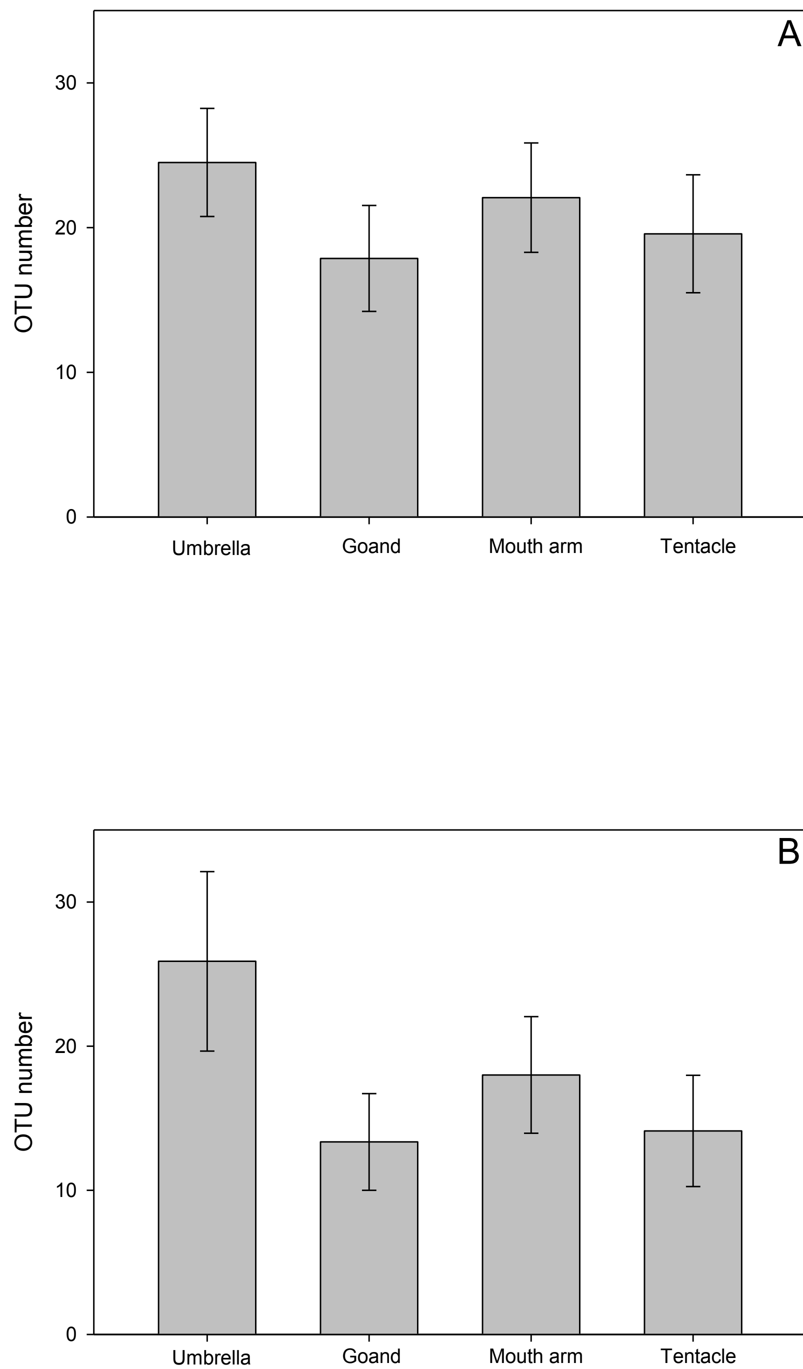
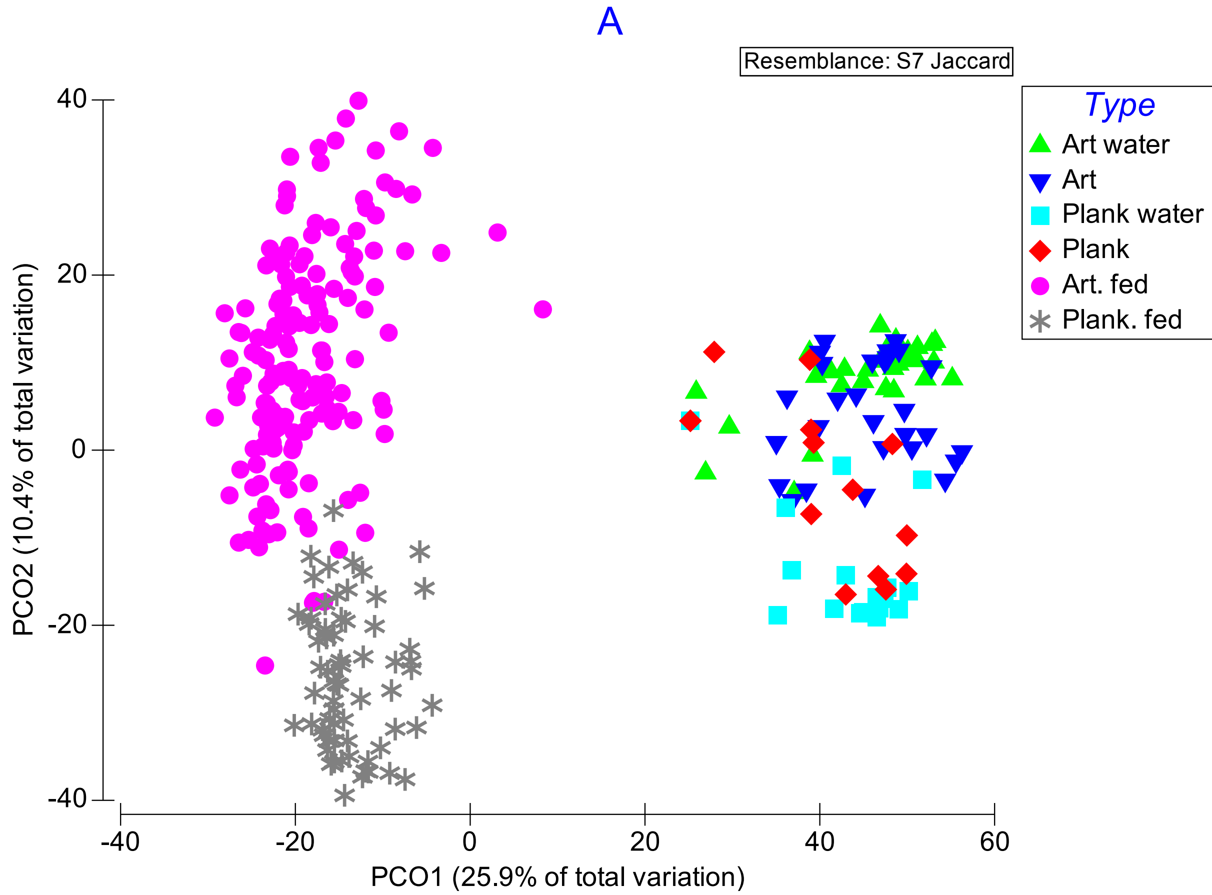


Figure 3. Bar chart of means of bacterial OTU number (alpha diversity) for different body parts of two scyphomedusae species obtained by ARISA fingerprint (A: *Cyanea lamarckii* and B: *Chrysaora hysoscella*). Whiskers indicate 95% confidence intervals.


A
Resemblance: S7 Jaccard
Type
Art water
Art
Plank water
Plank
Art. fed
Plank. fed
PCO2 (10.4% of total variation)
PCO1 (25.9% of total variation)
40
20
0
-20
-40
-40
-20
0
20
40
60

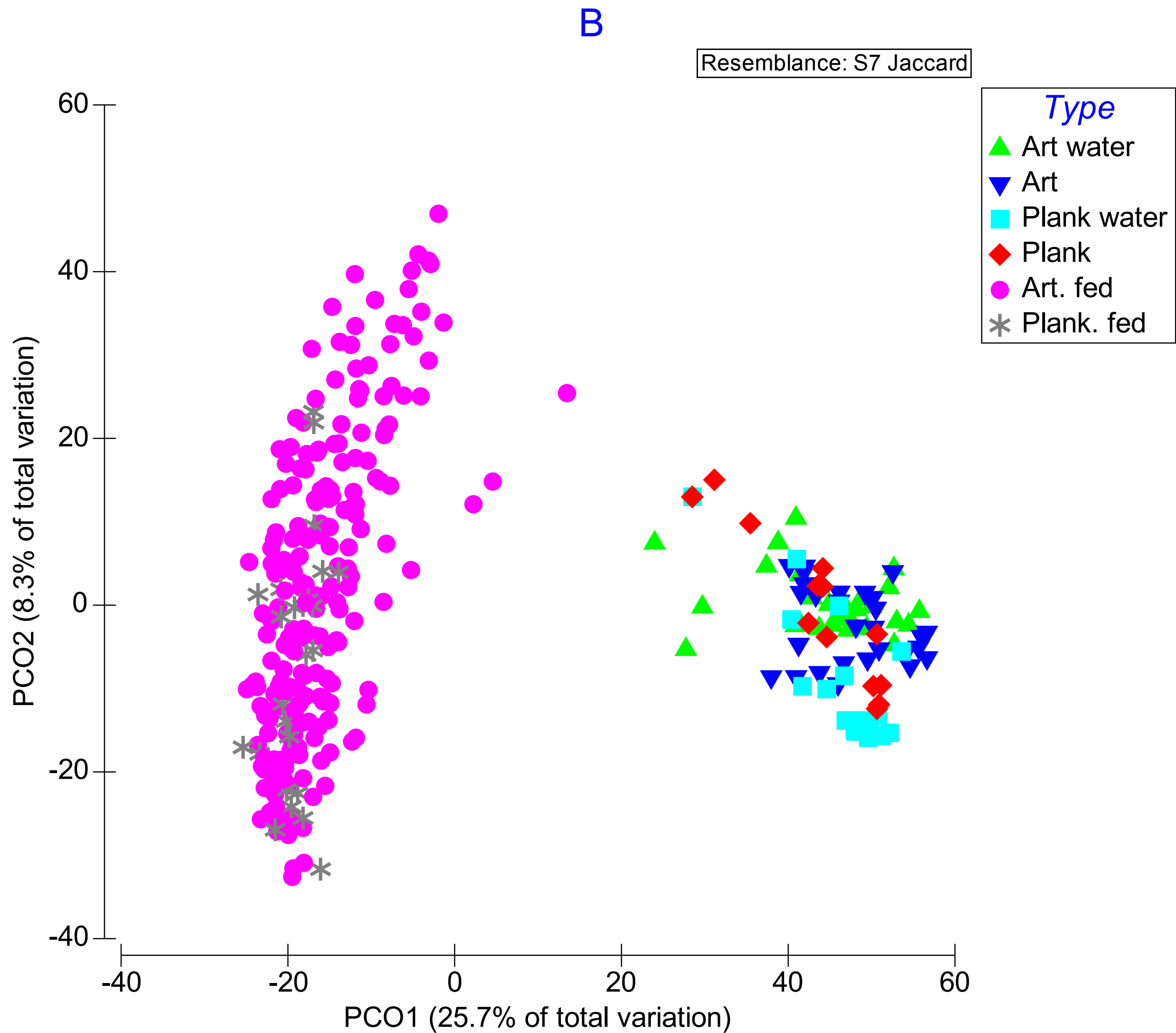


Figure 4. Principal coordinate (PCO) analysis presenting the bacterial communities associated with polyps of two scyphozoan species (A: *Cyanea lamarckii* and B: *Chrysaora hysoscella*) (including fed with *A. salina* plankton) and food resource (including attached community of food itself: *A. salina* and plankton, and free-living community of food: *A. salina* water and plankton water) based on Jaccard coefficient from ARISA profiles.

Table 5. PERMANOVA main tests of bacterial community structure of polyps of *C. lamarckii* and food source based on Jaccard dissimilarities of ARISA profiles.

Significant results (p (perm) <0.05) are highlighted in bold. Displayed are tests for the factor 'food type' and the partitioning of multivariate variation. p-values were obtained using type III sums of squares.

d.f.: degrees of freedom, SS: sums of squares, Sq. root: square root of the component of variation attributable to that factor in the model, in units of Jaccard dissimilarities.

Source of variation	d.f.	SS	*pseudo F*	*p (perm)*	Perms	Sq. root
food type	5	3.5405E5	35.232	**0.001**	999	40.065
Res	311	6.2506E6				44.831
Total	316	9.7911E5				

Table 6. PERMANOVA pair-wise comparisons of bacterial community structure of polyps of *C. lamarckii* and food source based on Jaccard dissimilarities of ARISA profiles.

Significant results (p (perm) <0.05) are highlighted in bold. Displayed are pair-wise comparisons of the factor "food type".

Comparison	t (perm)	*p (perm)*	Perms
Art water vs. Art	1.7823	**0.001**	998
Art water vs. Plank water	3.502	**0.001**	996
Art water vs. Plank	2.3867	**0.001**	995
Art water vs. Art. fed polyp	7.0902	**0.001**	999
Art water vs. Plank. fed polyp	7.3864	**0.001**	998
Art vs. Plank water	3.1951	**0.001**	998
Art vs. Plank	2.2315	**0.001**	996
Art vs. Art.fed polyp	7.031	**0.001**	998
Art vs. Plank. fed polyp	7.2344	**0.001**	999
Plank water vs. Plank	1.9237	**0.003**	997
Plank water vs. Art. fed polyp	6.3406	**0.001**	998
Plank water vs. Plank. fed polyp	6.3436	**0.001**	999
Plank vs. Art. fed polyp	5.0099	**0.001**	999
Plank vs. Plank. fed polyp	5.1833	**0.001**	999
Art. fed polyp vs. Plank. fed polyp	5.9727	**0.001**	998

Table 7. PERMANOVA main tests of bacterial community structure of polyps of *Ch. hysoscella* and food source based on Jaccard dissimilarities of ARISA profiles.

Significant results (p (perm) <0.05) are highlighted in bold. Displayed are tests for the factor 'food type' and the partitioning of multivariate variation. p-values were obtained using type III sums of squares.

d.f.: degrees of freedom, SS: sums of squares, Sq. root: square root of the component of variation attributable to that factor in the model, in units of Jaccard dissimilarities.

Source of variation	d.f.	SS	*pseudo F*	*p (perm)*	Perms	Sq. root
food type	5	3.1079E5	28.798	**0.001**	998	40.541
Res	332	7.1661E5				46.459
Total	337	1.0274E6				

Table 8. PERMANOVA pair-wise comparisons of bacterial community structure of polyps of *Ch. hysoscella* and food source based on Jaccard dissimilarities of ARISA profiles.

Significant results (p (perm) <0.05) are highlighted in bold. Displayed are pair-wise comparisons of the factor "food type".

Comparison	t (perm)	*p (perm)*	Perms
Art water vs. Art	1.7823	**0.001**	996
Art water vs. Plank water	3.502	**0.001**	999
Art water vs. Plank	2.3867	**0.001**	999
Art water vs. Art. fed polyp	6.7487	**0.001**	997
Art water vs. Plank. fed polyp	6.084	**0.001**	999
Art vs. Plank water	3.1951	**0.001**	998
Art vs. Plank	2.2315	**0.001**	998
Art vs. Art.fed polyp	6.8831	**0.001**	999
Art vs. Plank. fed polyp	6.3082	**0.001**	998
Plank water vs. Plank	1.9237	**0.003**	999
Plank water vs. Art. fed polyp	6.0758	**0.001**	996
Plank water vs. Plank. fed polyp	6.5636	**0.001**	998
Plank vs. Art. fed polyp	4.8174	**0.001**	999
Plank vs. Plank. fed polyp	4.9443	**0.001**	999
Art. fed polyp vs. Plank. fed polyp	2.7681	**0.001**	999

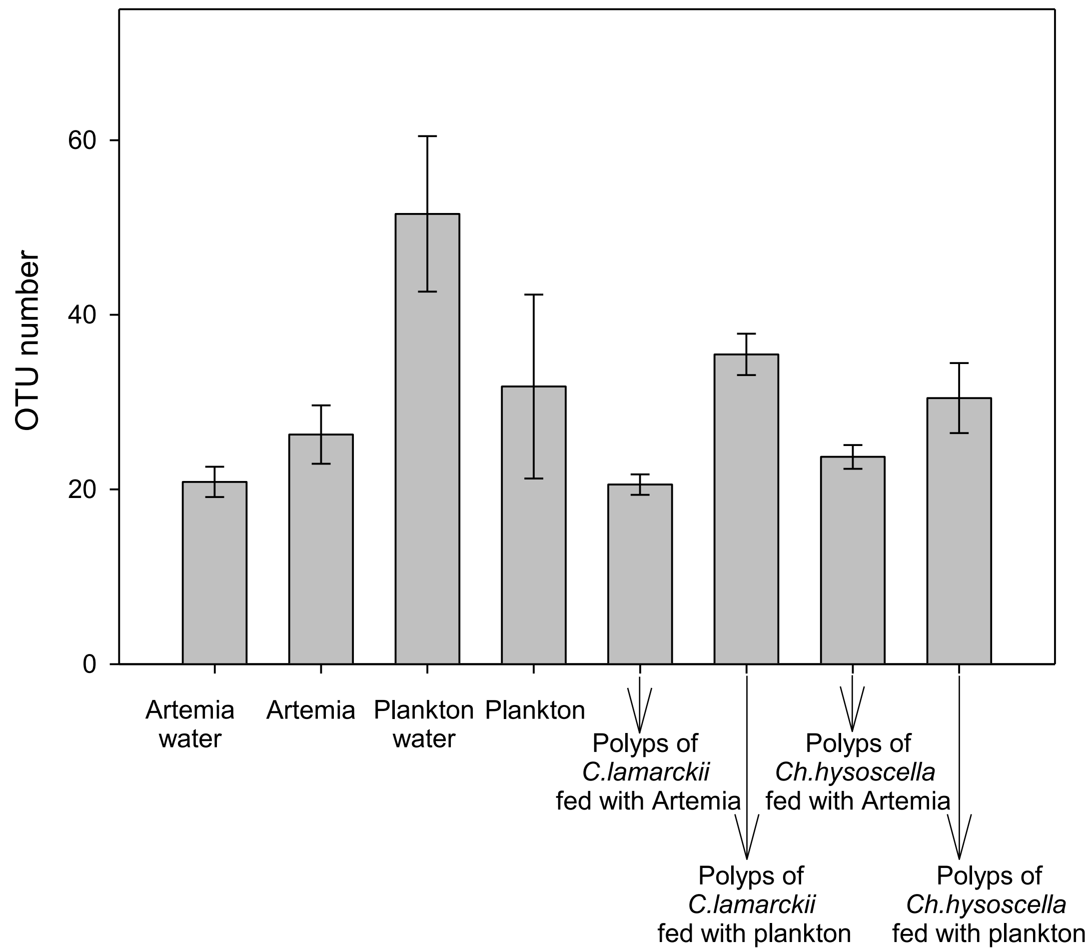
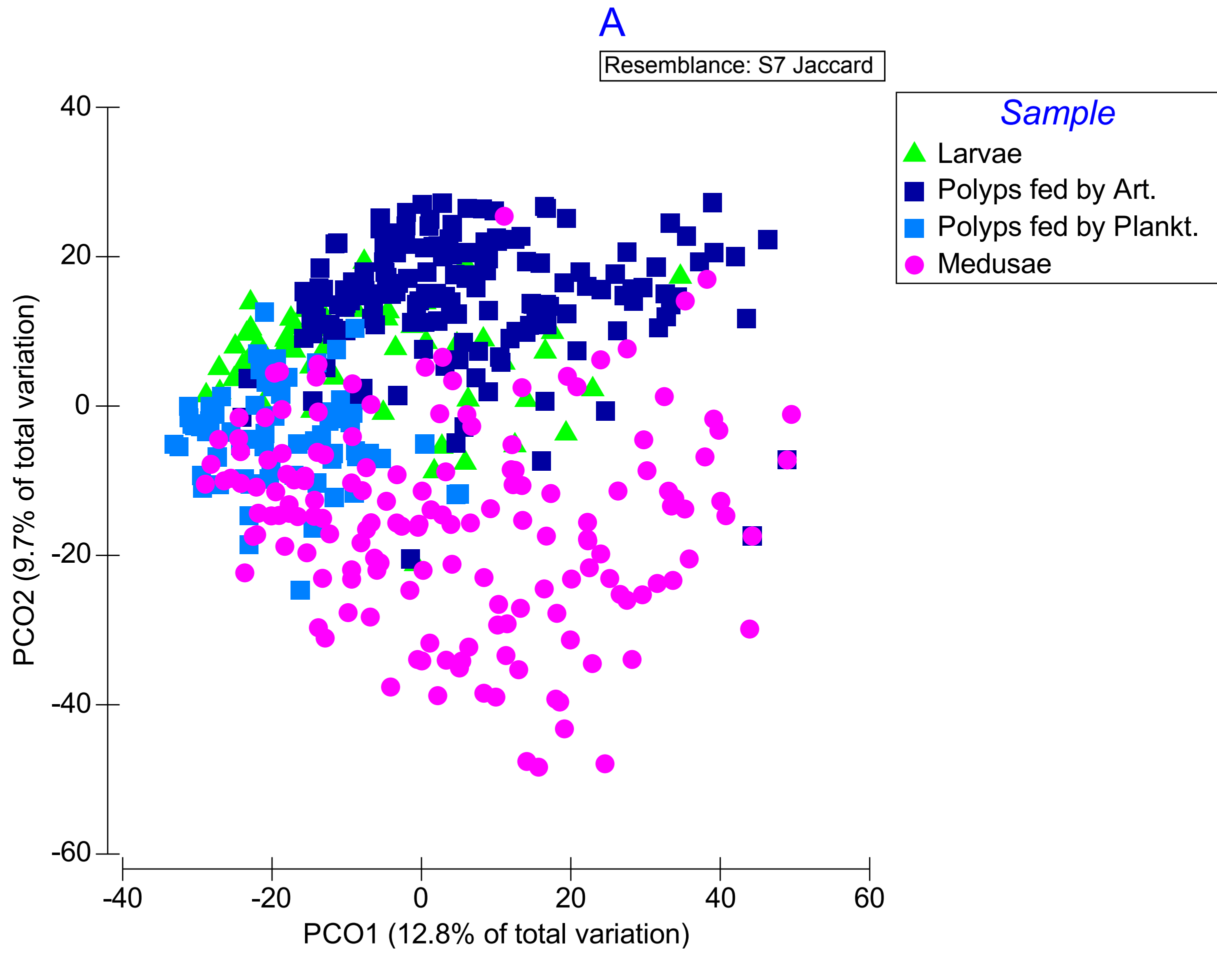


Figure 5. Bar chart of means of bacterial OTU number (alpha diversity) for polyps fed with different foods of two scyphomedusae species and food itself obtained by ARISA fingerprint. Whiskers indicate 95% confidence intervals.


A
Resemblance: S7 Jaccard
Sample
Larvae
Polyps fed by Art.
Polyps fed by Plankt.
Medusae
40
20
0
-20
-40
-60
PCO2 (9.7% of total variation)
-40
-20
0
20
40
60
PCO1 (12.8% of total variation)

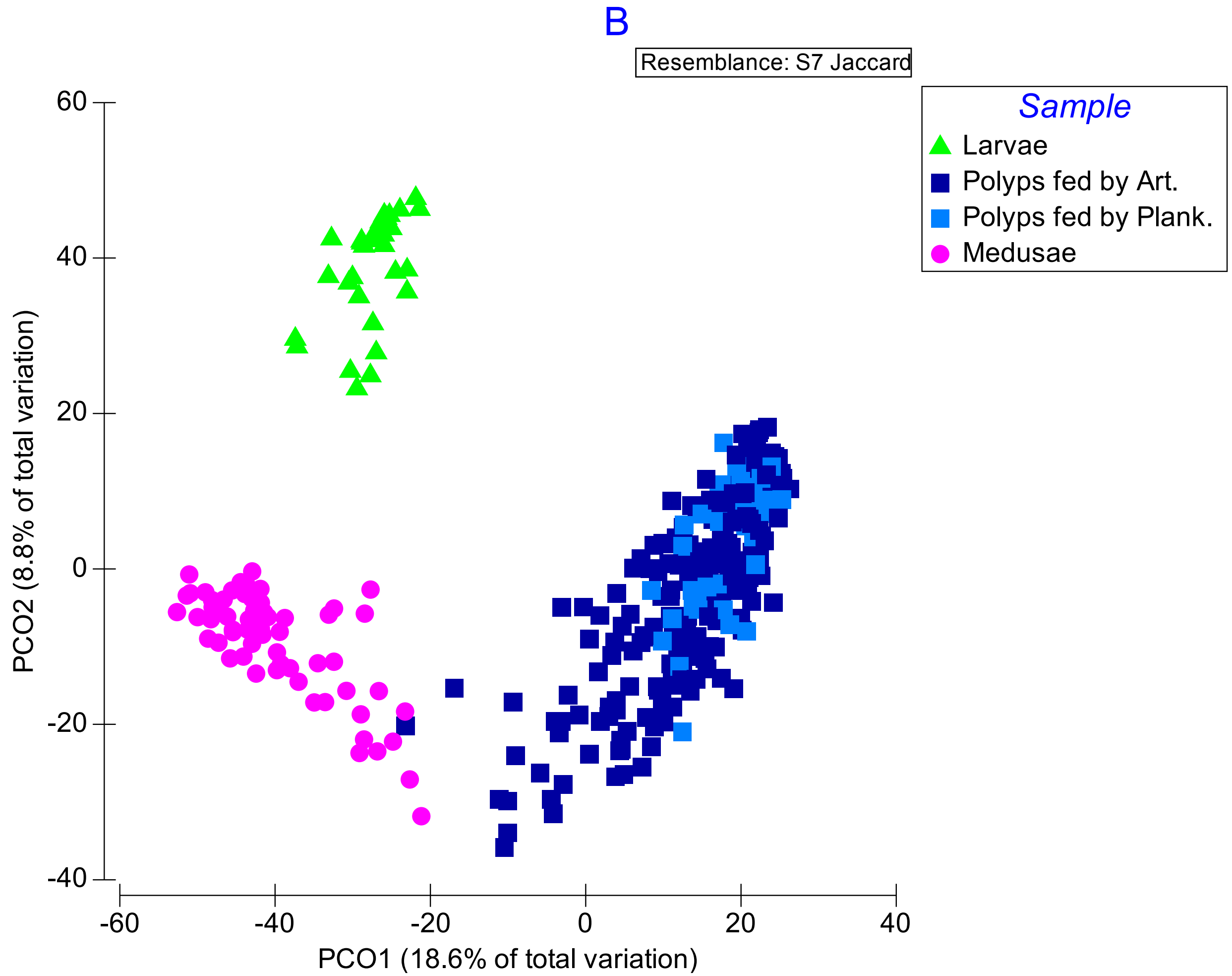


Figure 6. Principal coordinate (PCO) analysis presenting the bacterial communities associated with different life stages of scyphomedusae (A: *Cyanea lamarckii* and B: *Chrysaora hysoscella*) based on Jaccard coefficient from ARISA profiles.

Table 9. PERMANOVA main tests of bacterial community structure of different life stages of *C. lamarckii* based on Jaccard dissimilarities of ARISA profiles.

Significant results (p (perm) <0.05) are highlighted in bold. Displayed are tests for the factor 'sample' and the partitioning of multivariate variation. p-values were obtained using type III sums of squares.

d.f.: degrees of freedom, SS: sums of squares, Sq. root: square root of the component of variation attributable to that factor in the model, in units of Jaccard dissimilarities.

Source of variation	d.f.	SS	*pseudo F*	*p (perm)*	Perms	Sq. root
Sample	3	1.8504E5	26.29	**0.001**	998	23.937
Res	439	1.03E6				48.438
Total	442	1.215E6				

Table 10. PERMANOVA pair-wise comparisons of bacterial community structure of different life stages of *C. lamarckii* based on Jaccard dissimilarities of ARISA profiles.

Significant results (p (perm) <0.05) are highlighted in bold. Displayed are pair-wise comparisons of the factor "sample".

Comparison	t (perm)	*p (perm)*	Perms
Larva vs. Polyp fed with Art.	4.6219	**0.001**	999
Larva vs. Polyp fed with Plank.	5.4448	**0.001**	997
Larva vs. Medusa	4.0088	**0.001**	997
Polyp fed with Art. vs. Polyp fed with Plank.	5.8659	**0.001**	998
Polyp fed with Art. vs. Medusa	5.6428	**0.001**	996
Polyp fed with Plank. vs. Medusa	4.9969	**0.001**	998

Table 11. PERMANOVA main tests of bacterial community structure of different life stages of *Ch. hysoscella* based on Jaccard dissimilarities of ARISA profiles.

Significant results (p (perm) <0.05) are highlighted in bold. Displayed are tests for the factor 'sample' and the partitioning of multivariate variation. p-values were obtained using type III sums of squares.

d.f.: degrees of freedom, SS: sums of squares, Sq. root: square root of the component of variation attributable to that factor in the model, in units of Jaccard dissimilarities.

Source of variation	d.f.	SS	*pseudo F*	*p (perm)*	Perms	Sq. root
Sample	3	2.7552E5	40.386	**0.001**	999	38.36
Res	339	7.709E5				47.687
Total	342	1.0464E6				

Table 12. PERMANOVA pair-wise comparisons of bacterial community structure of different life stages of *Ch. hysoscella* based on Jaccard dissimilarities of ARISA profiles.

Significant results (p (perm) <0.05) are highlighted in bold. Displayed are pair-wise comparisons of the factor "sample".

Comparison	t (perm)	*p (perm)*	Perms
Larva vs. Polyp fed with Art.	7.1071	**0.001**	999
Larva vs. Polyp fed with Plank.	6.8027	**0.001**	998
Larva vs. Medusa	5.7475	**0.001**	999
Polyp fed with Art. vs. Polyp fed with Plank.	2.7725	**0.001**	999
Polyp fed with Art. vs. Medusa	7.9804	**0.001**	999
Polyp fed with Plank. vs. Medusa	5.3087	**0.001**	999

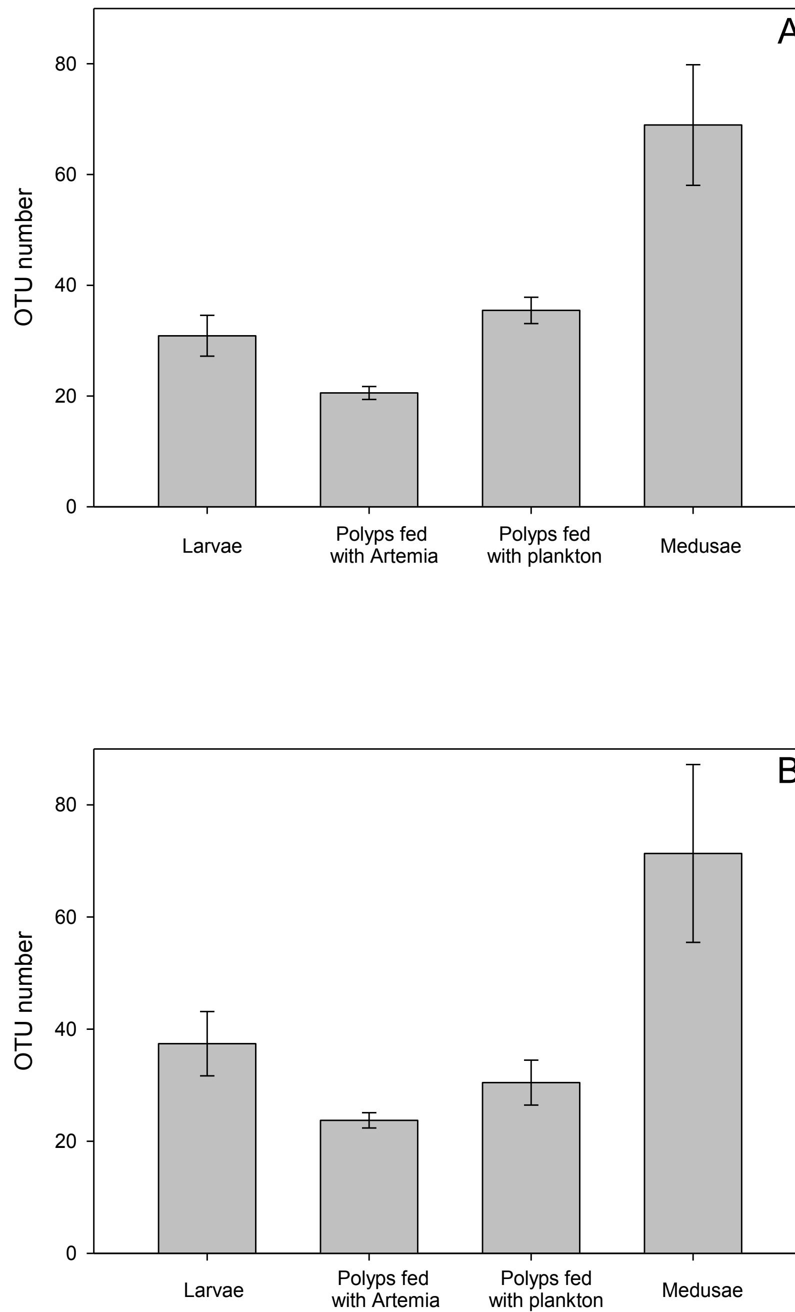
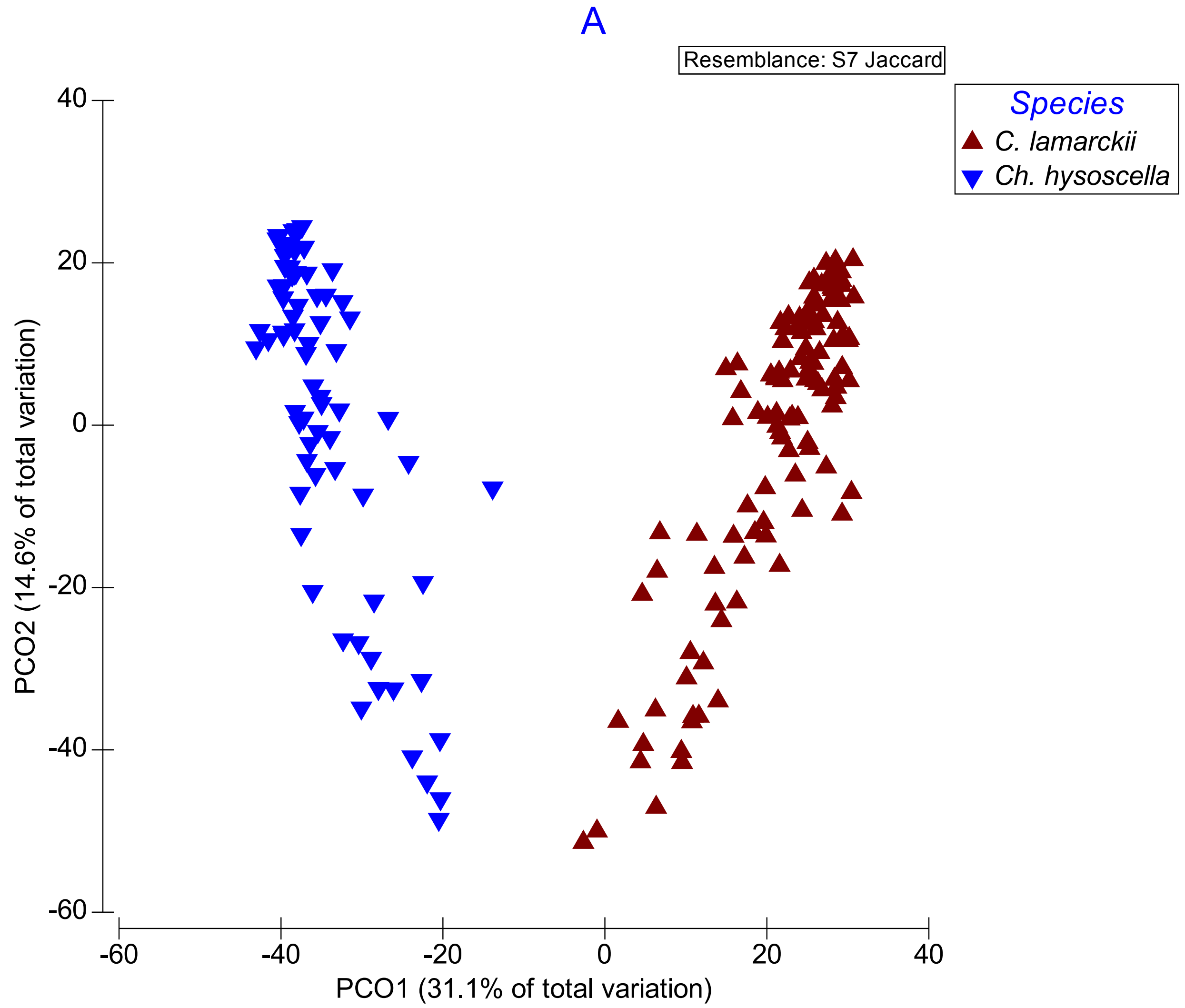
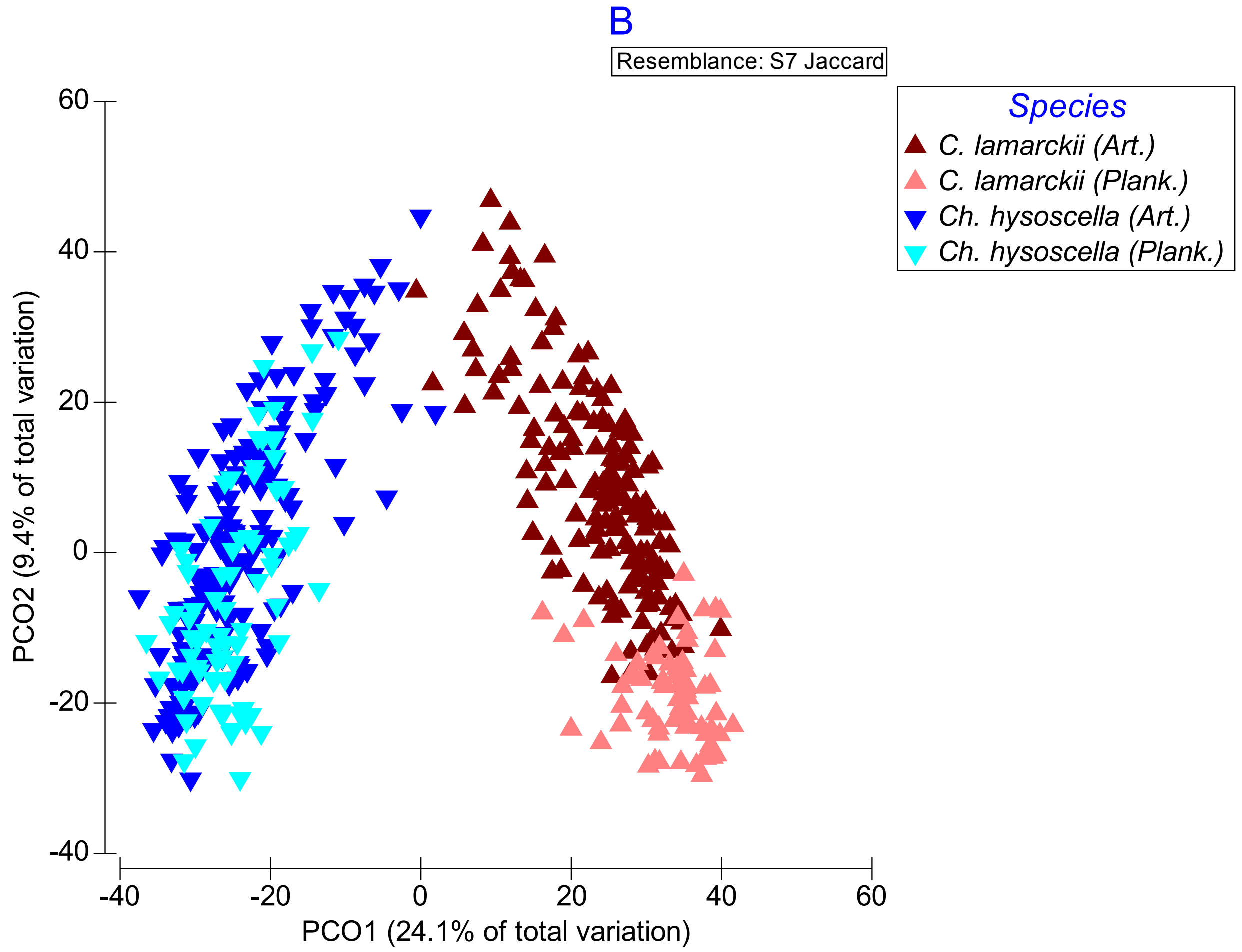


Figure 7. Bar chart of means of bacterial OTU number (alpha diversity) for different life stages of two scyphomedusae species obtained by ARISA fingerprint (A: *Cyanea lamarckii* and B: *Chrysaora hysoscella*). Whiskers indicate 95% confidence intervals.


A
Resemblance: S7 Jaccard
Species
C. lamarckii
Ch. hysoscella
PCO2 (14.6% of total variation)
PCO1 (31.1% of total variation)
40
20
0
-20
-40
-60
-60
-40
-20
0
20
40


B
Resemblance: S7 Jaccard
Species
C. lamarckii (Art.)
C. lamarckii (Plank.)
Ch. hysoscella (Art.)
Ch. hysoscella (Plank.)
PCO2 (9.4% of total variation)
PCO1 (24.1% of total variation)
60
40
20
0
-20
-40
-40
-20
0
20
40
60

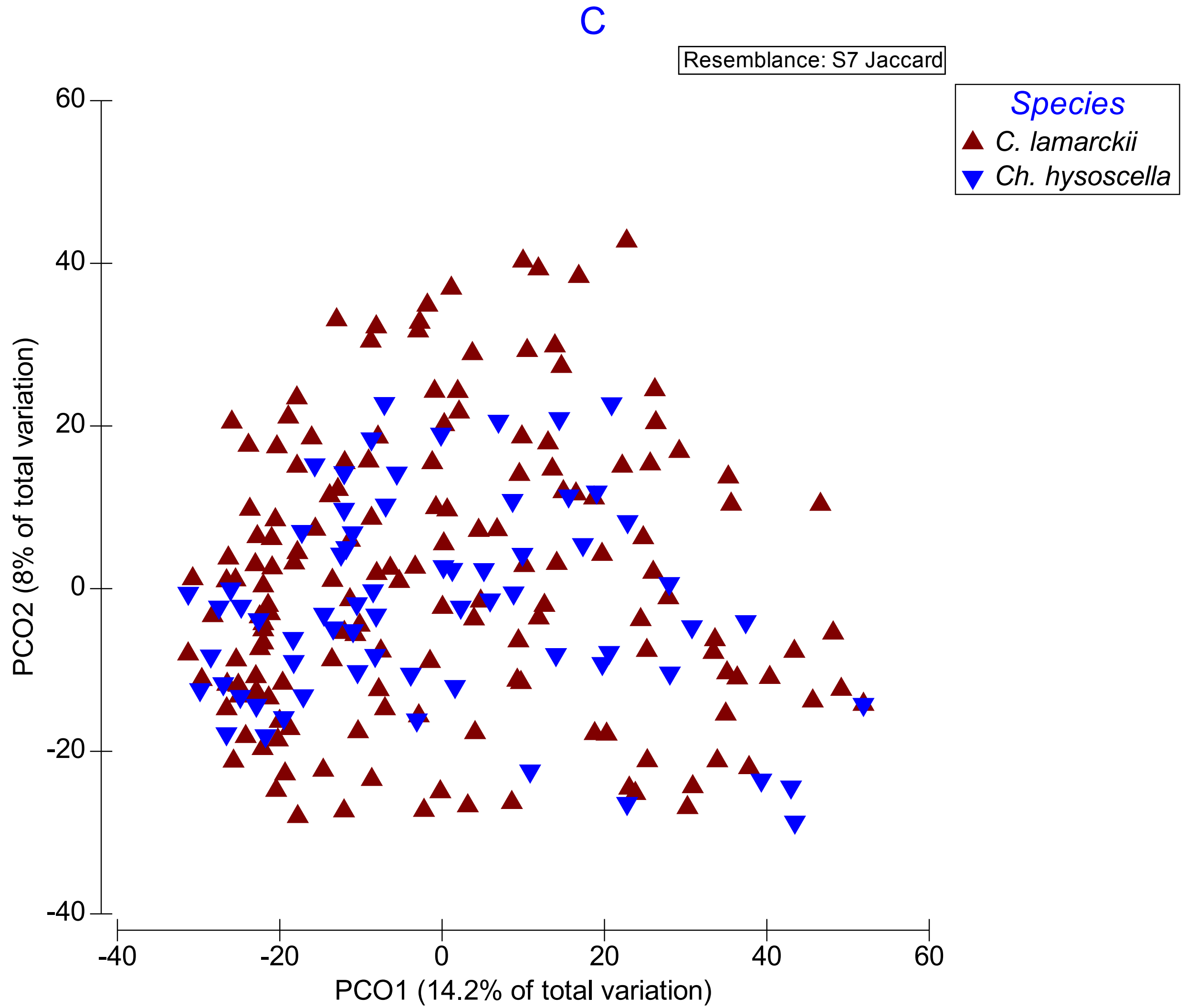


Figure 8. Principal coordinate (PCO) analysis presenting the bacterial communities associated with different life stages (A: Larvae, B: Polyps, C: Medusae) between two scyphomedusae species (*C. lamarckii* and *Ch. hysoscella*) based on Jaccard coefficient from ARISA profiles.

Table 13. PERMANOVA main tests of bacterial community structure of larvae based on Jaccard dissimilarities of ARISA profiles.

Significant results (p (perm) <0.05) are highlighted in bold. Displayed are tests for the factor 'species' and the partitioning of multivariate variation. p-values were obtained using type III sums of squares.

d.f.: degrees of freedom, SS: sums of squares, Sq. root: square root of the component of variation attributable to that factor in the model, in units of Jaccard dissimilarities.

Source of variation	d.f.	SS	*pseudo F*	*p (perm)*	Perms	Sq. root
Spe	1	1.4141E5	77.928	**0.001**	999	39.404
Res	188	3.4114E5				42.598
Total	189	4.8254E5				

Table 14. PERMANOVA main tests of bacterial community structure of polyps based on Jaccard dissimilarities of ARISA profiles.

Significant results (p (perm) <0.05) are highlighted in bold. Displayed are tests for the factor 'species' and the partitioning of multivariate variation. p-values were obtained using type III sums of squares.

d.f.: degrees of freedom, SS: sums of squares, Sq. root: square root of the component of variation attributable to that factor in the model, in units of Jaccard dissimilarities.

Source of variation	d.f.	SS	*pseudo F*	*p (perm)*	Perms	Sq. root
Species	1	1.8153E5	86.654	**0.001**	998	37.912
Food	1	37302	17.807	**0.001**	999	16.793
Sp×Fo	1	32021	15.286	**0.001**	998	21.896
Res	473	9.9085E5				45.769
Total	476	1.3853E6				

Table 15. PERMANOVA main tests of bacterial community structure of medusae based on Jaccard dissimilarities of ARISA profiles.

Significant results (p (perm) <0.05) are highlighted in bold. Displayed are tests for the factor ‘species’ and the partitioning of multivariate variation. p-values were obtained using type III sums of squares.

d.f.: degrees of freedom, SS: sums of squares, Sq. root: square root of the component of variation attributable to that factor in the model, in units of Jaccard dissimilarities.

Source of variation	d.f.	SS	*pseudo F*	*p (perm)*	Perms	Sq. root
Spe	1	12589	4.0264	**0.001**	998	10.028
Res	223	6.9724E5				55.916
Total	224	7.0983E5				

Discussion

Bacterial communities associated with scyphozoan regarding to different body parts were firstly analyzed in the present study. According to the ARISA fingerprints, bacterial community associated with umbrella was significantly different from those with tentacle and gonad with constant diversity in both species based on multivariate analysis (PERMANOVA) (Tab. 1-4). Scyphomedusae have a muscular saucer-shaped or hemispherical umbrella which propels the medusae through the water by contracting and expelling water behind. The umbrella of scyphozoan species is relative thick mesoglea containing epidermis and gastrodermis (Larson, 1976.). Gonads occur on fold of the gastrodermis. The four long oral arms surround the mouth end. They are ribbon-like or curtain-like and their ciliated epithelia transport the prey to the mouth. Long threadlike tentacles covered by stinging cells (nematocysts) arise from the umbrella and are used to capture prey (Arai, 1996). We analyzed the whole mesoglea of umbrella fraction including the surface and aboral surface. The mesoglea of scyphomedusae seems to be the main metabolically active tissues (Thuesen *et al.*, 2005). There is clear decline on the oxygen concentrations from the outer umbrella surface to the gonad (Thuesen *et al.*, 2005). The different characteristics of oxygen concentrations within different body parts might influence the colonization of the associated bacterial community.

Interestingly, from the studies in *Hydra*, the bacterial communities were significantly modulated in response to the changes in the epithelia cell composition by removing the interstitial stem cell (Fraune *et al.*, 2009). These results indicate a direct interaction between cellular tissue composition and microbiota (Fraune *et al.*, 2009). In jellyfish, different types of cells are described in different body parts (Lesh-Laurie & Suchy, 1991). For example, the epidermal gland cells are surrounding the nematocyst clusters in the tentacle of *C. lamarckii* with an antibody activity (Elofsson & Carlberg, 1989). The tentacle of scyphomedusae contains numerous nematocyst toxins which are generally cytolytic, hemolytic and neurotoxic (Bailey *et al.*, 2003; Helmholz *et al.*, 2010; Lassen *et al.*, 2010). Schuett & Doepke (2010) found species-specific endobiotic bacteria in the tentacle of scyphomeduesa. Titelman *et al.*(2006) reported the inhibition of bacterial growth depending on the different body fractions of jellyfish *Periphylla periphylla*. The umbrella had the strongest inhibitory effect on bacterial community while the weakest in the tentacle (Titelman *et al.*, 2006). All this makes it obvious, it’s necessary to take

the different body parts into account in future study regarding to bacterial community associated with scyphozoan.

To the best of our knowledge, bacterial communities associated with different life stages of the two scyphozoan species *Cyanea lamarckii* and *Chrysaora hysoscella* were first investigated in the present study. We mainly focused on three representative life stages: planula larva, polyp and adult medusa. The bacterial communities structures associated with three life stages were significantly different among each other in both scyphozoan species (Fig. 6, Tab. 9-12). The BCCs associated with three life stages of *C. lamarckii* significantly separated (Tab. 9 and 10) from different stages indicating a kind of transition from larvae to polyps until medusae (Fig. 6 A). Fraune *et al*. (2010) investigated the bacterial colonization during early embryogenesis in *Hydra* and they found significantly different bacterial communities associated with early embryos from that with later developmental stages. Furthermore, different bacterial communities between embryo and polyp were observed in their study. Bacterial community of early cleavage embryo in *Hydra* has lower richness than the later stages. In contrast for scyphozoan, the bacterial communities associated with the early stage larvae (S=31 and 37, respectively in *C. lamarckii* and *Ch. hysoscella*) present higher diversity than that in the later stage polyp (S=21 and 24, fed with *A. salina* respectively in *C. lamarckii* and *Ch. hysoscella* in both species (Fig. 7), whereas the diversity of the bacterial community associated with the adult stage medusa"dramatically increased in *C. lamarckii* (S=69) and *Ch. hysoscella* (S=71). Apparently, the early planula stages are colonized by a limited number of bacterial colonizers compared with medusa. During later life cycle stages (polyp and medusa), the structure and richness of bacterial community changed. For both scyphozoan species, the bacterial associations displayed more diverse community in the medusa adult stage. It might indicate that different life stages offer different resources and may display different niches. Although the richness of bacterial community associated with three life stages display similar patterns (Fig. 7), the bacterial community structures are interestingly strong distinct (Fig. 6). For *C. lamarckii*, each stage appears to be a passive substrate colonized by a different and more diverse bacterial community presenting a dispersive community among three stages (Fig. 6 A). For *Ch. hysoscella*, the bacterial communities of each stage show a strong selective processes of bacterial colonization with a highly assembled and separated community structure (Fig. 6 B).

Widersten (1965) and Holst (2012 b) described the histology and morphology of the gonads and the development of planulae in different scyphozoan species. In *C. lamarckii*, ciliary activity transports the embryos from ovary to the folds of the mouth arms and embryos develop in the mouth arms of female medusae, whereas in *Ch. hysoscella* the embryos remain in the hermaphrodite gonads and develop into planulae (Widersten, 1965; Holst *et al.*, 2007). However, the bacterial communities associated with planula larvae were significantly different from the communities of mouth arm in *C. lamarckii* as well as to the gonad in *Ch. hysoscella* (data not shown). It might indicate that there is no transformation between the larvae and the body parts either from mouth arm in *C. lamarckii* or from the gonads in *Ch. hysoscella* regarding their associated bacterial community.

The early planula stage might be capable of controlling their bacterial colonizer. Fraune *et al.* (2010) demonstrated that *Hydra* embryos are protected by a maternally produced antimicrobial peptide (AMP) of the periculin peptide family, which controls the establishment of the microbiota during embryogenesis. Antimicrobial peptides (Chapman *et al.* 2010) represent the major defense system against microbial infection in marine invertebrates (Otero-González *et al.*, 2010). They are known as prominent effecter of the innate immune system that often get secreted in response to external stimulation (Bosch, 2013). With over expressed periculin in polyps, it caused not only decreases in the number of associated bacteria but also changes in the composition of community (Fraune *et al.*, 2010). The novel antimicrobial peptide Aurelin is another examples of such genes has been found in other scyphozoa species *Aurelia aurita* (Ovchinnikova *et al.*, 2006). This Aurelin peptide exhibited activity against gram-positive and gram-negative bacteria (Ovchinnikova *et al.*, 2006). Franzenburg *et al.* (2013) stated that species-specific antimicrobial peptides shape species-specific bacterial associations. Although there is no available data regarding to the antimicrobial peptides in *C. lamarckii* and *Ch. hysoscella*, we speculate that the colonized bacterial community may adapt to different AMP repertoires of scyphomedusae species resulting in specific associations. This need to be investigated in the future.

The associated bacterial communities of polyps of two scyphozoan species were investigated under lab condition and the impact of food on the BCCs was first investigated in the present study. In Cnidarians, pelagic larvae undergo metamorphosis to a sessile polyp (Woollacott & Hadfield, 1996; Leitz, 1997; Holst & Jarms, 2010). It is becoming increasingly clear that specific

cues inducing settlement and/or metamorphosis emanate from the substrates (Fletcher, 1994) which is comprised by biofilms in marine environments. Within the biofilms certain bacteria are suggested to deliver the metamorphosis-inducing stimulus (Clare *et al.*, 1998; Müller & Leitz, 2002). It was shown that axenic cultures of buds and larvae of *Cassiopea andromeda* do not undergo metamorphosis. Interestingly the metamorphosis was induced by incubation with *Vibrio* sp. isolated from aquarium material found to induce metamorphosis of *Cassiopea* (Hofmann *et al.*, 1978). However, the larvae spontaneously settled and have undergone the metamorphosis to polyps in the current study, supplied only with sterile seawater, without adding additional inducers. This also happened in the experiment carried out by Holst & Jarms (2006; 2010; Holst, 2012 a and b) Taken together, larvae and polyps perhaps harbor their own bacteria which they need for growth and development in later stages. These innate set of bacteria are particular species-specific by using gene-encoded mechanism to protect and survive independent of the body parts in which they develop (Fraune *et al.*, 2010). The only chance of polyps to interact with external environment is the different kinds of food sources during feeding process. Based on the ARISA fingerprints, the bacterial communities of food sources clearly separated from the communities associated with polyps. However, the bacterial communities associated with polyps fed with *A. salina* were significantly different from the one fed with plankton in two scyphozoan species (Fig. 4 and Fig. 8B). Regarding to richness of the bacterial community, we found that bacterial community associated with polyps fed with plankton were more diverse than the one fed with *A. salina*. This might indicate that the bacterial community of the food, either the free-living or the attached community do have an impact on the bacterial community associated with polyps, but not in the process of the selecting and shaping the bacterial communities associated with polyps in both scyphozoan species. Polyps might react differently in response to different food source, for example, with distinct metabolic activity, which may play a pivotal role for the selection and formation of the bacterial community associated with polyps.

Conclusion

In the present study, the bacterial communities associated with two scyphomedusae species were firstly investigated at Helgoland Roads (North Sea, Germany). Significant differences show in both scyphomedusae species not only in respect to different body parts but also in different life stages. With the significant differences between umbrella and tentacle in different parts, at this point, bacterial community associated with scyphomedusae should analyzed with different parts to better understanding these bacterial associations' ecological role and functions. For the different life stages, completely different patterns present in both scyphomedusae species. A passive substrate colonized by diverse bacterial community presenting a dispersive community among three stages in *C. lamarckii*, while a strong selected processed of bacterial colonization in each life stage presenting a highly separated community structure in *Ch. hysoscella*. To interpret the variability of bacterial community associated with different life stages, additional stages such as strobilation and ephyra need to be taking into account. Moreover, significantly different bacterial community associated with three life stages was revealed compared between two scyphomedusae species. This might indicate that the BCCs of different life stages of scyphomedusae are species-specific. Interestingly the diversity of BCC associated with polyps is influenced by different sources of food, but also in the bacterial community composition, where bacterial community associated with polyps presented significant differences in response to different food source. To provide a comprehensive picture of the selection of bacterial community in each life stage of scyphomedusae, hatching process of polyp in the field experiment, cellular tissue composition, functional genes and links between innate immune system need to be analyzed in later study.

Chapter III

Bacterial communities respond to the excretion of DOM released by live jellyfish

Wenjin Hao[1], Antje Wichels[1], Bernhard Maximilian Fuchs[2], Gunnar Gerdts[1]

[1] Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Biologische Anstalt Helgoland, 27498 Helgoland, Germany

[2] Max-Planck-Institute for Marine Microbiology, Department Molecular Ecology, Celsiusstr. 1, 28359 Bremen, Germany

Key words: Bacterial community structure, bacterial community composition, DOM, jellyfish, ARISA, CARD-FISH

Abstract

Jellyfish blooms have increased around the world and these outbreaks of jellyfish population not only impact the food web structures by voracious predation, but also play an important role in the dynamics of nutrients and oxygen in planktonic food webs. So far, the impacts of dead jellyfish biomass on bacterial growth and microbial community composition were quantified. However, it remains unclear if the colloidal and dissolved organic matter released by live jellyfish is specific carbon compounds and whether such compounds have the potential to shape bacterial community composition. In this study, we focused on the compositional succession of bacterioplankton community in response to the DOM released by live scyphomedusae (*Cyanea lamarckii* and *Chrysaora hysoscella*) at Helgoland Roads in the German Bight of the North Sea. The bacterial communities were significantly stimulated by the DOM released by live jellyfish with different dominant phylotypes regarding to different scyphomedusae species. Bacterial community structure determined via automated ribosomal intergenic spacer analysis (ARISA) significantly differed regarding to different DOM sources including jellyfish treatment, “Kabeltonne” seawater and artificial seawater (DOC-free). Based on catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH) analysis, we observed a rapid shift in community composition for the jellyfish treatment with distinct differences in respect to the two scyphozoan species. Gammaproteobacteria dominated the community conducted with *C. lamarckii*, while Gammaproteobacteria and Bacteroidetes dominated the community within *Ch. hysoscella* in equal amounts. These significant differences in the bacterial community composition and succession indicate that the DOM released by different jellyfish genera might consist of different compounds which are species specific.

Introduction

The ocean contains one of earth's largest bioactive dissolved organic carbon (DOC) pools (Hedges, 1992). This large reservoir of carbon and nutrients largely derives from phytoplankton biomass, zooplankton grazing activities and viral lysis (Nagata, 2000). Bacterioplankton play a key role in assimilating and transforming this source of reduced carbon (Kujawinski, 2011), whereby energy and nutrients are channeled to higher trophic levels (Azam *et al.*, 1983). A great diversity of uptake mechanisms and metabolic pathways for different carbon compounds have evolved among phylogenetic diverse bacteria to utilize DOC (Hopkinson & Barbeau, 2012). Gómez-Consarnau (2012) reported that different low-molecular-weight organic compounds (e.g. amino acids, glucose, dimethylsulphoniopropionate, acetate or pyruvate) differentially stimulated bacterial growth in most cases and triggered the development of unique combinations of dominant phylotype. These results emphasize that bacteria substantially differ in their abilities to utilize specific carbon compounds, with some bacteria being specialists and others having a more generalist strategy (Martinez *et al.*, 1996; Cottrell & Kirchman, 2000; Riemann & Azam, 2002). Studies on the variability of bacterial populations in time and space also indicate the role of resources in determining population dynamics. The appearance of Roseobacter clade bacteria and Flavobacteria has been linked to the organic matter released during phytoplankton blooms (González *et al.*, 2000; Pinhassi *et al.*, 2004; Teeling *et al.*, 2012). Additional characteristics, such as the substrate affinity or efficiency of carbon processing, may also vary substantially among bacterial taxa, suggesting that the quality of available compounds could be a strong selective force on bacterioplankton community composition (Riemann *et al.*, 2000; Condon *et al.*, 2011; Kujawinski, 2011).

Jellyfish blooms have occurred in many estuarine, coastal and open sea ecosystems worldwide during the past decades (Brodeur *et al.*, 2002; Parsons & Lalli, 2002; Billett *et al.*, 2006; Doyle *et al.*, 2008). Jellyfish acquire C, N and P by assimilating organic compounds from ingested prey and take up small amounts of dissolved organic material (Pitt *et al.*, 2009). Jellyfish are also known to release organic matter (Hansson & Norrman, 1995) by several mechanisms such as sloppy feeding or excretion of fecal material or mucus (Pitt *et al.*, 2009). Large live medusae accumulatively damaged and gradually broken down in the water column throughout the season (Mills, 2001). This process of decomposition may support microbial production. Meanwhile,

inorganic N and P regenerated by excretion may support algal production. Therefore, jellyfish play an important role in the dynamic of nutrients in planktonic food webs via their excretion of inorganic nutrients, primarily as ammonium (NH_4^+) and phosphate (PO_4^{3-}), and by release of dissolved organic matter (DOM) (Condon *et al.*, 2010).

Bacteria thrive in the dissolved organic carbon released by jellyfish which has been described both in the laboratory (Hansson & Norrman, 1995) and in the field (Riemann*,* *et al.*, 2006). Tinta *et al.*(2010; 2012) observed an increase in bacterial abundance and production and a rapid shift in community composition from unculturable Alphaproteobacteria to culturable species of Gammaproteobacteria and Flavobacteria coupled with NH_4^+ accumulation and oxygen consumption (Tinta *et al.*, 2012). Condon *et al.* (2011) reported that jellyfish released substantial quantities of extremely labile C-rich DOM, which was quickly metabolized by bacterioplankton. Jelly-DOM not only support the rapid growth and dominance of specific bacterial phylogenetic groups (primarily Gammaproteobacteria) (Titelman *et al.*, 2006; Condon *et al.*, 2011) that were rare in ambient waters, but also detour the pathway of C towards bacterial CO_2 production and away from higher trophic levels (Condon *et al.*, 2011). Previous studies quantified the impacts of the dead jellyfish biomass on bacterial growth and microbial community composition by modifying carbon and nutrient conditions (Martinez, 1996) through release of nutrients and bio-available dissolved organic matter (Hansson & Norrman, 1995; Titelman *et al.*, 2006). However, it remains unclear if particular bacteria preferentially utilize specific carbon compounds released by jellyfish during metabolism and whether such compounds have the potential to shape the bacterial community composition.

In this study, we focused on the compositional succession of bacterioplankton community in response to the DOM released by scyphomedusae at Helgoland Roads in the Germany Bight. We conducted incubation experiments to evaluate the influence of native bacterial communities in response to DOM released by live scyphomedusae *Cyanea lamarckii* and *Chrysaora hysoscella*. *C. lamarckii* and *Ch. hysoscella* (Russell, 1970) are common medusa species at Helgoland Roads in the German Bight and usually occur during summer (Möller, 1980; Hay *et al.*, 1990; Barz & Hirche, 2007). These scyphomedusae occur worldwide in numerous coastal and shelf sea environments (Lucas, 2001) frequently forming large blooms (Hamner & Dawson, 2009).

Materials and methods

Sample collection and preparation

Sampling of jellyfish *Cyanea lamarckii* and *Chrysaora hysoscella* was performed separately in July and August 2012 at Helgoland Roads station in the Germany Bight (54˚11.3′N, 7˚54.0′E). Jellyfish used in the experiment were collected by handling bucket from surface water and immediately transported to the laboratory. Damaged animals were omitted from the analysis. Prior to the experiment, jellyfish were gently transferred into a sterile DOC free NaCl solution (PSU 30) (20L) for 15 min. This was repeated for three times to rinse the specimen and provided them time to clear their gut content. Potential confounding effects of sloppy feeding and leaching of DOM from fecal material during the experiment was reduced. In general, most animals appeared healthy and undamaged after washing.

Collection of DOM released by live medusae

15 individuals of each jellyfish species were incubated separately in 3 L beakers filled with 2.5 L DOC-free sterile artificial seawater (ASW) under in *situ* temperature in dark for 24h. Every beaker was covered with combusted aluminum foil. The DOC-free sterile ASW was prepared according to Kisand *et al.* (2008) (1L ASW, main elements: $FeCl_3 \times 6H_2O$, 0.11g; NaCl, 19.45g; $MgCl_2 \times 6H_2O$, 12.6g; $MgSO_4 \times 7H_2O$,6.63g; $CaCl_2 \times 2H_2O$, 2.38g; KCl, 0.55g; $NaHCO_3$, 0.16g; $Na_2HPO_4 \times 2H_2O$, 0.01g; trace elements: KBr, 0.08 g; $SrCl_2 \times 6H_2O$, 0.06 g; H_3BO_3, 0.02g; Na_4O_4Si, 0.007g; NaF, 0.002 g; $H_4N_2O_3$, 0.002g). During the incubation, jellyfish were not fed in order to avoid organic matter release due to sloppy feeding. After 24h, the jellyfish were gently removed from the ASW using laboratory gloves and a bent spoon. Each spoon was prepared with following steps: soaked with acidified Milli-Q water (pH 2) and rinsed twice with Milli-Q water then combusted at 400°C for 4h. Following jellyfish removal, the incubated ASW of all beakers was instantly processed. Five of the jelly-incubated media were collected for later experiment setup see below.

Experiment setup and sampling

The schematic overview of the experiment setup is present in Figure 1. The setup consisted of three treatments: jellyfish treatment, "Kabeltonne" seawater and artificial seawater. Each of the treatment included five replicates. Jellyfish incubated medium as described above, is artificial seawater containing dissolved organic matter released by live jellyfish. "Kabeltonne" seawater treatment served as control containing natural DOM. Artificial seawater treatment is DOC-free and served as blank. These three different media were filtered through GF/F filter (Whatman) and subsequently through 0.2µm filter (GTTP polycarbonate filters, Millipore) to get 2 L (final volume) of sterile medium containing different dissolved organic matter both in concentration and in composition. The entire experiment was carried out twice with two different scyphomedusae species in July and Aug. 2012, respectively (Tab. 1). Therefore, both treatments of the control (Kabeltonne seawater) and blank (artificial seawater) were performed twice in corresponding with different scyphomedusae species.

Experimental treatments were inoculated with 2 ml fresh seawater from station Helgoland Roads in the Germany Bight (54˚11.3′N, 7˚54.0′E). Seawater was filtered through 3µm filter before inoculation to remove larger organisms. After set up of the experiments, all treatments were incubated under in *situ* temperature in the dark. Samples were collected at the following time points: 6h, 12h, 24h and every 24h (48h, 72h, 96h, 120h, and 144h) until the final sampling point 168h, to analyse the bacteria abundance and composition. Each water sample from the different treatments was collected with a sterile syringe in a rubber tube. Before sampling the incubated water was fully stirred. Flow cytometry was applied to measure the bacteria abundance. CARD-FISH was applied to investigate the bacterial composition. At the end of each experiment (at 168h), except for collecting the abundance and composition samples, DNA samples were collected for the analyses of bacterial community structure by ARISA fingerprint.

To avoid DOM contamination all the containers and material (sterile syringe and rubber tube) were first washed and soaked overnight with acidified (pH 2) Milli-Q water and rinsed twice with Milli-Q water then sealed with combusted aluminum foil.

Bacterial enumeration by flow cytometry

For flow cytometry analyses, 500 µl of a sample was stained directly after sampling with 10 µl of a freshly prepared 400× SYBR Green (invitrogen™, Life Technologies, Paisley, UK) solution in sterile filtered dimethyl sulfoxide (DMSO) for 10 min at room temperature and in the dark. 10 µl of a diluted solution of Fluoresbrite® Polychromatic Red Microspheres 1.0 µm (Polysciences Europe, Eppelheim, Germany) as an internal counting standard (final concentration of about 10% of the expected number of cells) was directly added to the sample prior to staining. Samples were analyzed samples with an Accuri C6 flow cytometer (BD Accuri Cytometers, Ann Arbor, MI, USA) with the fluidics setting "slow" for 1.5 min. To reduce noise, a threshold on FL1-H of 550 for all treatment was applied. The actual flow through was calibrated with BD Trucount™ Controls (BD Biosciences, San Jose, CA, USA).

DNA extraction

Bacterial biomass was collected on 0.2 µm Isopore™ Membrane Filters (GTTP-type, 47 mm diameter, Millipore) and DNA extraction was performed as previously described (Sapp *et al.*, 2007 b). All DNA extracts were dissolved in 30-50µl sterile water and served as template DNA to analyse the Bacterial community structure via ARISA fingerprinting. The quantity and quality of extracted DNA was determined microphotometrically using a Tecan Infinite 200 NanoQuant.

Bacterial community structure

To characterize the differences of bacterial community structure in response to different DOM sources, ARISA fingerprint were performed at the end of the experiments according to Krause *et al*. (2012) as described previously (described in details in Chapter I).

Fixation and CARD-FISH

For the composition of bacterial community, catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH) was performed according to Pernthaler *et al.* (2004) with modifications. Samples for CARD-FISH were fixed with 37% formaldehyde solution (final concentration 1% v/v) at 4°C overnight. Water samples of 10 ml were filtered onto

polycarbonate filters (type GTTP, 0.2 μm pore size, 47 mm diameter, Millipore) and were stored at -20°C until further analyses.

According to the growth curves of each treatment (flow cytometry), the inoculation and four representing time points of the treatments including the initial point (24h) and the exponential growth phase (48h), the beginning of stationary phase (96h) and the ending of experiment (168h) were chosen for the bacterial community composition analyses.

For the jellyfish treatment, permeabilization was conducted with 10 mg/ml Lysozyme in 50mM EDTA, 100 mM Tris/HCl for 35 min at 37°C, while the samples from "Kabeltonne" seawater and artificial seawater treatments were permeated for 1h at the same temperature. The hybridization was carried out for 2.5 h in three treatments with horseradish peroxidase (HRP)-labeled oligonucleotide probes at varying formamide concentrations depending on the probes (Table 2). The fluorescein-labeled tyramide was used for signal amplification (Pernthaler *et al.*, 2004) which was carried out for 30 min in all three treatments. The filter sections were washed twice in 96% ethanol, dried and embedded on microscope slides with 4:1 (v/v) Citifluor (Citifluor Ltd., London, UK) and VectaShield (Vector Laboratories Inc., Burlingame, CA, USA) antifading reagents.

For the quantification of total microbial cell numbers, the cells were stained with DAPI (1μg/mL) and were partly quantified manually on an Axioplan II Imaging epifluorescence microscope (Carl Zeiss MicroImaging GmbH, Göttingen, Germany) and partly enumerated automatically with the Zeiss Axio Imager.Z2 (Carl Zeiss MicroImaging GmbH). For automated cell quantification, the software package AxioVision 7.6 (Carl Zeiss MicroImaging GmbH) was used in conjunction with the macro MPISYS and the ACMEtool 0.75 software (Zeder *et al.*, 2011).

Based on the basic knowledge of the composition of free-living bacterial community and the associated bacterial community of jellyfish, three general probes, including Gam42 and CF319a and Ros537 were applied on all three treatments at four time points (mentioned above) with five replicates. According to the results obtained by applying general probes, no difference among the five replicates was observed in respect to the bacterial community composition, therefore another four more specific probes including Alt1413, Psa184, Pol1740 and Ulv995 were applied to one replicate for each treatment. Alt1413, Psa184, Pol1740 and Ulv995 were applied for the time point of inoculation (0h) and jellyfish treatment at both 24h and 168h. Two specific probes

Alt1413 and Psa184 were applied on "Kabeltonne" seawater and artificial seawater treatments at both 24h and 168h. All probes are list in the Table 2.

Statistical analysis

Analysis of ARISA data

ARISA fingerprints were analyzed by using BioNumerics 6.6 software (Applied Maths, Sint-Martens-Latem, Belgium). Normalization of band patterns and binning to band classes was performed according to Kovacs *et al*. (2010) and Brown (2005) as described previously (described in details in Chapter I).

The alpha diversity (OTU richness) (operational taxonomic units) of each sample obtained from ARISA fingerprints was calculated by summing the total number of remaining bands. ARISA-OTUs were analyzed based on a constructed binary table. Differences between the groups were tested by one-way analysis of variance (ANOVA) in the software package Statistica 9.

Growth kinetics of bacterial community in response to different DOM source

Growth kinetics of bacterial community in jellyfish medium, "Kabeltonne" seawater and artificial seawater were compared by the construction of growth curves. The growth kinetics parameters including lag time (LT) and specific growth rate (SGR) were determined by the modified Gompertz equation using GraphPad Prism 4.0 (GraphPad Software, San Diego, CA, USA) (Kim *et al.*, 2012). The equation used was as follows:

$$Y = N_0 + C \times \exp(-\exp((2.718 \times SGR/C) \times (Lag - t) + 1))$$

In this equation, Y represents the viable cell count (log cell/ml), N_0 is the initial log number of cells, C is the difference between the initial and final cell numbers, SGR is the maximum specific growth rate (log N/ml), LT is the lag time before growth and t is the sampling time. The goodness-of-fit of the data was evaluated based on the coefficient of determination (R^2), which was provided by GraphPad Prism.

Multivariate analyses

For multivariate statistical analyses the software package PRIMER v.6 and the add-on PERMANOVA+ (both PRIMER-E Ltd, Plymouth, UK) was used. The permutational

multivariate analysis of variance (PERMANOVA) with fixed factor was applied to investigate the difference of bacterial community composition (BCC) in two scyphomedusae species regarding different DOM treatments based on Jaccard coefficient. Principal co-ordinate analysis (PCO) was performed to visualize patterns of the bacterial community in response to different DOM treatments.

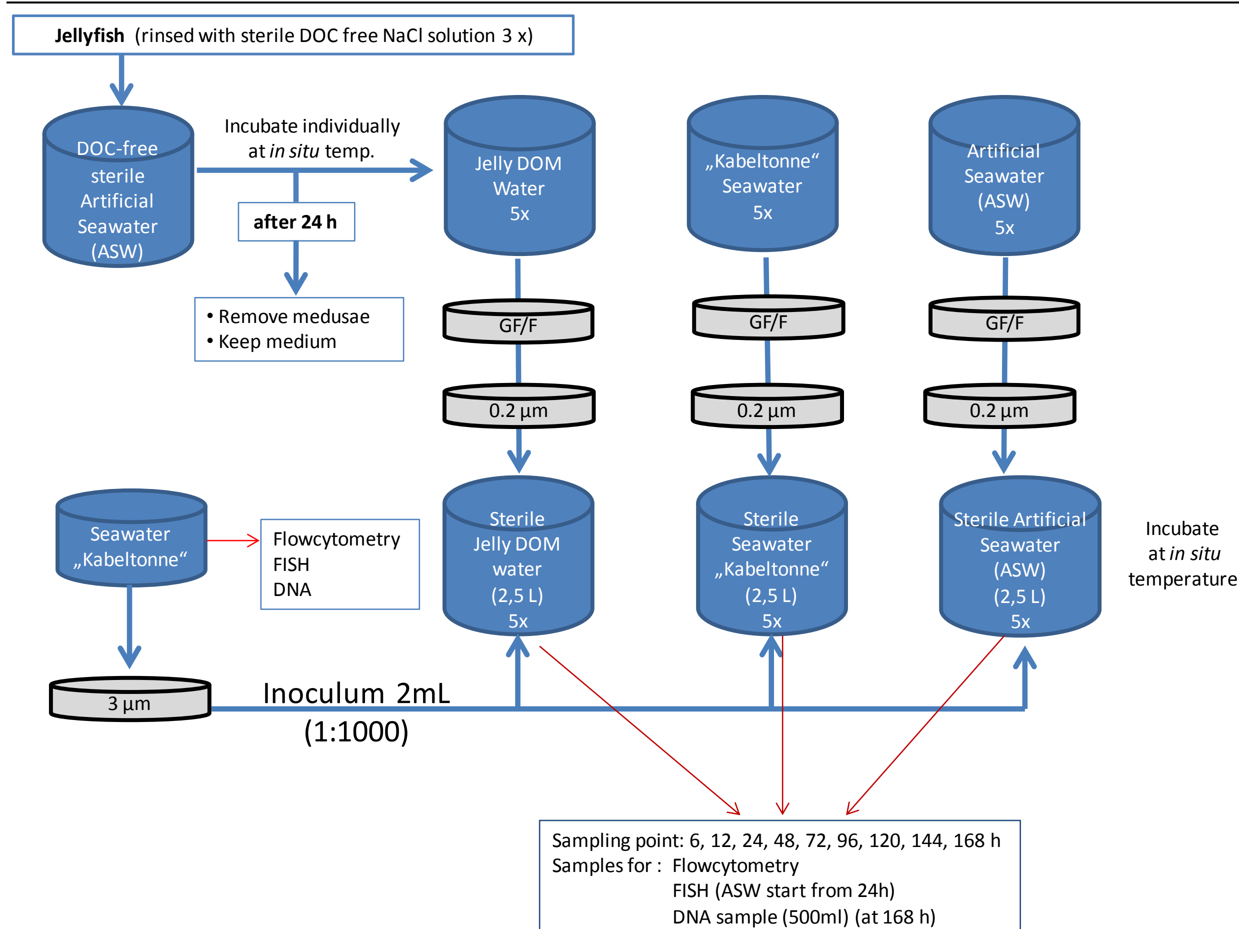


Figure 1. The flow diagram of the experiment setup is indicated with the thick blue line. The sampling schedules are indicated with the thin red line. Each of the treatment contains five replicates. The same setup was performed twice each on different dates (July and Aug. 2012, respectively) with two scyphomedusae species (*Cyanea lamarckii* and *Chrysaora hysoscella*). (For details on preparation and sampling see the material and methods part.)

Table 1. Bacterial abundances of inoculation and incubation temperature for each sampling date, and jellyfish species

Jellyfish species	Jellyfish sampling day	Inoculation's sampling day	Bacterial abundance of inoculation (N/ml)	Incubation temperature (°C)
C. lamarckii	18.07.2012	19.07.2012	1.1×10^{6}	15
Ch. hysoscella	30.08.2012	31.08.2012	1.9×10^{6}	17

Table 2. List of applied probes including target groups, probe sequences, required formamide concentrations (FA%) and references (The specific probes marked with different symbols belong to the general probes marked with the same symbol)

Probe	Target group	Probe sequence (5' to 3')	FA%	Reference
Eub338	Bacteria	GCTGCCTCCCGTAGGAGT	35	(Amann *et al.*, 1990)
Gam42a	γ-Proteobacteria *	GCCTTCCCACATCGTTT	35	(Manz *et al.*, 1992)
CF319a	Bacteroidetes #	TGGTCCGTGTCTCAGTAC	35	(Manz *et al.*, 1996)
Ros537	Roseobacter clade	CAACGCTAACCCCCTCC	35	(Eilers *et al.*, 2001)
Alt1413	Alteromonas/ Colwellia *	TTTGCATCCCACTCCCAT	40	(Eilers *et al.*, 2000)
Psa184	*Pseudoalteromonas* spp. *	CCCCTTTGGTCCGTAGAC	30	(Eilers *et al.*, 2000)
Pol740	*Polaribacter* spp. #	CCCTCAGCGTCAGTACAT ACGT	35	(Malmstrom *et al.*, 2007)
Ulv995	*Ulvibacter* spp. #	TCCACGCCTGTCAGACTA CA	35	(Teeling *et al.*, 2012)
Ulv995Comp1	Competitor 1 to Ulv995	TCCACTCCTGTCAGACTAC A		(Bennke *et al.*, 2013)
Ulv995Comp2	Competitor 2 to Ulv995	TCCACCCCTGTCAGACTAC A		(Bennke *et al.*, 2013)

Results

Growth kinetics of bacterial community

In the present study, we investigated the response of the planktonic bacterial community to different DOM sources including a jellyfish treatment with jelly-DOM released by live jellyfish, a “Kabeltonne” seawater containing natural DOM source and a DOC-free artificial seawater treatment. This study was conducted twice with two different scyphomedusae species (*Cyanea lamarckii* and *Chrysaora hysoscella*). The wet weight and dry weight of two scyphomedusae specimens were shown in Table 3. Wet weight and dry weight does not correspond with each other and the specimen with the highest wet weight does not show highest dry weigh. One of the first views, dry weight varied depending on the water content in different specimen. Interestingly, macroscopic visible aggregates were also observed to correlate with the highest dry weight individual (*C. lamarckii*: replicate B and *Ch. hysoscella*: replicate A).

The growth curves of the first set of treatments conducted with *C. lamarckii* well fitted to the Gompertz equation (R^2 = 0.972 to 0.983). Lag time (LT), specific growth rate (SGR) and maximum population density (MPD) were compared among the treatments, jellyfish treatment *C. lamarckii*, “Kabeltonne” seawater and artificial seawater (Tab. 4). Concerning the jellyfish treatment and “Kabeltonne” seawater treatment, the bacterial communities had no lag time, they started to grow immediately after inoculation and showed similar growth rates (Fig. 2), In contrast, the bacteria showed a long lag phase in the artificial seawater treatment and started to grow only after 48h. The highest growth rate of bacterial community was observed in the artificial seawater treatment (Tab. 4). This might be due to fast growth of bacteria in exponential growth phase (from 48h to 72h) of the artificial seawater treatment (Fig. 2). Therefore, the value of growth rate (Tab. 4 SGR) might not be able to fully represent the real situation of bacterial growth in all treatments. Bacterial community within *C. lamarckii* and “Kabeltonne” seawater treatments entered into the beginning of stationary phase at the same time around 48h~72h but with different abundance. The maximum bacterial population densities (MPD) varied among the three treatments. The highest MPD was detected in the *C. lamarckii* treatment (4.4×10^6 N/ml), followed by the “Kabeltonne” seawater treatment (1.2×10^6 N/ml). Although bacteria rapidly grew in the artificial seawater treatment after 48h, they went into stationary phase within one day

(72h). The bacteria reached the stationary phase with lowest abundance (8×10^5 N/ml). Significant differences ($F_{8, 138} = 92.73$, $p<0.0001$) of the bacterial growth kinetics in global test were observed regarding to different treatments conducted with *C. lamarckii*.

The growth curves of the second set of treatments conducted with *Ch. hysoscella* also well fitted to the Gompertz equation (R^2 = 0.928 to 0.979). The values of LT, SGR and MPD were compared among three treatments (Tab. 5). In this experiment, for the *Ch. hysoscella* DOM treatment, bacterial community had a short lag phase and entered into exponential growth phase after 12h (Fig. 3), whereas bacteria of "Kabeltonne" seawater treatment started to grow without lag time. Compared with the treatment in artificial seawater, bacteria had a long lag time (20h). Among these three treatments, bacterial community within jellyfish treatment displayed lower growth rate than that in "Kabeltonne" seawater and entered the stationary phase after 72h with highest cell abundance (1.0×10^7 N/ml). Bacteria in the "Kabeltonne" seawater treatment showed the fastest growth rate but they reached the stationary phase earlier (at 48h) than in the other two treatments. The maximum abundance at the end was low (3.7×10^5 N/ml). The bacteria in artificial seawater grew slowly and reached the maximum density at 72h with the lowest amount (3.6×10^5 N/ml). Significant differences ($F_{8, 138} = 88.52$, $p<0.0001$) of the bacterial growth kinetics in global test were observed regarding to different treatment conducted with *Ch. hysoscella*.

Bacterial community structure

Automated ribosomal intergenic spacer analysis (ARISA) was performed at the end of both experiments to characterize the differences of the bacterial community structure in response to different DOM sources. Based on the ARISA fingerprints, the PCO plot (Fig. 4) depicted the bacterial communities of different DOM treatments with the first set performed with *C. lamarckii*. The first two axes of the PCO captured 48% of the total variation. The bacterial communities in *C. lamarckii* treatment dispersed, as well as in the artificial seawater treatment. Compared with the communities from "Kabeltonne" seawater, they all assembled together. However, the communities in respect to different treatments all separated from each other presenting a clear separation between the jellyfish treatment and the "Kabeltonne" and artificial seawater, respectively, regarding to the bacterial community structure. The PERMANOVA main test revealed significant differences among all samples regarding to different treatments (p=0.001,

Tab. 6). For the posteriori PERMANOVA pair-wise comparisons, the Monte Carlo test was applied due to the small size of unique permutation (<999) shown in Table 7. The bacterial community of *C. lamarckii* treatment presented a different structure from that in both “Kabeltonne” and artificial seawater treatments (p=0.021 and 0.026). The bacterial community structure of “Kabeltonne” seawater treatment significantly differed from the community of artificial seawater (p=0.002).

For the second set of experiment conducted with *Ch. hysoscella*, according to the ARISA fingerprints, the PCO plot (Fig. 5) revealed the bacterial community structure in response to different DOM treatments. The first two axes of the PCO captured 48% of the total variation. There was apparent separation among the assemblages regarding to different DOM treatments and each treatment clustered tightly (Fig. 5). Consistent with the PERMANOVA main test, bacterial communities significantly differed in respect to each DOM treatments (p=0.001, Tab. 8). For the PERMANOVA pair-wise comparisons based on the Monte Carlo test, bacterial community of *Ch. hysoscella* treatment was different from both “Kabeltonne” seawater and artificial seawater treatments (p=0.011, 0.013, respectively, Tab. 9). The community structure of “Kabeltonne” seawater significantly differed from that in the artificial seawater (p=0.001).

In addition, the bacterial community was significantly different (p=0.001, Tab. 10) regarding to DOM treatments comparing between two set of experiments (Fig. 6). Concerning the richness, we observed similar diversity in the initial seawater (inoculations) with 49 different ARISA band classes in both experiments (with *C. lamarckii* and *Ch. hysoscella*). For the first set of experiment with *C. lamarckii* (Fig. 7 A), the highest richness was observed in the “Kabeltonne” seawater (S=54), followed with the *C. lamarckii* treatment (S=46). The artificial seawater treatment showed the lowest richness with 37 band classes. According to the ANOVA, bacterial communities of all three different DOM sources displayed significant differences regarding the richness ($F_{2, 12}$=7.1632, p=0.009). Specifically, significant differences were observed between the “Kabeltonne” seawater and artificial seawater (p=0.007). For the second set of experiment with *Ch. hysoscella* (Fig. 7 B), there is no difference in richness regarding to different DOM treatments ($F_{2, 12}$=2.934, p=0.918). The highest richness was also observed in the “Kabeltonne” seawater treatment (S=48).

Bacterial community composition

We applied CARD-FISH to analyse the succession of specific bacterial groups in response to different DOM treatments. For the first set of experiment conducted with *C. lamarckii*, the bacterial community of initial inoculation of seawater mainly consisted of Bacteroidetes (55%), followed by Roseobacter clade (19%). Gammaproteobacteria were only present in minor ratio (9%) (Fig. 8 (A-C) 0h).

Bacterial community composition as revealed by different taxonomic groups significantly changed in all treatments after the inoculation (Fig. 8 (A-C)). In the treatment with jellyfish *C. lamarckii* DOM (Fig. 8 A), Gammaproteobacteria dominated the bacterial community after 24h (87%). In the "Kabeltonne" seawater treatment (Fig. 8 B), Gammaproteobacteria significantly increased and dominated the community after 24h (71%). For the treatment of artificial seawater (Fig. 8 C), Gammaproteobacteria also increased but with low ratio 43%. Regarding the Bacteroidetes, this group significantly decreased in all treatment after 24h. The relative abundance of Bacteroidetes was 5% in jellyfish (*C. lamarckii*) treatment and 11% and 21%, respectively in "Kabeltonne" seawater and artificial seawater treatments. The community of Roseobacter clade decreased compared with the initial inoculation, but the relative abundance was similar among the three treatments (8%-13%) after 24h.

For the *C. lamarckii* treatment (Fig. 8 A), the bacterial community composition has no difference between 24h and 48h, whereas the bacterial composition changed after 96h. The Bacteroidetes bacteria increased after 96h (31%). The Gammaproteobacteria decreased from 67% (after 48h) to 38% (after 96h). The community of Roseobacter clade increased from 4% (after 48h) to 11% (after 96h). Comparing the bacterial community in *C. lamarckii* treatment after 96h and 168h, the bacterial community composition with respect to these major groups showed the same abundance.

Regarding the "Kabeltonne" seawater treatment (Fig. 8 B), Gammaproteobacteria firstly reached their maximum (82%) after 48h and decreased (59%) after 96h. For the community of Bacteroidetes, they decreased (3%) after 48h and then increased (9%) after 96h. The community of Roseobacter clade increased from 6% (after 48h) to 12% (after 96h). At the end of the experiment, the community composition of "Kabeltonne" seawater treatment presented no difference compared with the community after 96h, Gammaproteobacteria dominated the whole

community (50%), and the community of Bacteroidetes and Roseobacter clade are presented with similar abundance 13% and 12%, respectively.

Regarding to the artificial seawater treatment (Fig. 8 C), the community of Gammaproteobacteria increased (66%) after 48h and then decreased (44%) after 96h. The community of Bacteroidetes and Roseobacter clade significantly decreased from 2% and 11% (after 48h) to 0.4% and 1.3% (after 96h), respectively. After 168h, Gammaproteobacteria remained the dominant group with 40%. Bacteria of the Roseobacter clade were only present in minor ratio 4% and Bacteroidetes disappeared at the end.

In general, the bacterial community composition significantly changed from the initial inoculation in response to different DOM treatments. Although Gammaproteobacteria dominated the whole community in all treatments, the relative abundance varied from each other (40%-50%). To get more information within the major groups, we investigated the composition of these dominant communities with specific probes (Fig. 9 (A-C)). For the initial inoculation (Fig. 9 A 0h), Gammaproteobacteria were composed of *Alteromonas* (4%) and a minor ratio of the *Pseudoalteromonas* (0.7%). The dominating Bacteroidetes were composed of *Polaribacter* (21%) and a minor content of *Ulvibacter* (1%). For the jellyfish *C. lamarckii* DOM treatment (Fig. 9 A), the community was highly dominated by *Alteromonas* (97%) after 24h. However, at the end (168h), the detectable group of Gammaproteobacteria was dominated by *Alteromonas* which decreased to 27% and *Pseudoalteromonas* which increased after 168h (1.7%). For the community of Bacteroidetes, *Polaribacter* were present with low ratio (4%). No *Ulvibacter* were detected at the end. For the "Kabeltonne" treatment (Fig. 9 B), *Alteromonas* dominated the community both at the beginning (24h) (69%) and the end (168h) (45%). *Pseudoalteromonas* was detected in minor ratio (1%) after 24h and nearly disappeared (0.6%) after 168h. For the artificial seawater treatments (Fig. 9 C), for the Gammaproteobacteria community, *Alteromonas* were only present (42%) after 24h, whereas, both *Alteromonas* (52%) and *Pseudoalteromonas* (44%) were present after 168h.

For the second set of experiment conducted with *Ch. hysoscella*, only 51% of the microbial community was detectable as bacteria in the initial inoculation. These bacteria mainly consisted of Bacteroidetes (18%), followed by Roseobacter clade (5%) and Gammaproteobacteria (4%) (Fig. 8 (D-F) 0h). For the jellyfish *Ch. hysoscella* DOM treatment (Fig. 8 D), Bacteroidetes dominated the bacterial community with 53% relative abundance. Compared with the initial

inoculation, the Bacteroidetes significantly increased after 24h and the community of Gammaproteobacteria also increased (25%). The abundance of Roseobacter clade bacteria did not change after inoculation and contributed 6% to the whole community. However, regarding the community of "Kabeltonne" seawater treatment (Fig. 8 E), Gammaproteobacteria significantly increased after 24h and contributed 64% to the whole community. The Bacteroidetes and Roseobacter clade decreased to 9% and 0.8%, respectively, compared with the initial inoculation. For the artificial seawater treatment (Fig. 8 F), Gammaproteobacteria and Roseobacter clade all increased with different ratio (27% and 10%, respectively). As compared with the initial inoculation, the community of Bacteroidetes still kept the original relative abundance (16%) after 24h.

For the treatment of *Ch. hysoscella* DOM treatment (Fig. 8 D), no difference between 48h and 24h in respect to the bacterial composition was observed. Bacteroidetes still dominated (39%) the community after 48h, whereas, Gammaproteobacteria increased from 23% (48h) to 33% after 96h, but there was no difference regarding to the relative abundance between community of Gammaproteobacteria and Bacteroidetes after 96h. The community of Roseobacter clade increased from 0.7% (48h) to 4% after 96h. At the end of the experiment (168h), Gammaproteobacteria and Bacteroidetes were present with equal amounts (22% and 25%, respectively). The relative abundance of Roseobacter clade surprisingly stayed the same after 168h (5%) as compared with the initial inoculation.

For the "Kabeltonne" seawater treatment (Fig. 8 E), Gammaproteobacteria were predominant with 74% after 48h. However, Bacteroidetes and Roseobacter clade recovered after 96h (17% and 4%, respectively) compared with the abundance after 48h (0.3% and 0.5%, respectively). At the end after 168h, Gammaproteobacteria significantly decreased from 56% (after 96h) to 14%. The abundance of Bacteroidetes and Roseobacter clade (12% and 6%, respectively) did not change even after 96h. In addition, the proportion of detectable bacteria decreased and was much lower (47%) after 168h than other time points.

For the treatment of artificial seawater (Fig. 8 F), the Gammaproteobacteria increased (57%) after 48h compared with the community after 24h. Bacteria of the Roseobacter clade decreased (0.3%) after 48h. The bacterial community composition significantly changed after 96h. Gammaproteobacteria highly dominated the whole community (77%). Bacteroidetes nearly disappeared (0.4%) and Roseobacter clade only contributed to a minor ratio (2%) after 96h. The

bacterial community composition after 168h was almost similar as compared with that after 96h. Gammaproteobacteria were still the dominant group with lower abundance (61%) as compared with that after 96h. Regarding to the abundance of Bacteroidetes and Roseobacter clade after 168h, they only contributed to 0.3% and 4%, respectively, of the whole community.

Furthermore, with more specific probes of the major bacterial groups, significant differences of the bacterial composition were shown in response to different DOM treatments (Fig. 9 (D-F)). As mentioned before, a low coverage of the total bacteria was revealed in the initial inoculation. Therefore, the ratio of specific probes was even lower. A large proportion remained unidentified. *Polaribacter* were present with a ratio of 4%. For the *Ch. hysoscella* DOM treatment (Fig. 9 D), the community of Gammaproteobacteria consisted of 17% *Alteromonas* and 3% *Pseudoalteromonas*. The dominant Bacteroidetes after 24h mainly consisted of 47% *Polaribacter*. At the end (168h), the Gammaproteobacteria consisted of 10% *Alteromonas* and 11% *Pseudoalteromonas*. The Bacteroidetes consisted of 39% *Polaribacter*. However, in the "Kabeltonne" seawater treatment (Fig. 9 E), the dominant Gammaproteobacteria consisted of *Alteromonas* both after 24h and 168h with varied abundance. 62% *Alteromonas* was detected at the beginning (24h) and they significantly decreased after 168h with 8%. For the artificial seawater treatment (Fig. 9 F), the dominant Gammaproteobacteria also consisted of *Alteromonas* both after 24h (25%) and 168h (6%) with different abundance. *Pseudoalteromonas* which could not be detected at the beginning of the experiment were found with 2% at the end (168h).

Table 3. Wet and dry weight of two different scyphomedusae species with five replicates in the incubation experiment.

	C. lamarckii		*Ch. hysoscella*	
	Wet weight (g)	Dry weight (g)	Wet weight (g)	Dry weight (g)
A	41.56	1.38	51.33	1.71
B	28.53	1.54	35.41	0.97
C	28.53	0.87	45.63	1.27
D	16.46	1.01	23.7	1.26
E	28.03	0.84	25.37	0.9

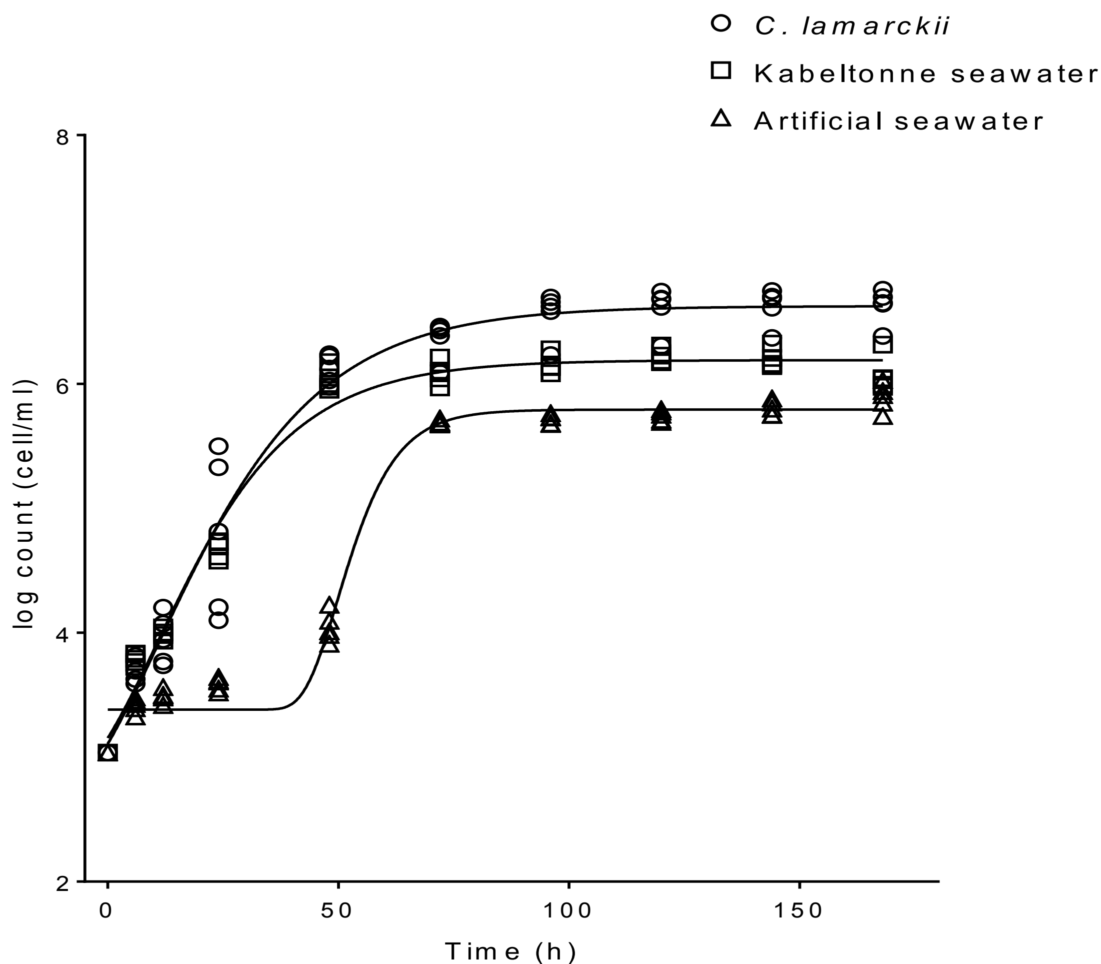


Figure 2. Growth curve of bacterial community under different DOM treatments shown as different symbols with *Cyanea lamarckii*. (R^2=0.972-0.983, $F_{8, 138}$ = 92.73, p<0.0001. Equation for *C. lamarckii* treatment: $Y = 2.379 + 4.247 \times \exp(-\exp(0.05 \times (-8.663 - t) + 1))$. Equation for Kabeltonne seawater: $Y = 2.622 + 3.569 \times \exp(-\exp(0.05 \times (-6.054 - t) + 1))$. Equation for Artificial seawater: $Y = 3.383 + 2.411 \times \exp(-\exp(0.14 \times (42.64 - t) + 1))$.)

Table 4. Lag time (LT), specific growth rate (SGR) and maximum population density (MPD) of three treatments in *C. lamarckii*.

	C. lamarckii	Kabeltonne seawater	Artificial seawater
LT (h)	-8.663±8.309	-6.054±4.862	42.64±1.379
SGR (log/h)	0.078±0.006	0.077±0.004	0.123±0.027
MPD (log)	6.625±0.058	6.192±0.034	5.794±0.036

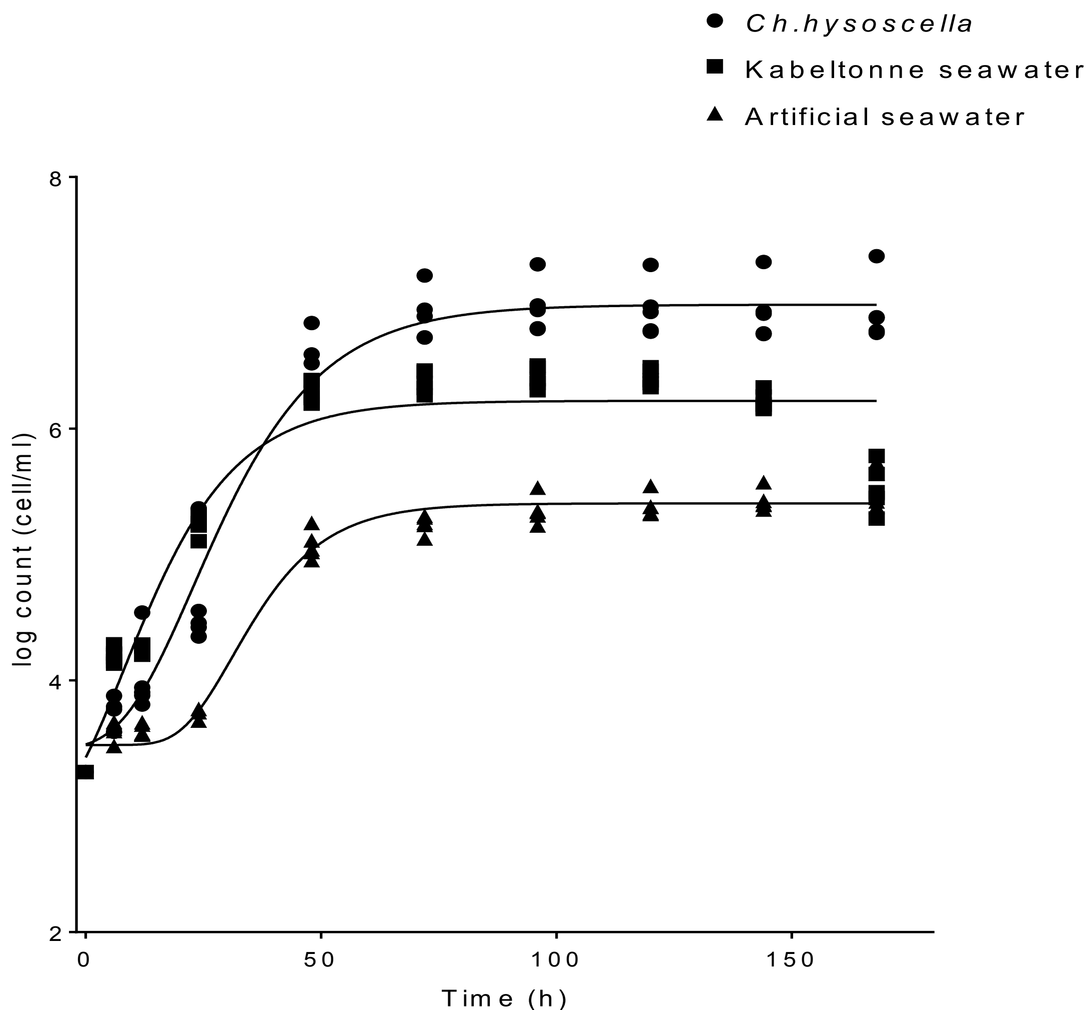


Figure 3. Growth curve of bacterial community under different DOM treatments shown as different symbols with *Chrysaora hysoscella*. (R^2=0.928-0.979, $F_{8, 138}$ = 88.52, p<0.0001. Equation for *Ch. hysoscella* treatment: $Y = 3.453 + 3.532 \times \exp(-\exp(0.05 \times (7.702 - t) + 1))$. Equation for Kabeltonne seawater: $Y = 2.859 + 3.363 \times \exp(-\exp(0.08 \times (-5.156 - t) + 1))$. Equation for Artificial seawater: $Y = 3.487 + 1.92 \times \exp(-\exp(0.08 \times (20.37 - t) + 1))$.)

Table 5. Lag time (LT), specific growth rate (SGR) and maximum population density (MPD) of three treatments in *Ch. hysoscella*.

	Ch. hysoscella	Kabeltonne seawater	Artificial seawater
LT (h)	7.702±3.074	-5.156±8.679	20.37±1.628
SGR (log/h)	0.085±0.008	0.093±0.011	0.065±0.006
MPD (log)	6.985±0.061	6.222±0.062	5.407±0.029

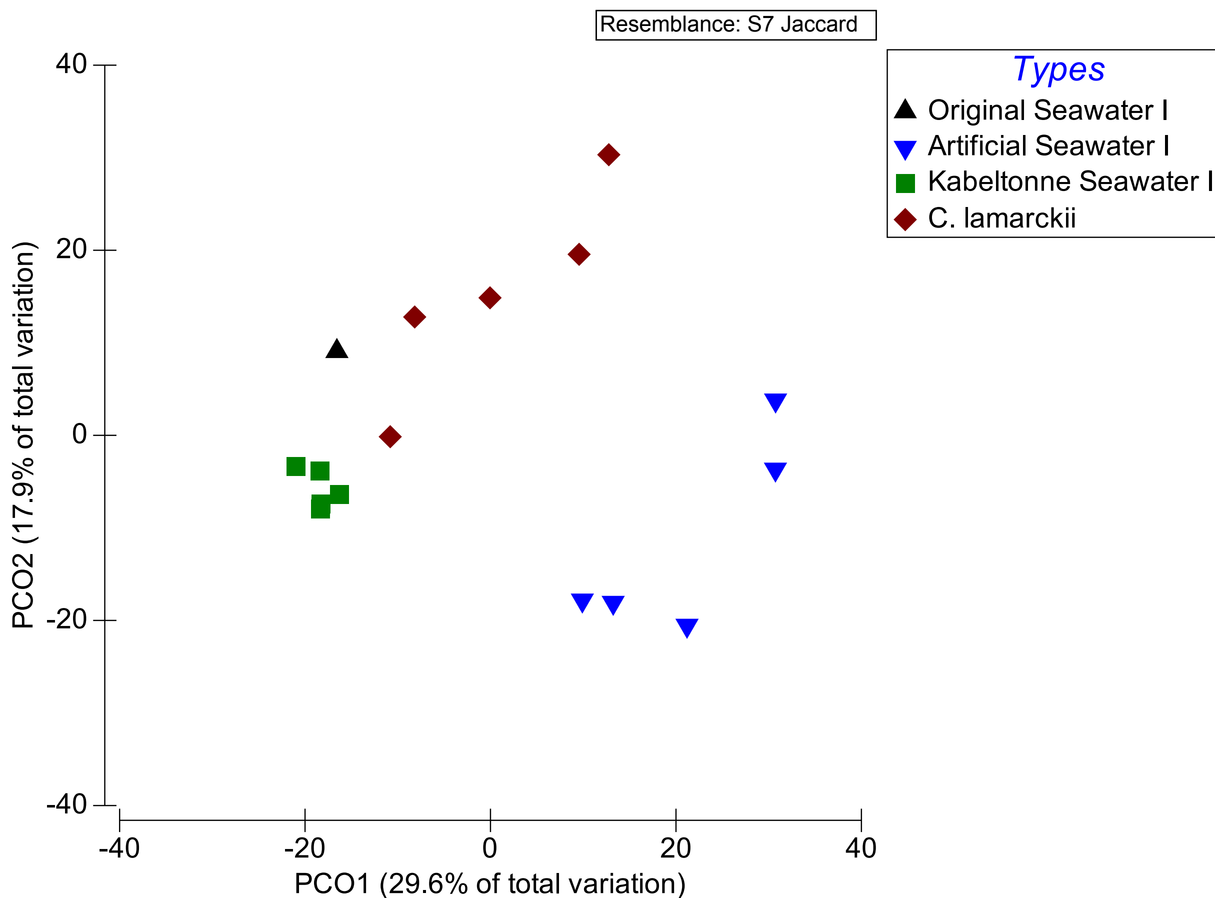


Figure 4. Principal coordinate (PCO) analysis presenting the bacterial communities of different DOM treatments including the initial inoculation (original seawater) with *Cyanea lamarckii* based on Jaccard coefficient from ARISA profiles.

Table 6. PERMANOVA main tests of bacterial community structure of different DOM treatments with *C. lamarckii* based on Jaccard dissimilarities of ARISA profiles.

Significant results (p (perm) <0.05) are highlighted in bold. Displayed are tests for the factor 'sample' and the partitioning of multivariate variation. p-values were obtained using type III sums of squares.

d.f.: degrees of freedom, SS: sums of squares, Sq. root: square root of the component of variation attributable to that factor in the model, in units of Jaccard dissimilarities.

Source of variation	d.f.	SS	*pseudo F*	*p (perm)*	Perms
Sam	3	7762.7	3.3594	**0.001**	998
Res	12	9242.9			
Total	15	17006			

Table 7. PERMANOVA pair-wise tests of bacterial community structure of different DOM treatments with *C. lamarckii* based on Jaccard dissimilarities of ARISA profiles.

Significant results (p (perm) <0.05) are highlighted in bold. Displayed are pair-wise comparisons of the factor "sample".

Comparison	*t (perm)*	p (MC)
Original Seawater vs. Artificial Seawater	1.4709	0.122
Original Seawater vs. Kabeltonne Seawater	3.8076	**0.004**
Original Seawater vs. *C. lamarckii*	1.0064	0.413
Artificial Seawater vs. Kabeltonne Seawater	2.7885	**0.002**
Artificial Seawater vs. *C. lamarckii*	1.6605	**0.026**
Kabeltonne Seawater vs. *C. lamarckii*	1.9384	**0.021**

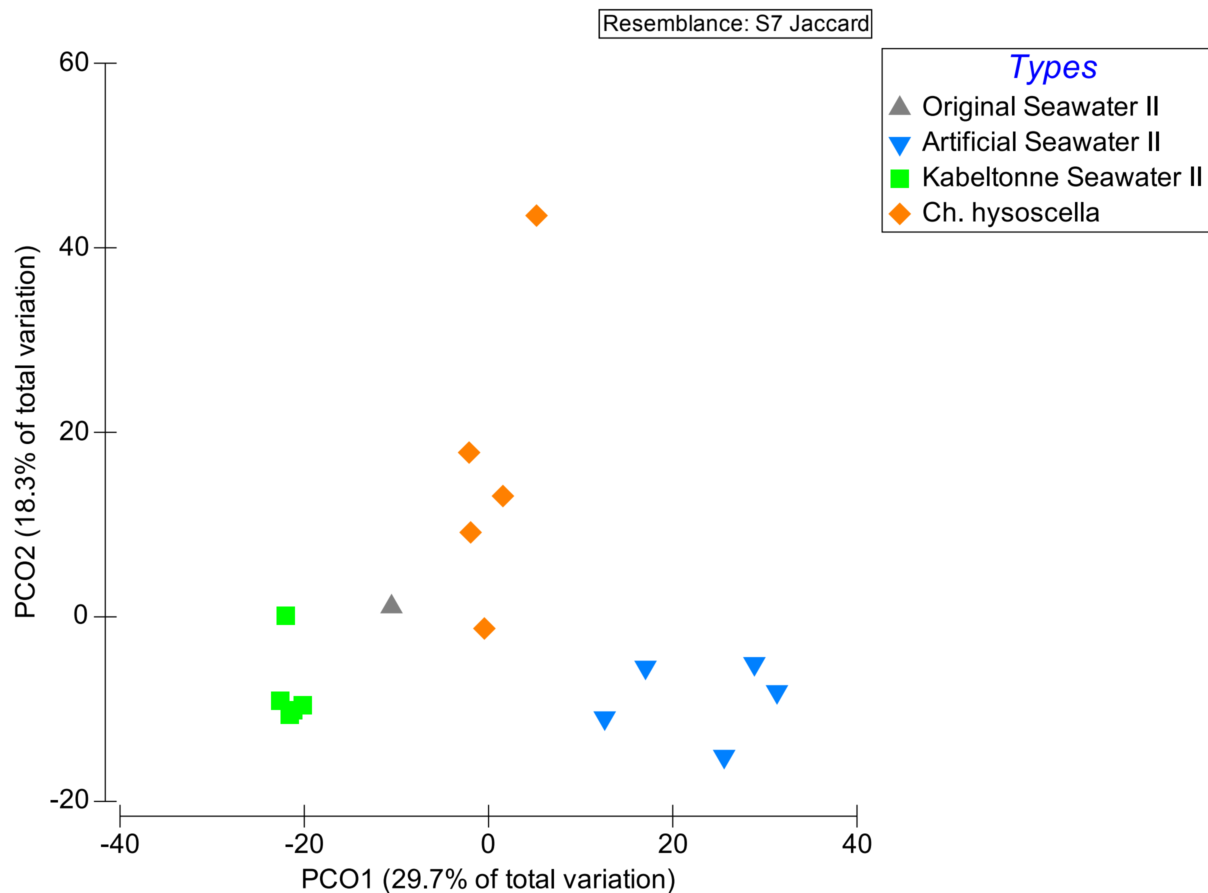


Figure 5. Principal coordinate (PCO) analysis presenting the bacterial communities of different DOM treatments including the initial inoculation (original seawater) with *Chrysaora hysoscella* based on Jaccard coefficient from ARISA profiles.

Table 8. PERMANOVA main tests of bacterial community structure of different DOM treatments with *Ch. hysoscella* based on Jaccard dissimilarities of ARISA profiles.

Significant results (p (perm) <0.05) are highlighted in bold. Displayed are tests for the factor 'sample' and the partitioning of multivariate variation. p-values were obtained using type III sums of squares.

d.f.: degrees of freedom, SS: sums of squares, Sq. root: square root of the component of variation attributable to that factor in the model, in units of Jaccard dissimilarities.

Source of variation	d.f.	SS	*pseudo F*	*p (perm)*	Perms
Sam	3	9275.3	4.182	**0.001**	996
Res	12	8871.5			
Total	15	18147			

Table 9. PERMANOVA pair-wise tests of bacterial community structure of different DOM treatments with *Ch. hysoscella* based on Jaccard dissimilarities of ARISA profiles.

Significant results (p (perm) <0.05) are highlighted in bold. Displayed are pair-wise comparisons of the factor "sample".

Comparison	*t (perm)*	p (MC)
Original Seawater vs. Artificial Seawater	2.0415	0.051
Original Seawater vs. Kabeltonne Seawater	1.7756	0.061
Original Seawater vs. *Ch. hysoscella*	1.1375	0.313
Artificial Seawater vs. Kabeltonne Seawater	3.236	**0.001**
Artificial Seawater vs. *Ch. hysoscella*	1.9648	**0.013**
Kabeltonne Seawater vs. *Ch. hysoscella*	1.9613	**0.011**

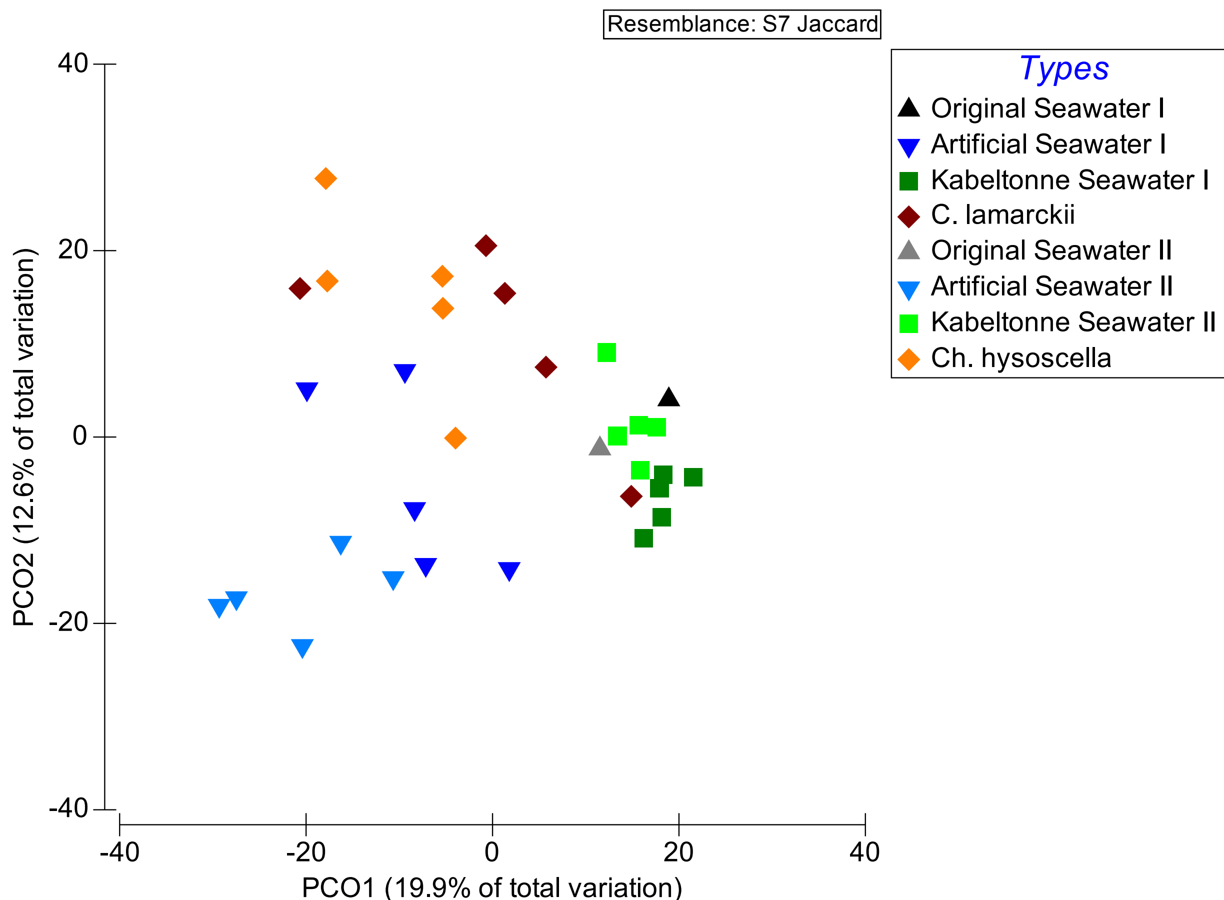


Figure 6. Principal coordinate (PCO) analysis presenting the bacterial communities of different DOM treatments including the initial inoculation (original seawater) with both scyphomedusae species (*Cyanea lamarckii* and *Chrysaora hysoscella*) based on Jaccard coefficient from ARISA profiles. (Original seawater I and II referred to the experiments conducted with *C. lamarckii* and *Ch. hysoscella*, respectively, as well as artificial and Kabeltonne seawater treatment.)

Table 10. PERMANOVA main tests of bacterial community structure of different DOM treatments between two scyphomedusae species (*C. lamarckii* and *Ch. hysoscella*) based on Jaccard dissimilarities of ARISA profiles.

Significant results (p (perm) <0.05) are highlighted in bold. Displayed are tests for the factor 'sample' and the partitioning of multivariate variation. p-values were obtained using type III sums of squares.

d.f.: degrees of freedom, SS: sums of squares, Sq. root: square root of the component of variation attributable to that factor in the model, in units of Jaccard dissimilarities.

Source of variation	d.f.	SS	*pseudo F*	*p (perm)*	Perms	Sq. root
Spe	7	20850	3.9463	**0.001**	999	23.901
Res	24	18114				27.473
Total	31	38964				

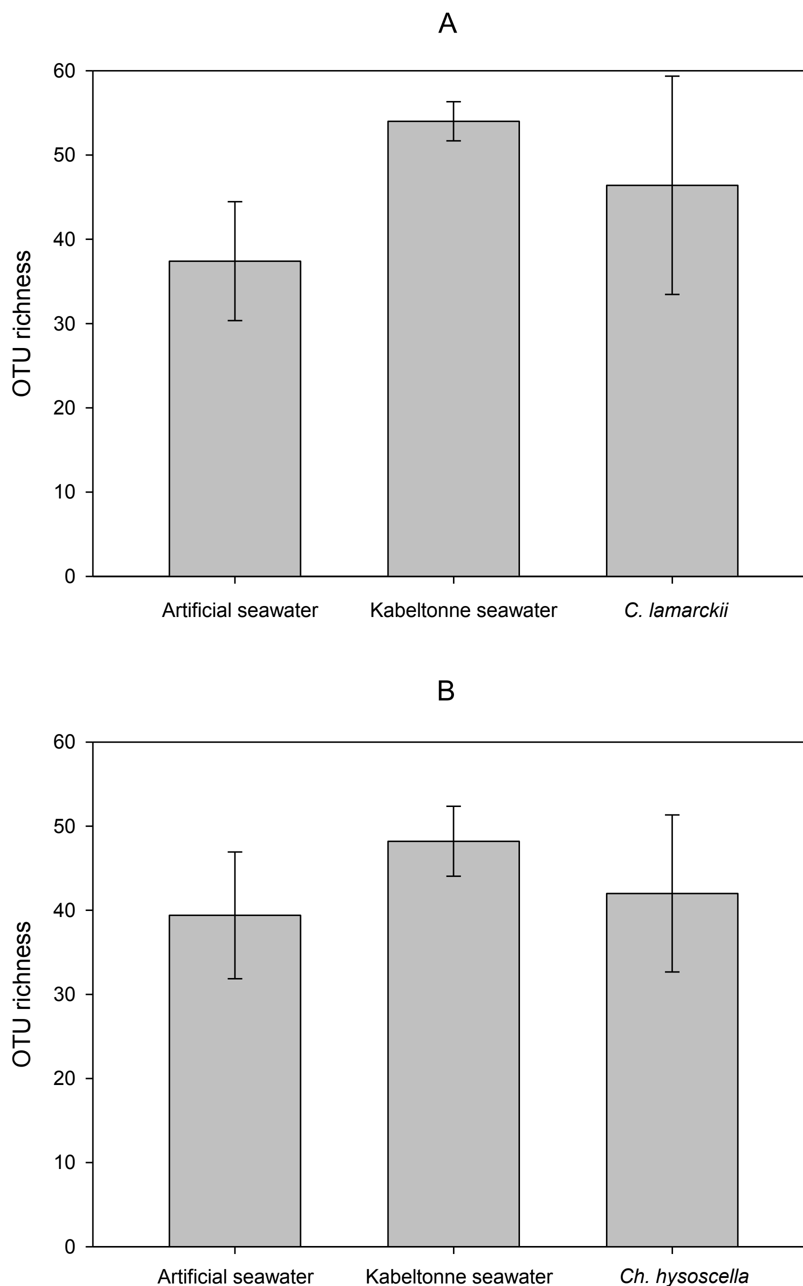


Figure 7. Bar chart of means of bacterial OTU number (richness) for different DOM treatments of two Scyphomedusae species (A: *Cyanea lamarckii* and B: *Chrysaora hysoscella*) obtained by ARISA fingerprint. Whiskers indicate 95% confidence intervals.

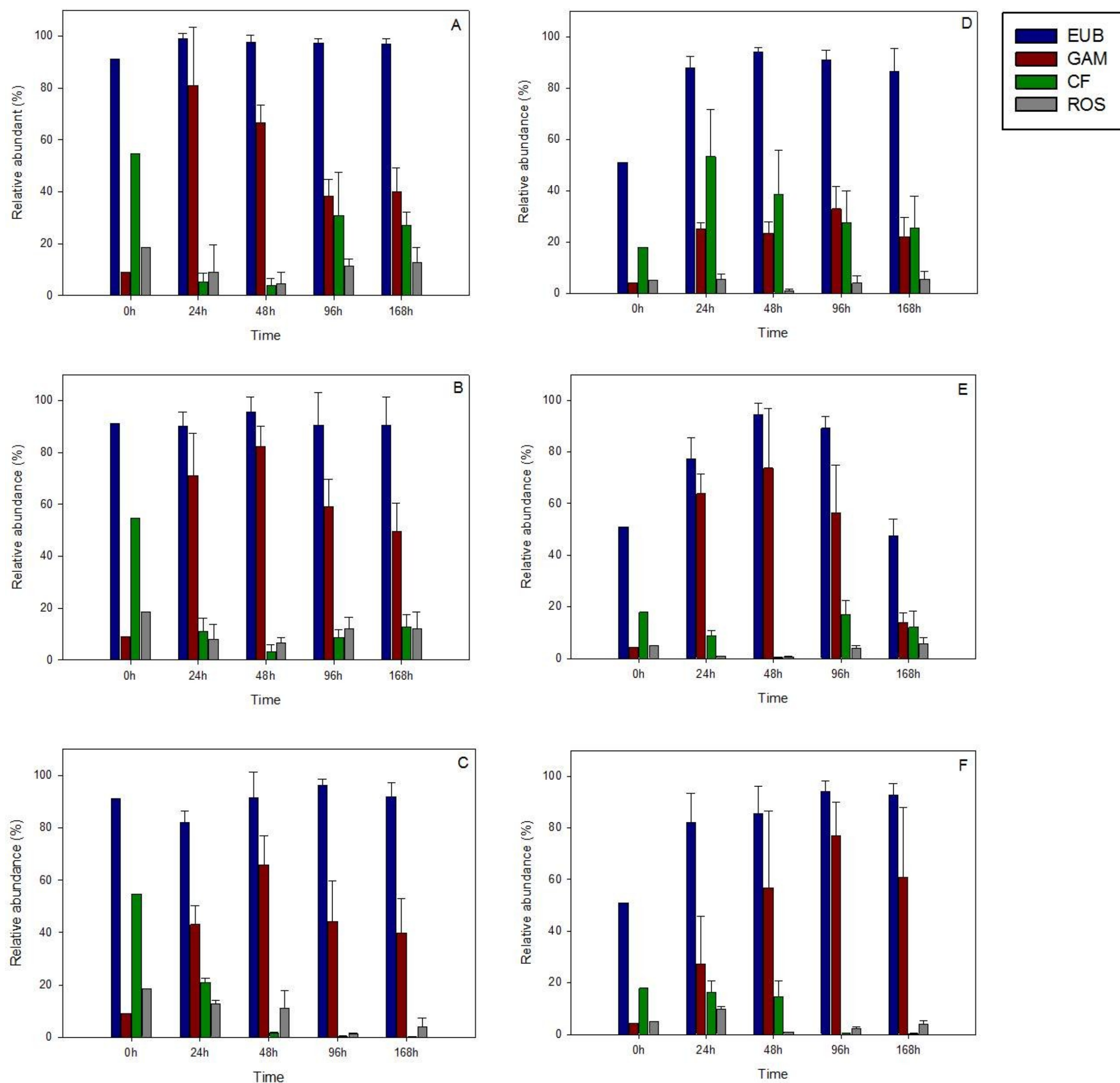


Figure 8. Relative abundance of bacterial community in response to different DOM treatments with general probes shown in the legend. (DOM treatments: DOM released by living jellyfish: A, *C. lamarckii* and D, *Ch. hysoscella*; "Kabeltonne" seawater: B and E; Artificial seawater (DOC free): C and F) (Error bars present the 95% confidence) (EUB: most bacteria, GAM: Gammaproteobacteria, CF: Bacteroidetes, ROS: Roseobacter clade)

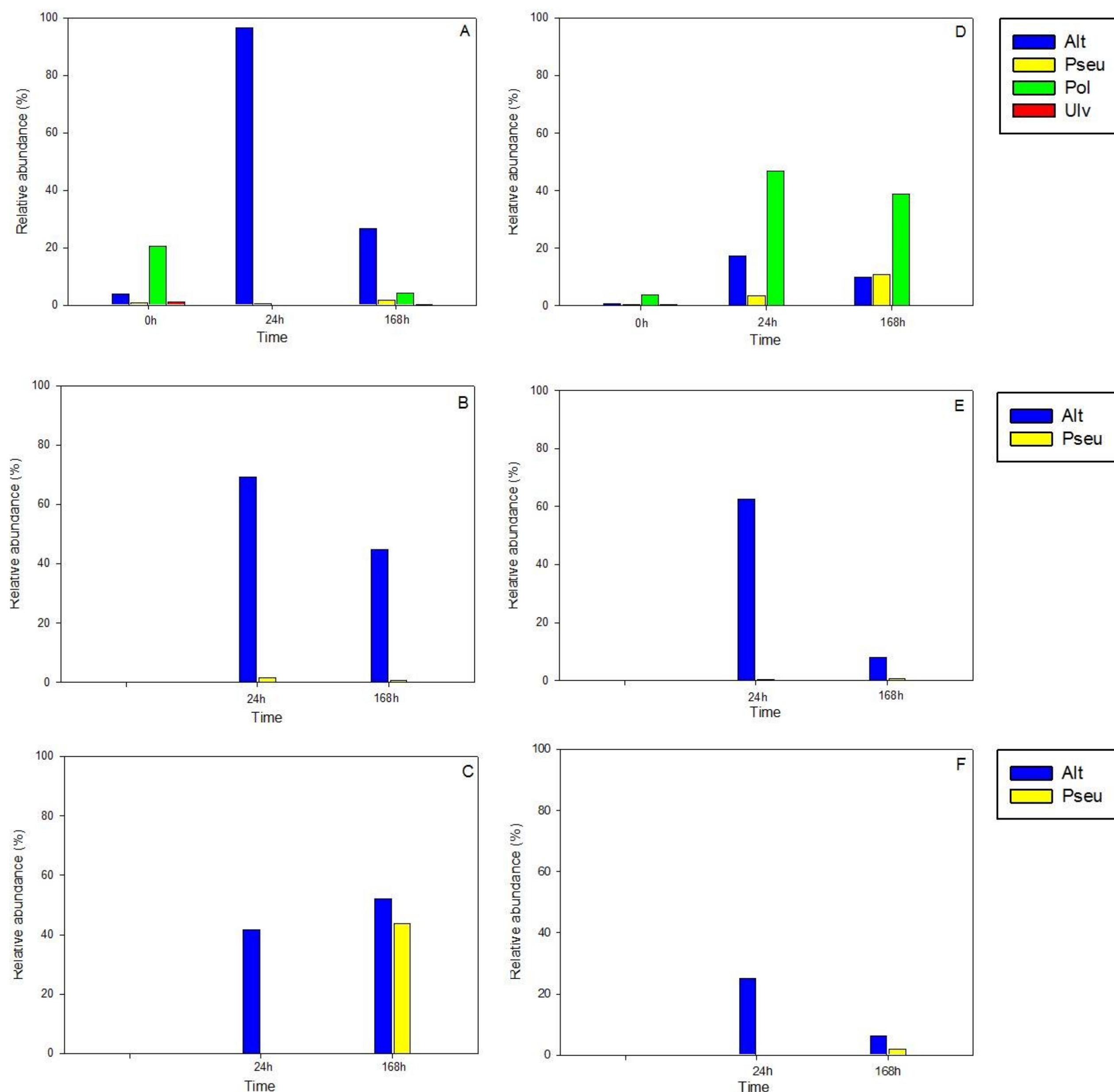


Figure 9. Relative abundance of bacterial community in response to different DOM treatments with specific probes shown in the legend. (DOM treatments: DOM released by living jellyfish: A, *C. lamarckii* and D, *Ch. hysoscella*; "Kabeltonne" seawater: B and E; Artificial seawater (DOC free): C and F) (Alt: Alteromonas, Pseu: *Pseudoalteromonas* spp., Pol: *Polaribacter* spp., Ulv: *Ulvibacter* spp.)

Discussion

We present the first study which assayed the DOM utilization released by live jellyfish. According to our results, the bacterial community showed significant activity which was displayed by the highest bacterial abundance in the jellyfish treatment compared with two other treatments ("Kabeltonne" seawater and artificial seawater) (Fig. 2 and 3). The "Kabeltonne" seawater treatment contains the natural DOM source available in the pelagic marine environment and the artificial seawater treatment is DOC-free. To our knowledge few studies have been performed showing the degree of changes in bacterial diversity depending on dead jellyfish biomass (Titelman *et al.*, 2006; Tinta *et al.*, 2010; Condon *et al.*, 2011; Tinta *et al.*, 2012). The DOC released during decomposition of jellyfish supports bacterioplankton production (Titelman *et al.*, 2006). Increases in bacterial abundances resulted in growth of specific bacterial phylotypes indicate that jellyfish tissues stimulated the growth of specific bacteria (Titelman *et al.*, 2006). In our study, the bacterial community was significantly different regarding to different DOM treatments based on the ARISA fingerprint (Fig. 4 and 5, Tab. 6-9). Apparently, the composition of the different DOM sources is the main factor which modulate the bacterial community in present study. Based on the CARD-FISH analysis, the succession of the bacterial community composition was revealed with certain bacterial groups (Fig. 8 and 9), it might be speculate that DOM as the main controlling factor for the bacterial community in current study.

The initial inoculum was dominated by the *Cytophaga-Flavobacteria* which belongs to Bacteroidetes based on CARD-FISH in both experiments conducted with *C. lamarckii* and *Ch. hysoscella* in July and August 2012, respectively. The *Cytophaga-Flavobacteria* cluster is the most abundant group of all bacterial communities in many oceanic habitats (Llobet-Brossa *et al.*, 1998; Glöckner *et al.*, 1999; Simon *et al.*, 1999; Cottrell & Kirchman, 2000; Eilers *et al.*, 2000) accounting for as much as half of all bacteria potentially identified by FISH. However, due to many gaps occurred in the bacterial community composition of the initial inoculums, to determine the detailed composition of the whole community, more probe need to be analyzed in later study.

In the jellyfish treatment, the bacterial community composition was dominant by Gammaproteobacteria and Bacteroidetes in the *Cyanea lamarckii* treatment coupling with a clear succession (Fig. 8 A). Gammaproteobacteria (particular represented by *Alteromonas*) was

consistently dominant throughout the whole experiment with an obvious decreasing abundance at the end (from 97% to 27%), whereas Bacteroidetes decreased at the beginning and recovered at the end of the experiment. However, in the jellyfish treatment conducted with *Chrysaora hysoscella*, Bacteroidetes were consistently abundant throughout the whole experiment and Gammaproteobacteria were equally abundant with Bacteroidetes at the end of that experiment (Fig. 8 D). Gammaproteobacteria are frequently observed in high abundances not only in incubation experiments (Eilers *et al.*, 2000; Pinhassi & Berman, 2003), but also in previous studies addressed the specific utilization of DOM in the bacterial communities (Eiler *et al.*, 2003; Alonso-Sáez & Gasol, 2007; Landa *et al.*, 2013). Bacteroidetes, in particular, representatives of the class Flavobacteria are presumed to play an important role in the degradation of complex organic matter (OM) (Kirchman, 2002). This was identified in present study in the jellyfish treatment by *Polaribacter* specifically. As a member of Flavobacteria, *Polaribacter* was abundant both at the beginning and the end of the experiments conducted with *Ch. hysoscella*, while it composed of a minor portion at the end of the experiment conducted with *C. lamarckii*. Gómez-Consarnau*, et al.* (2012) revealed that some bacterial phylotypes were highly abundant specific to enrichment with specific carbon compounds (eg. *Acinetobacter* sp. B1-A3 with acetate and *Psychromonas* sp. B3-U1 with glucose). The clear difference of bacterial community composition in response to the DOM released by different jellyfish species might indicate that the DOM released by jellyfish might be consisted of different compounds which are species specific.

A rapid shift (after 24h) in community composition from Bacteroidetes dominated to culturable species of Gammaproteobacteria and Bacteroidetes were revealed in response to the DOM released by jellyfish in the current study (Fig. 8A and 8D). Particularly, *Alteromonas* and *Polaribacter* are favored in response to DOM released by live jellyfish (Fig. 9A and 9D). *Alteromonas macleodii* exhibits hydrolytic ectoenzyme activities e.g., amylases, gelatinases, and lipases (Baumann *et al.*, 1972). Thus, it should be well equipped to degrade major components of mucus released by jellyfish. Investigation of the bacterial taxa in response to the addition of mucus from the coral *Fungia* sp. (Allers *et al.*, 2008), the author found that the bacterial communities were stimulated showing a steep increases of numbers of Gammaproteobacteria during short term incubations (50h), particularly, *Alteromonas* was the dominant phylotype. *Alteromonas* spp., in particular can utilize the monomers, such as hexoses, disaccharides, sugar

acids, amino acids, and ethanol (Baumann *et al.*, 1972). In addition, another dominant group, the *Polaribacter* is able to form microcolonies within aggregates indicating active growth and production of extracellular polysaccharides (Gómez-Pereira *et al.*, 2012). The versatile metabolism of these microorganisms may help them exploit rapid changes in the supply of a complex substrate source, such as bioavailable DOM released by live jellyfish.

Tina *et al.* (2010; 2012) also confirmed a shift in community composition from unculturable Alphaproteobacteria to culturable species of Gammaproteobacteria and Flavobacteria in response to the addition of dead jellyfish biomass (Tinta *et al.*, 2012). Dinasquet *et al.* (2013) examined the bacterial utilization and community responses to bioavailable DOC obtained from different time points during a mesocosm experiment manipulated with the ctenophore species *Mnemiopsis leidyi*, they identified bacteria of the order Alteromonadales belonging to the Gammaproteobacteria to be the predominant at the beginning in the treatment with *M. leidyi*, whereas it was less prevalent at the end and was replaced by Oceanospirillales (Dinasquet *et al.*, 2013). Alteromonadales seem to be adapted to utilize freshly available DOC (Allers *et al.*, 2008), specialized in utilizing carbohydrates (Dinasquet *et al.*, 2013). Based on the analysis of ectoenzymes activities from their study, authors hypothesize that there might be preferential degradation of protein-rich compounds by bacteria which switch to carbohydrate-rich DOC when proteins are depleted (Dinasquet, *et al.*, 2013). Taken these studies (Titelman *et al.*, 2006; Tinta *et al.*, 2010; Condon *et al.*, 2011; Tinta *et al.*, 2012) and the present study together, the bacterioplankton community is not only influenced by dead jellyfish biomass, but also presented a strong succession in response to the metabolic process of live jellyfish.

In the treatment of the "Kabeltonne" seawater, bacterial community was dominated by Gammaproteobacteria after the inoculation in both experiments (Fig. 8B and 8E). Gammaproteobacteria play a role in amino acid and glucose assimilation, consistent with the view of them as opportunistic organisms (Alonso & Pernthaler, 2006; Alonso-Sáez & Gasol, 2007). They are also capable of degrading high molecular weight organic compounds (McBride *et al.*, 2009), due to their genes encoding hydrolytic enzymes having a preference for polymeric carbon sources and a distinct capability for surface adhesion (Bauer *et al.*, 2006). Specifically, in the current study, *Alteromonas* was predominant both at the beginning and the end of the first experiment (Fig. 9 B), while this phylotype steeply decreased in the second experiment (Fig. 9 E). Rapid growth of *Alteromonadaceae* has been observed for incubation of marine waters from

various habitats from the North Sea, the Mediterranean Sea, and the Red Sea (Eilers *et al.*, 2000; Pinhassi & Berman, 2003; Allers *et al.*, 2007). The proliferation of different assemblages of Gammaproteobacteria in the different sets of "Kabeltonne" seawater treatments probably due to the different compositional inoculum were added from different time period. It suggests that DOM availability significantly affected the community succession.

The composition of DOM released by live jellyfish might be different from that in the "Kabeltonne" seawater treatment. Unfortunately, no DOM and DOC data released by jellyfish can be presented here currently. Jellyfish are renowned for producing large quantities of mucus (Heeger & Möller, 1987; Arai, 1997; Graham *et al.*, 2001). Copious amounts of mucus released by jellyfish are colloidal in nature (Wells, 2002). Colloidal material is defined as particles with a linear dimension between the size range from 0.001 to 1 μm (Hiemenz & Rajagopalan, 1997). A large fraction of dissolved colloids (0.001–0.2 μm) was included in our jellyfish treatments. Niggl *et al.* (2010) quantified organic matter released by jellyfish *Cassiopea* sp. from the Northern Red Sea. The collection of the DOM released by jellyfish was performed with the similar method as our study except that the jellyfish was incubated for 12h in our study while it was 6h in their study. The mean mucoid particulate organic matter release in that study was 21.2±9.4 mg POC and 2.3±1.1 PN m^{-2} jellyfish surface area h^{-1} (Niggl *et al.*, 2010). The fraction (0.001–0.2 μm) of colloids as we performed in our experiments was measured by the DOC quantification in Niggl *et al*. (2010). Release rates of DOC by *Cassiopea* sp. determined in study of Niggl *et al*. (2010) were highly variable with 44% of all investigated specimens exhibiting net DOC uptake. According to the observation in our study, particulate aggregates are macroscopic in the jellyfish treatment of both species which conducted with the higher dry weight individuals (*C. lamarckii*: replicate B and *Ch. hysoscella*: replicate A) (Tab. 3). Consistent with this observation, highest bacterial abundance was detected in these replicates derived from the highest dry weight jellyfish individuals (data not shown). This indicates that the amount of DOM released by live jellyfish might correlate with the dry weight of jellyfish specimen. In other studies by Hansson & Norrman (1995) and Condon *et al.* (2011), dry weight were used to caculate the cabon weights of jellyfish and normalize the rate of DOM released by jellyfish, respectively. Unfortunately, no DOM and DOC data released by jellyfish can present here currently, these points need to be addressed in further studies to demonstrate this speculation. In addition, as proteins constitute the greatest proportion of organic biomass of pelagic

coelenterates, products of protein metabolism are likely to constitute the bulk of DON excreted by jellyfish (Pitt *et al.*, 2009). Dissolved primary amines (DPA) compose 21% and 46%, respectively, of the total N and DON excreted by *M. leidyi* (Kremer, 1977). Likewise, dissolved free amino acids (DFAA) are also excreted in large amounts by jellyfish, with glycine and alanine being the most abundant DFAA components (Webb & Johannes, 1967). Release of glycoproteins, which are also produced by scyphomedusae and ctenophores, would either directly or indirectly contribute to DOC and DON pools (Condon *et al.*, 2010). Other sources of DOM released from jellyfish include the leaking of digestive enzymes to DOC and DON pools. Possible enzyme compositions include trypsin and amylase have high activities in *A. aurita* medusae (Båmstedt, 1988).

In marine environments, primary production by phytoplankton is the ultimate source of marine organic matter (Ogawa & Tanoue, 2003). The fraction and composition of photosynthetic production released as DOM are highly variable among species and growth conditions (Carlson *et al.*, 2000). In addition, microbes may also release compounds during nutrient acquisition, such as predation by protozoa or bacteria, and chemical defense (Nagata, 2008; Kujawinski, 2011). The DOM applied in the "Kabeltonne" treatment of present study was directly collected from fresh natural surface seawater. It was found that the high molecular weight (HMW) fraction was abundant in the surface compared with the deep water (Ogawa & Tanoue, 2003). Although HMW DOM is the minor fraction of DOM (~30%), HMW DOM was more available for bacterial utilization than low molecular weight (LMW) DOM (Amon & Benner, 1994) which is the major size fraction of DOM throughout the whole water column in the ocean (Ogawa & Tanoue, 2003), but requires enzymatic digestion prior to uptake (Hoppe, 1991). In the surface ocean the labile organic matter is composed mainly of polysaccharides (Benner *et al.*, 1992), proteins, lipids (Ogawa & Tanoue, 2003), and bacterial cell wall components such as peptidoglycan (McCarthy *et al.*, 1998). Although we do not have the data concerning the composition of DOM in our study currently, the bacterial community of "Kabeltonne" seawater treatment in response to the HMW DOM was highly dominated by Gammaproteobacteria based on CARD-FISH, particularly by the genus *Alteromonas*.

For the artificial seawater treatment, it was served as a blank without any DOC in present study. But this treatment was inoculated with 2 ml of bacterial community of 3 µm filtrated seawater. The distinct differences between other two treatments (jellyfish and "Kabeltonne") and the

artificial seawater treatment are the relative long lag phase and the lowest maximum population density performed with both scyphozoan species (Fig. 2 and 3). The long lag phase perhaps caused by no available DOM in the artificial seawater for the initial inoculated bacteria to utilize at the beginning of the experiment. The low abundance could be linked to the low DOC concentration due to no DOM addition in this treatment. In addition, bacterial community structure significantly differed in the artificial seawater treatment from that in the jellyfish and "Kabeltonne" seawater treatments (Fig. 4 and 5, Tab. 6-9) according to ARISA fingerprints. Consistent with the ARISA results, clear different bacterial community composition was revealed by CARD-FISH in both experiments conducted with two scyphozoan species (*C. lamarckii* and *Ch. hysoscella*). Gammaproteobacteria were the dominant community, particularly by the *Alteromonas* at the beginning and *Pseudoalteronomas* occurred at the end of the experiment. Bacteroidetes was decreased and could not be detected at all at the end of both experiments. *Alteromonas* and *Pseudoalteromonas* as the cultivable genera were frequently isolated from coastal and pelagic regions of the Pacific Ocean as well as from the North Sea using low nutrient media (Eilers *et al.*, 2000; Cho *et al.*, 2007). The presence of Gammaproteobacteria in the artificial seawater treatment likely reflects their capability to rapidly adapt to the culture condition rather than to the DOM input.

Conclusion

Our observations, based on the CARD-FISH of the bacterial community composition, show that significant shifts in bacterial community composition and increases in bacterial activities were observed regarding to different DOM treatments in current study. In both experiments (*Cyanea lamarckii* and *Chrysaora hysoscella*), bacterial communities were more active in the jellyfish and "Kabeltonne" seawater treatments than that in the blank (artificial seawater treatment). Bacterial abundance was significantly stimulated by the DOM released by live jellyfish. According to the ARISA fingerprints, the bacterial communities were significantly different regarding to different DOM treatments. This was confirmed by CARD-FISH with some specific groups that Gammaproteobacteria and Bacteroidetes were consistent present in the experiment conducted with *Ch. hysoscella*, while Bacteroidetes decreased at the beginning and recovered at the end of the experiment conducted with *C. lamarckii*, indicating the different capacity of bacterial phyla to utilize specific carbon compounds (Cottrell & Kirchman, 2000; Gómez-Consarnau *et al.*, 2012). Species-specific DOM released by jellyfish was revealed in the current study which was utilized by significant different bacterial communities. Last but not the least, this study clearly showed that the bacterioplankton community is not only influenced by dead jellyfish biomass, but also strongly impacted by DOM released from the excretion processes of live jellyfish. Although being an appealing idea, a confident linkage between certain taxa and specific carbon compounds cannot be established here because of the lacking of both chemical characterization of the DOM pools and a complete analysis of bacterial groups in different treatments. Therefore, composition of DOM as well as the bacterial functional, such as ectoenzymatic activities and growth efficiency need to be investigated in further studies. In addition, as many gaps presented in the bacterial communities in current study, a further study needs to be conducted to reveal the complete and detailed bacterial community composition in each treatment for understanding the detailed successions of the bacterial community in response to different DOM compounds.

GENERAL DISCUSSION

Jellyfish blooms are increasing in many coastal areas around the world. As the key elements in marine ecosystem, the expansions of jellyfish have significantly impacted not only on the zooplankton standing stocks but also on the commercial fisheries. But the consequences for the marine bacterial community remain poorly understood. The first prerequisite of this study was to determine the bacterial community associated with jellyfish. Therefore, we investigated the bacterial community associated with ctenophores and scyphomedusae respectively in this thesis addressing several different aspects. Firstly, the natural bacterial communities associated with four ctenophore species were revealed. Secondly, the bacterial communities associated with scyphomedusae were determined regarding to i) different body parts, ii) different life stages and iii) the specific impact of the food on the polyps. Thirdly, the response of the bacterial community to the DOM released by live jellyfish was investigated.

Bacterial communities associated with jellyfish

This is the first time to characterize the bacterial communities associated with jellyfish including four ctenophore species (Chapter I) and two scyphomedusae species (Chapter II) respectively at Helgoland Roads in the German Bight. Although jellyfish are composed of 95% water, ctenophore and scyphomedusae contain significantly distinct bacterial communities ($p=0.001$) (Fig. 1). Significant differences among the associated bacterial communities of four ctenophore species (*Mnemiopsis leidyi*, *Beroe* sp., *Bolinopsis infundibulum* and *Pleurobrachia pileus*) were observed based on ARISA fingerprints. Daniels & Breitbart (2012) also demonstrated that each ctenophore genus (*Mnemiopsis leidyi* and *Beroe ovata*) contained a unique and low diversity microbiota by T-RFLP which were distinct from the surrounding water in the Tampa Bay of Florida. Additionally, a clear separation of the BCC between summer and the winter mouth were observed in the species *M. leidyi* confirmed by the PCO plot and PERMANOVA test (main and pair-wise comparisons) in the present study. This indicated a significant seasonal variation in the bacterial community of *M. leidyi*. Consistent with our results, Daniels & Breitbart (2012) also observed temporal variation in the bacterial community associated with *M. leidyi* in the Tampa Bay.

The temporal variation observed in the *M. leidyi* communities might indicate that, on the one hand, the microbial associates of ctenophores change over time probably to adapt to environmental conditions. The variable bacterial community may be in the best interest of this pelagic animal. Further multivariate statistical analyses are necessary to evaluate the biotic and abiotic factors which drive the associated bacterial community of ctenophore. Bacterioplankton dynamics are governed by seasonal changes in abiotic and biotic factors (Pinhassi & Hagstrom, 2000; Gerdts *et al.*, 2004). Sapp *et al.* (2007 b) reported that several factors contributed to the winter-spring succession in the bacterial community especially by temperature and phytoplankton, as well as nutrients (nitrite). Oberbeckmann *et al.* (2012) found that high water temperature and low salinity best explained the proliferation of *Vibrio* spp. at Helgoland Roads. However, little is known about factors driving the bacterial community associated with jellyfish. Hence, the quantification of these associated bacterial community and their response to biotic and abiotic parameters need to be assessed in further studies. On the other hand, it might be possible that different populations were sampled. Beside our study, Daniels & Breitbart did not distinguish the ctenophore population between adult and larval specimen but showed a variation of the associated bacterial community from the whole-body extracts over the sampling period. Daniels & Breitbart (2012) observed that the ctenophore-associated bacterial communities varied significantly over time. This type of community flux has been described by Littman *et al.* (2009) in juvenile acroporid corals, however, adult corals and most sponges ultimately contain conserved bacterial communities (Hentschel *et al.*, 2006; Taylor *et al.*, 2007; Littman *et al.*, 2009).

To confirm the differences of these associated bacterial communities between the adult and larval specimen, different life stages of scyphomedusae were investigated in current study (Chapter II). We found that the structures of the bacterial communities associated with three life stages (planula larva, polyp and adult medusa) were significantly different among each other. This is true for both scyphozoan species (*Cyanea lamarckii* and *Chrysaora hysoscella*) based on the multivariate analysis of ARISA fingerprints. For *C. lamarckii*, with a transition from larvae to polyps until medusae, all stages appeared to be a passive substrate colonized by a diverse bacterial community presenting a dispersive community structure. For *Ch. hysoscella*, the bacterial communities of each stage showed a strong selective processes of bacterial colonization with a highly separated community structure. Unlike other sessile invertebrates (corals and

sponges), scyphozoan display a metagenetic life cycle, therefore, the changing bacterial community of each stage may represent a life cycle strategy to take advantage of the available bacteria from the environment, rather than spending resources for maintenance and protection of a conserved consortia (Daniels & Breitbart, 2012). Although the OTU richness varied over time, it is possible that the different bacterial communities contain members fulfilling set of metabolic functions for the ctenophore. Therefore, the analysis of the bacterial community present at different life stages of jellyfish need to be take into account in further studies.

Dinasquet *et al*. (2012) found that the gut community was different from the community present on the surface of *M. leidyi*. Titelman *et al.*(2006) reported the inhibition of bacterial growth depending on the different body fractions of jellyfish *Periphylla periphylla*. This point was also addressed in the present study and the bacterial communities associated with two scyphomedusae (*C. lamarckii* and *Ch. hysoscella*) were individually analyzed regarding to different body parts. According to the ARISA fingerprints, bacterial community associated with umbrella was significantly different from those with tentacle and gonad with constant diversity in both species based on multivariate analysis (PERMANOVA). It might indicate that the associated bacterial communities are related to the biological functions regarding to different body parts. The umbrella represents a larger colonization surface than other parts and highly contact with the surrounding seawater. This might result in the highest OTU richness among four parts (Chapter II Fig. 3 A and B). Due to the more contact between mouth arms and the tentacles with prey, bacterial communities associated with these two body parts might be more influenced by the prey items. However, Schuett & Doepke (2010) observed a species-specific endobiotic bacteria in the tentacle of scyphomeduesa. Although tentacle of scyphomedusae contains numerous nematocyst toxins which are generally cytolytic, hemolytic and neurotoxic (Bailey *et al.*, 2003; Helmholz *et al.*, 2010; Lassen *et al.*, 2010), Titelman *et al.*(2006) found the umbrella had the strongest inhibitory effect on bacterial community while the weakest in the tentacle (Titelman *et al.*, 2006). In addition, Fraune *et al.* (2009) reported that direct interaction between cellular tissue composition and bacterial community composition. In jellyfish, different types of cells are described in different body parts (Lesh-Laurie & Suchy, 1991). For example, the epidermal gland cells are surrounding the nematocyst clusters in the tentacle of *Cyanea lamarckii* with an antibody activity (Elofsson & Carlberg, 1989). Therefore, the different cellular composition might be another factor resulting in the different bacterial communities associated with different

body parts. Taken together, all make it obvious that the different body parts need to be taken into account in future study regarding to the bacterial community especially associated with scyphozoan.

The distribution of *M. leidyi* populations is linked to temperature and food availability, and their life span is on the order of months (Ghabooli *et al.*, 2011). Cydippid larvae of *M. leidyi* primarily feed on protists prey, however, the morphological transition of *M. leidyi* from cydippid to lobate involves a shift from a microplanktonic diet to a metazoan-based diet including copepods, cladocera, and larvae of fish and mollusks (Rapoza *et al.*, 2005). Therefore, Daniels & Breitbart (2012) speculated that the composition of the surrounding bacterial community in the water column or total food availability as well as dietary composition may influence the ctenophores' bacterial community. However, Dinasquet *et al*. (2012) reported that specifically the gut community of starved *M.leidyi* was dominated by the genus *Tenacibaculum,* a Flavobacterium related to the fish pathogen *Tenacibaculum maritimum* with strong proteolytic activity (Bernardet & Nakagawa, 2006; Ferguson *et al.*, 2010; Delannoy *et al.*, 2011; Mitchell *et al.*, 2013). And they stated that these communities originate from the gut rather than from prey items *per se* (Dinasquet *et al.*, 2012). In addition, members of Bacteroidetes phylum were also present in *M.leidyi* (10%) and *B.ovata* (25%) at Tampa Bay (Daniels & Breitbart, 2012). Bacteria of the Bacteroidetes phylum usually occur in coastal and marine environments throughout the year, partly dominating the bacterioplankton in the North Sea especially during or after phytoplankton blooms (Teeling *et al.*, 2012). Interestingly, the Bacteroidetes seem to play a minor role with < 1% occurrence in all ctenophore species in present study. Based on the approach applied in both two studies (Daniels & Breitbart 2013; Dinasquet *et al.*, 2013), as well as ours, unfortunately, it is not possible to determine to what extent the detected community originate from bacteria associated with prey, colonizing free-living bacteria, or from a more permanent symbiotic gut microflora. However, according to our findings, the absence of Bacteroidetes in our study can be taken as evidence for successful defecation and cleaning of the ctenophores before further processing ctenophore biomass since Bacteroidetes are commonly found to be associated with small plankton organisms, potential food of ctenophores at Helgoland Roads.

To reveal the influence of food on the bacterial community associated with jellyfish, one stage of scyphomedusae “polyp” was individually hatched from larvae and fed with two kinds of food sources (*A. salina* and plankton) under lab condition (Chapter II). The bacterial communities

associated with polyps of two scyphozoan species, as well as prey were analyzed to determine whether diet is driving the BCCs of polyps. For both scyphomedusae (*C. lamarckii* and *Ch. hysoscella*), the bacterial communities associated with two kinds of food (including attached and free-living community) were clearly separated from the communities associated with polyps (Chapter II, Fig. 4 A and B). However, the bacterial communities associated with polyps do react differently to different food source. The bacterial communities associated with polyps of *C. lamarckii* fed with different food were clearly distinguishable from each other (Fig. 4 A). For *Ch. hysoscella*, the communities of polyps fed with *A. salina* were overlapped and not easily distinguished from the bacterial communities associated with polyps fed with plankton (Fig. 4 B). This might indicate that the bacterial community of the food, either the free-living or the attached community do have an impact on the bacterial community associated with polyps, but not in the process of the selecting and shaping the bacterial communities associated with polyps in both scyphozoan species. Polyps might react differently in response to different food source, for example, with different metabolic activity, which may play a pivotal role for the selection and formation of the bacterial community associated with polyps.

Fraune *et al*. (2010) demonstrated that *Hydra* embryos are protected by a maternally produced antimicrobial peptide (AMP) of the periculin peptide family, which controls the establishment of the microbiota during embryogenesis. Antimicrobial peptides (Chapman *et al*. 2010) represent the major defense system against microbial infection in marine invertebrates (Otero-González *et al.*, 2010). They are known as prominent effector of the innate immune system that often get secreted in response to external stimulation (Bosch, 2013). With over expressed periculin in polyps, it caused not only decreases in the number of associated bacteria but also changes in the composition of community (Fraune *et al.*, 2010). The novel antimicrobial peptide Aurelin is another examples of such substance that have been found in scyphozoa species *Aurelia aurita* (Ovchinnikova *et al.*, 2006). This Aurelin peptide exhibits activity against gram-positive and gram-negative bacteria (Ovchinnikova *et al.*, 2006). Franzenburg *et al.* (2013) stated that species-specific antimicrobial peptides shape species-specific bacterial associations. Although there is no available data regarding to the antimicrobial peptides in *C. lamarckii* and *Ch. hysoscella*, we speculate that the colonized bacterial community may adapt to different AMP repertoires of scyphomedusae species resulting in specific associations. This need to be investigated in the future.

Furthermore, a clear species-specific character was revealed in the present study regarding to the bacterial community associated with jellyfish. For ctenophore, it was demonstrated both based on automated ribosomal intergenic spacer analysis (ARISA) fingerprinting (Chapter I Fig.1) and ribosomal amplicon pyrosequencing (Chapter I Fig.4). This was in agreement with other two recent studies on the BCC of ctenophore (Daniels & Breitbart, 2012; Dinasquet *et al.*, 2012) performed in the Tampa Bay, Gulf of Mexico and the Gulmar Fjord. For example, the occurrence of closely related *Marinomonas* species (Chapter I Fig.5) from different geographical origins gives probably evidence to suggest that *Marinomonas* is a common member of the bacterial community in ctenophora on a more global scale, but this need to be proven in further studies. If this is the case, the functional role of this genus in this consortium has to be elucidated (symbiosis, commensalism and pathogen). For scyphomedusae, distinct species-specific patterns were revealed in both aspects, different body parts (data not shown) and different life stages (Chapter II Fig. 8) based on ARISA fingerprints. Regarding to different body parts, according to PERMANOVA main test, bacterial communities associated with mouth arm and tentacle were different between two scyphomedusae species ($p=0.023$ and 0.016, respectively), except for the gonad, while bacterial community associated with umbrella was significantly different between two scyphomedusae species ($p=0.009$). Regarding to different life stages, the BCCs associated with three life stages (larvae, polyps and medusae) were all significantly differed between *C. lamarckii* and *Ch. hysoscella*. In general, current study first described the bacterial community associated with jellyfish including ctenophore and scyphomedusae at Helgoland Roads in German Bight which is species-specific. With numerous studies focused on marine invertebrates, such as corals (Sunagawa *et al.*, 2009) and *Hydra* (Bosch, 2012) reported that bacterial community were different in respect to different species among each organism (Friedrich *et al.*, 2001; Hentschel *et al.*, 2001; Rohwer *et al.*, 2002; Ritchie, 2006; Fraune & Bosch, 2007; Bosch, 2012). Summarizing these results, it appears that associated microbial communities are indeed unique and at least partially organism specific.

In the present study, we applied ribosomal amplicon pyrosequencing to reveal the bacterial community composition among four ctenophore species. The bacterial communities of all ctenophore species were dominated by Proteobacteria (Chapter I). Although Alphaproteobacteria and Gammaproteobacteria are dominant bacterial classes in ctenophore's community, these groups contain various bacteria with high phenotypic and metabolic diversity. In the present

study, three major groups Oceanospirillaceae, Pseudoalteromonadaceae and Moraxellaceae were detected in these four ctenophore species, in particular, *Marinomonas*, *Pseudoalteromonas* and *Psychrobacter*. *Marinomonas* highly dominated the community of *M. leidyi*, *Beroe* sp. and *B. infundibulum* in our study. Interestingly, in two recent studies on the BCC of ctenophore (Daniels & Breitbart, 2012; Dinasquet *et al.*, 2012) performed in the Tampa Bay, Gulf of Mexico and the Gulmar Fjord, *Marinomonas* was also identified as the prominent genus. Our pyrosequencing results showed that *Pseudoalteromonas* occurred in *B. infundibulum* and *P. pileus*. Bacteria of the genus *Psychrobacter* were observed only in *P. pileus*. Alphaproteobacteria, another important group in the associated bacterial community of ctenophores, dominated the communities of *Beroe* sp. (~94%) and *B. infundibulum* (~47%). The genus *Thalassospira* is the most prominent group. The bacterial community of *B. ovate* from Tampa Bay was also dominated by members of this genus which is in accordance with our findings (Daniels & Breitbart, 2012). In contrast, Dinasquet *et al*. (2012) found *Thalassospira* neither in tissue nor in gut samples. Bacteria of the Rhodobacteraceae dominated the Alphaproteobacteria associated with *M. leidyi* in the Gullmar fjord (Dinasquet *et al.*, 2012) which is in agreement with our findings that Rhodobacteraceae was the major group in Alphaproteobacteria associated with *M. leidyi* at Helgoland Roads even though they only account for 1% of the whole community. In general, *Marinomonas*, *Thalassospira*, *Pseudoalteromonas* and *Psychrobacter* were identified as predominant genera associated with ctenophores. Our results indicate that the bacterial community associated with ctenophores is highly species-specific. This suggests that either specific bacteria are selected by a particular ctenophore genus, or that certain bacteria selectively colonize the ctenophore.

Marinomonas contain multifunctional polyphenol oxidases which are able to oxidize a wide range of substrates (Solano & Sanchez-Amat, 1999) and are involved in a series of secondary metabolism and biodegradative processes (Sanchez-Amat *et al.*, 2001). *M. mediterranea* synthesizes an antibacterial protein with activity against both gram-positive and gram-negative bacteria (Lucas-Elio *et al.*, 2005). Bacteria of genus *Pseudoalteromonas* are known to produce a variety of highly bioactive compounds, including extracellular enzymes, exopolysaccharides and a range of different molecular weight compounds with antimicrobial, anti-fouling, algicidal and various pharmaceutically relevant activities (Holmström & Kjelleberg, 1999; Bowman, 2007). *Thalassospira* can utilize hydrocarbons, carbohydrates, organic acids or amino acids as sole

carbon sources for growth and degrade polycyclic aromatic hydrocarbons (PAHs) in oil-contaminated seawater (Kodama *et al.*, 2008). Hence, these capabilities might successfully enable them to compete for nutrients and colonize surfaces (Holmström *et al.*, 2002). It is possible that such antibacterial activities might prevent the colonization of other bacterial groups resulting in the lower diversity in the community of ctenophore compared with other organisms.
Additionally, the detection of genus *Tenacibaculum*, *a Flavobacterium* related to the fish pathogen *Tenacibaculum maritimum* with strong proteolytic activity (Bernardet & Nakagawa, 2006; Ferguson *et al.*, 2010; Delannoy *et al.*, 2011; Mitchell *et al.*, 2013), were both reported in *Beroe ovata* tissues (Daniels & Breitbart, 2012) and the gut community of starved *M.leidyi* (Dinasquet *et al.*, 2012). It might indicate that this particular ctenophore may be a vector for pathogens. However, the Bacteroidetes seem to play a minor role with < 1% occurrence in all ctenophore species in the present study. But bacteria of the genus *Vibrio* were found in *Beroe* sp. (19%) and *M.leidyi* (4%) in the present study and probably serve as potentially pathogenic bacteria. The community of *Vibrio* spp. were quantified associated with free-living, plankton-attached and shellfish at Helgoland Roads and *V. alginolyticus* was revealed as the dominant *Vibrio* species at the current study location (Oberbeckmann *et al.*, 2011). Therefore, additional information on metabolic functions or activities by transcription proteome analysis concerning the interaction between pathogen and ctenophore need to be established in future.

Impact of Jelly-DOM on food webs

Concerning the accidental introductions and subsequent expansions of jellyfish have become more frequent over the past few decades (Mills, 2001; Brodeur *et al.*, 2002; Billett *et al.*, 2006; Brotz *et al.*, 2012), it has significantly altered food web structure and heavily impacted commercial fisheries because jellyfish are voracious predators (Purcell & Decker, 2005). Voracious jellyfish predation impacts food webs by converting large quantities of carbon (C), fixed by primary producers and consumed by secondary producers, into gelatinous biomass, which restrict C transfer to higher trophic levels because jellyfish have few natural predators (Condon *et al.*, 2011). Therefore their carcasses at the termination of a bloom represent an organic-rich substrate that supports rapid bacterial growth, and may have a large impact on the surrounding environment. Recently, a few studies explored whether jellyfish biomass have an impact on bacterial community phylotype selection (Hansson & Norrman, 1995; Riemann *et al.*,

2006; Titelman *et al.*, 2006; Tinta *et al.*, 2010; Condon *et al.*, 2011; Tinta *et al.*, 2012; Dinasquet *et al.*, 2013). But little is known about the impact of the dissolved organic matter released by live jellyfish on the microbial community.

The results presented in this thesis were the first study assayed the impact of jelly-DOM released by live jellyfish on the selection of bacterial community (Chapter III). The focus of present study was to explore whether particular bacteria preferentially utilize specific carbon compounds released by live jellyfish during excretion and if such compounds have the potential to shape bacterial community composition. Bacterial community was significantly stimulated by the DOM released by jellyfish which was obviously present with the highest abundance in the treatments conducted with *C. lamarckii* and *Ch. hysoscella* (Chapter III Fig. 2 and 3). Tinta *et al.* (2010; 2012) observed an increase in bacterial abundance and production in response to dead jellyfish biomass coupled with NH_4^+ accumulation and oxygen consumption. According to ARISA fingerprints, bacterial community structure in the jellyfish treatment was significantly different from that in the “Kabeltonne” seawater and artificial seawater (DOC-free) treatments based on PERMANOVA test in both sets of experiments (Chapter III Fig. 4 and 5). According to catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH) analysis, a rapid and different shift from Bacteroidetes dominated to culturable species of Gammaproteobacteria and Bacteroidetes were revealed in the current study in response to the DOM released by different jellyfish species (Fig. 8A and 8D). Gammaproteobacteria dominated the community instead of the Bacteroidetes group conducted with *C. lamarckii*, while Gammaproteobacteria and Bacteroidetes dominated the community within *Ch. hysoscella*. Tina *et al.* (2012) reported a shift in community composition from unculturable Alphaproteobacteria to culturable species of Gammaproteobacteria and Flavobacteria due to the addition of the jelly-biomass. Jelly-DOM also favored the rapid growth and dominance of specific bacterial phylogenetic groups (primarily γ-proteobacteria) that were rare in ambient waters (Condon *et al.*, 2011). The significant differences in the bacterial community composition and succession found in the present study indicate that the DOM released by jellyfish might consist of different compounds which are species specific reflecting the utilization by significant different bacterial communities in respect to two scyphomedusae species. Unfortunately, because of the lacking of chemical characterization of the DOM pools for all different treatments, a confirmed linkage between certain taxa and specific carbon compounds cannot be established here, detailed data of chemical

composition of DOM and DOC need to be analyzed later. Taken together, the present study clearly shows that the bacterioplankton community is not only influenced by the degradation of jellyfish biomass, but also strongly affected by DOM released during the metabolic process of live jellyfish.

Condon *et al.* (2011) report that jellyfish released substantial quantities of extremely labile C-rich DOM, relative to nitrogen (25.6 ± 31.6 C:1N), which was quickly metabolized by bacterioplankton. It was shunted toward bacterial respiration instead of production, bacterial growth efficiencies were significantly reduced by 10% to 15% when jelly-DOM was consumed (Condon *et al.*, 2011). Condon *et al.* (2011) suggest major shifts in microbial structure and function associated with jellyfish blooms accompanying a channel of C toward bacterial CO_2 production and away from higher trophic levels. These results further suggest fundamental transformations in the biogeochemical functioning and biological structure of food webs associated with jellyfish blooms (Condon *et al.*, 2011). Niggl *et al.* (2010) investigated the impact of organic matter release by jellyfish *Cassiopea* from on planktonic microbes and zooplankton. They demonstrated the uptake of *Cassiopea*-derived organic matter by the zooplanktonic mysids *Idiomysis tsurnamali*. DOC and dissolved free amino acids released by jellyfish have been described as easily accessible C and N sources for bacteria and other marine sapotrophs (Webb & Johannes, 1967; Hansson & Norrman, 1995). These findings suggest that jellyfish-derived organic matter may function as a newly discovered trophic pathway for organic matter from the benthic environment to pelagic food chains in marine ecosystems.

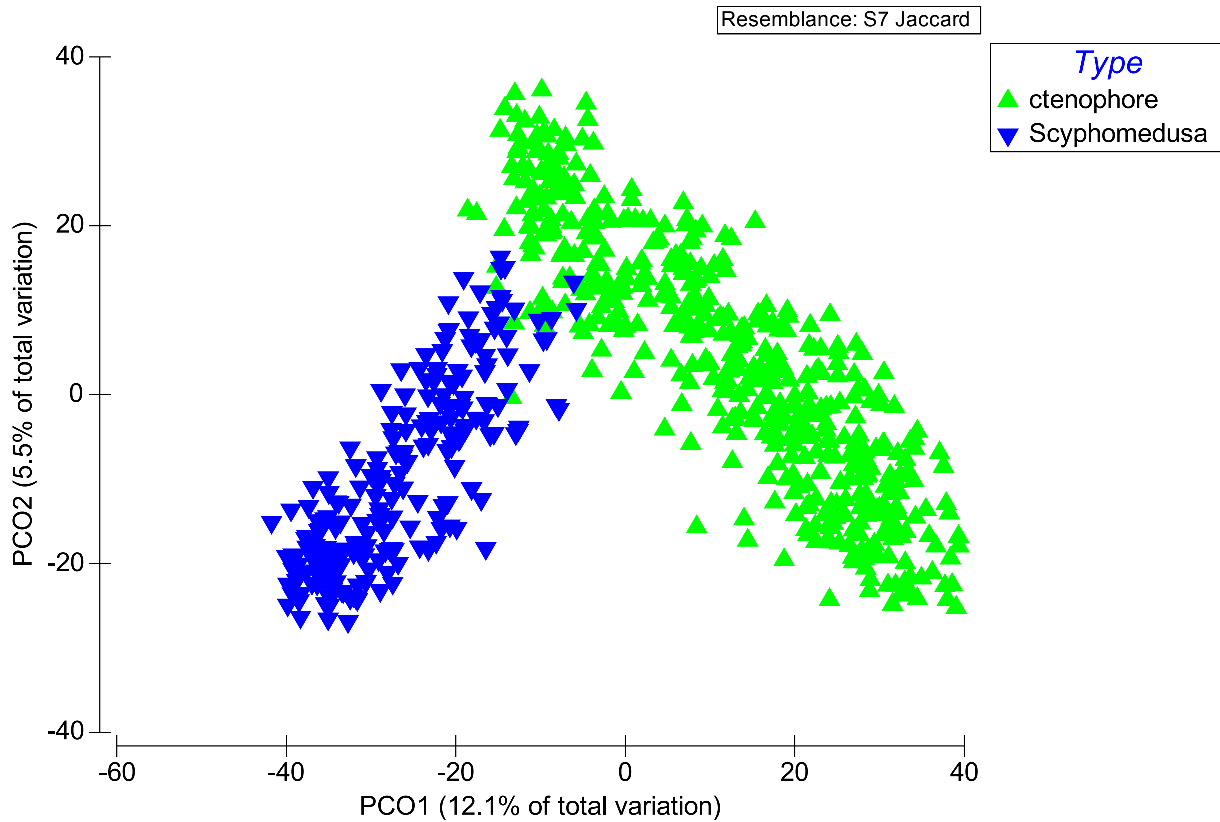


Figure 1. Principal coordinate (PCO) analysis presenting the bacterial communities associated with ctenophores and scyphomedusae based on Jaccard coefficient from ARISA profiles.

REFERENCES

Allers E, Niesner C, Wild C & Pernthaler J (2008) Microbes enriched in seawater after sddition of coral mucus. *Applied and Environmental Microbiology* 74: 3274-3278.

Allers E, Gómez-Consarnau L, Pinhassi J, Gasol JM, Šimek K & Pernthaler J (2007) Response of Alteromonadaceae and Rhodobacteriaceae to glucose and phosphorus manipulation in marine mesocosms. *Environmental Microbiology* 9: 2417-2429.

Allgaier M, Uphoff H, Felske A & Wagner-Döbler I (2003) Aerobic anoxygenic photosynthesis in Roseobacter clade bacteria from diverse marine habitats. *Applied and Environmental Microbiology* 69: 5051-5059.

Alonso-Sáez L & Gasol JM (2007) Seasonal variations in the contributions of different bacterial groups to the uptake of low-molecular-weight compounds in Northwestern Mediterranean coastal waters. *Applied and Environmental Microbiology* 73: 3528-3535.

Alonso C & Pernthaler J (2006) Roseobacter and SAR11 dominate microbial glucose uptake in coastal North Sea waters. *Environmental Microbiology* 8: 2022-2030.

Amann RI, Binder BJ, Olson RJ, Chisholm SW, Devereux R & Stahl DA (1990) Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. *Applied and Environmental Microbiology* 56: 1919-1925.

Amon RMW & Benner R (1994) Rapid cycling of high-molecular-weight dissolved organic matter in the ocean. *Nature* 369: 549-552.

Arai MN (1996) Functional biology of Scyphozoa. *Springer*.

Arai MN (1997) A Functional Biology of Scyphozoa. *Chapman & Hall, London.*

Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA & Thingstad F (1983) The ecological role of water-column microbes in the sea. *Marine Ecology Progress Series* 10: 257-263.

Bailey PM, Little M, Jelinek GA & Wilce JA (2003) Jellyfish envenoming syndromes: unknown toxic mechanisms and unproven therapies. *Medical journal of Australia* 178: 34-37.

Båmstedt U (1988) Interspecific, seasonal and diel variations in zooplankton trypsin and amylase activities in Kosterfjorden, western Sweden. *Marine Ecology Progress Series* 44: 15-24.

Båmstedt U (1990) Trophodynamics of the scyphomedusae *Aurelia aurita*. Predation rate in relation to abundance, size and type of prey organism. *Journal of Plankton Research* 12: 215-229.

Banin E, Israely T, Kusmaro A, Loya Y, Orr E & Rosenberg E (2000) Penetration of the coral-bleaching bacterium *Vibrio shiloi* into *Oculina patgonica*. *Applied and environmental microbiology* 66: 3031-3036.

Barz K & Hirche H-J (2007) Abundance, distribution and prey composition of scyphomedusae in the southern North Sea. *Marine Biology* 151: 1021-1033.

Bauer M, Kube M, Teeling H, Richter M, Lombardot T, Allers E, Würdemann CA, Quast C, Kuhl H, Knaust F, Woebken D, Bischof K, Mussmann M, Choudhuri JV, Meyer F, Reinhardt R, Amann RI & Glöckner FO (2006) Whole genome analysis of the marine Bacteroidetes'*Gramella forsetii*' reveals adaptations to degradation of polymeric organic matter. *Environmental Microbiology* 8: 2201-2213.

Baumann L, Baumann P, Mandel M & Allen RD (1972) Taxonomy of aerobic marine eubacteria. *Journal of Bacteriology* 110: 402-429.

Baxter E, Rodger H, McAllen R & Doyle T (2011) Gill disorders in marine-farmed salmon: investigating the role of hydrozoan jellyfish. *Aquacult Environ Interact* 1: 245-257.

Beaugrand G (2004) The North Sea regime shift: Evidence, causes, mechanisms and consequences. *Progress in Oceanography* 60: 245-262.

Beaugrand G, Reid PC, Ibañez F, Lindley JA & Edwards M (2002) Reorganization of North Atlantic marine copepod biodiversity and climate. *Science* 296: 1692-1694.

Benner R, Pakulski JD, Mccarthy M, Hedges JI & Hatcher PG (1992) Bulk chemical characteristics of dissolved organic matter in the ocean. *Science* 255: 1561-1564.

Bennke CM, Neu TR, Fuchs BM & Amann R (2013) Mapping glycoconjugate-mediated interactions of marine Bacteroidetes with diatoms. *Systematic and Applied Microbiology* 36: 417-425.

Bergh Ø (1995) Bacteria associated with early life stages of halibut, *Hippoglossus hippoglossus* L., inhibit growth of a pathogenic *Vibrio* sp. *Journal of Fish Diseases* 18: 31-40.

Bernardet J-F & Nakagawa Y (2006) An introduction to the family Flavobacteriaceae. In *The Prokaryot* (Dworkin M, Falkow S, Rosenberg E, Schleifer K-H & Stackebrandt E, ed.eds.), pp. 455-480. Springer New York.

Billett DSM, Bett BJ, Jacobs CL, Rouse IP & Wigham BD (2006) Mass deposition of jellyfish in the deep Arabian Sea. *Limnology and Oceanography* 5: 2077-2083.

Boero F, Bouillon J, Gravili C, Miglietta MP, Parsons T & Piraino S (2008) Gelatinous plankton: irregularities rule the world (sometimes). *Marine Ecology Progress Series* 356: 299.

Boersma M, Malzahn AM, Greve W & Javidpour J (2007) The first occurrence of the ctenophore *Mnemiopsis leidyi* in the North Sea. *Helgoland Marine Research* 61: 153-155.

Bosch TCG (2009) *Hydra* and the evolution of stem cells. *BioEssays* 31: 478-486.

Bosch TCG (2012) Understanding complex host-microbe interactions in *Hydra*. *Gut Microbes* 3: 345-351.

Bosch TCG (2013) Cnidarian-microbe interactions and the origin of innate immunity in metazoans. *Annual Review of Microbiology* 67: 499-518.

Bourne DG & Munn CB (2005) Diversity of bacteria associated with the coral *Pocillopora damicornis* from the Great Barrier Reef. *Environmental Microbiology* 7: 1162-1174.

Bowman J (2007) Bioactive compound synthetic capacity and ecological significance of marine bacterial genus *Pseudoalteromonas*. *Marine Drugs* 5: 220-241.

Brewer RH (1976) Larval settling behavior in *Cyanea capillata* (Cnidaria:Scyphozoa). *The Biological Bulletin* 150: 183-199.

Brewer RH (1984) The influence of the orientation, roughness and wettability of solid surfaces on the behavior and attachment of planulae of Cyanea (Cnidaria:Scyphozoa). *The Biological Bulletin* 166: 11-21.

Brodeur RD (1998) In situ observations of the association between juvenile fishes and scyphomedusae in the Bering Sea. *Marine Ecology Progress Series* 163: 11-20.

Brodeur RD, Sugisaki H, Hunt J & George L (2002) Increases in jellyfish biomass in the Bering Sea: implications for the ecosystem. *Marine Ecology Progress Series* 233 89-103.

Brodeur RD, Decker MB, Ciannelli L, Purcell JE, Bond NA, Stabeno PJ, Acuna E & Hunt Jr GL (2008) Rise and fall of jellyfish in the eastern Bering Sea in relation to climate regime shifts. *Progress in Oceanography* 77: 103-111.

Brotz L, Cheung WL, Kleisner K, Pakhomov E & Pauly D (2012) Increasing jellyfish populations: trends in large marine ecosystems. *Hydrobiologia* 690: 3-20.

Brown M (2005) Coupling 16S-ITS rDNA clone libraries and automated ribosomal intergenic spacer analysis to show marine microbial diversity development and application to a time series. *Environmental microbiology* 7: 1466-1479.

Brusca RC & Brusca GJ (1990) Invertebrates (Vol. 2). *Sunderland, MA: Sinauer Associates.*

Byern J, Mills C & Flammang P (2010) Bonding tactics in ctenophores — morphology and function of the colloblast system. *Biological Adhesive Systems,*(Byern J & Grunwald I, ed.eds.), pp. 29-40. Springer Vienna.

Campbell BJ, Stein JL & Cary SC (2003) Evidence of chemolithoautotrophy in the bacterial community associated with *Alvinella pompejana*, a hydrothermal vent polychaete. *Applied and Environmental Microbiology* 69: 5070-5078.

Carlson CA, Hansell DA, Peltzer ET & Smith Jr WO (2000) Stocks and dynamics of dissolved and particulate organic matter in the southern Ross Sea, Antarctica. *Deep Sea Research Part II: Topical Studies in Oceanography* 47: 3201-3225.

Carman KR & Dobbs FC (1997) Epibiotic microorganisms on copepods and other marine crustaceans. *Microscopy Research and Technique* 37: 116-135.

Cary SC & Giovannoni SJ (1993) Transovarial inheritance of endosymbiotic bacteria in clams inhabiting deep-sea hydrothermal vents and cold seeps. *Proceedings of the National Academy of Sciences* 90: 5695-5699.

Cavanaugh CM (1994) Microbial symbiosis: patterns of diversity in the marine environment. *American Zoologist* 34: 79-89.

Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T, Rattei T, Balasubramanian PG, Borman J, Busam D, Disbennett K, Pfannkoch C, Sumin N, Sutton GG, Viswanathan LD, Walenz B, Goodstein DM, Hellsten U, Kawashima T, Prochnik SE, Putnam NH, Shu S, Blumberg B, Dana CE, Gee L, Kibler DF, Law L, Lindgens D, Martinez DE, Peng J, Wigge PA, Bertulat B, Guder C, Nakamura Y, Ozbek S, Watanabe H, Khalturin K, Hemmrich G, Franke A, Augustin R, Fraune S, Hayakawa E, Hayakawa S, Hirose M, Hwang JS, Ikeo K, Nishimiya-Fujisawa C, Ogura A, Takahashi T, Steinmetz PRH, Zhang X, Aufschnaiter R, Eder M-K, Gorny A-K, Salvenmoser W, Heimberg AM, Wheeler BM, Peterson KJ, Bottger A, Tischler P, Wolf A, Gojobori T, Remington KA, Strausberg RL, Venter JC, Technau U, Hobmayer B, Bosch TCG, Holstein TW, Fujisawa T, Bode HR, David CN, Rokhsar DS & Steele RE (2010) The dynamic genome of *Hydra*. *Nature* 464: 592-596.

Chimetto LA, Cleenwerck I, Brocchi M, Willems A, De Vos P & Thompson FL (2011) *Marinomonas brasilensis* sp. nov., isolated from the coral *Mussismilia hispida*, and reclassification of *Marinomonas basaltis* as a later heterotypic synonym of *Marinomonas*

communis. *International Journal of Systematic and Evolutionary Microbiology* 61: 1170-1175.

Cho J-C, Stapels MD, Morris RM, Vergin KL, Schwalbach MS, Givan SA, Barofsky DF & Giovannoni SJ (2007) Polyphyletic photosynthetic reaction centre genes in oligotrophic marine Gammaproteobacteria. *Environmental Microbiology* 9: 1456-1463.

Clare AS, Fusetani N & Jones MB (1998) Introduction: Settlement and metamorphosis of marine invertebrate larvae. *Biofouling* 12: 1-2.

Clarke A, Holmes LJ & Gore DJ (1992) Proximate and elemental composition of gelatinous zooplankton from the Southern Ocean. *Journal of Experimental Marine Biology and Ecology* 155: 55-68.

Compère P, Martinez A-S, Charmantier-Daures M, Toullec J-Y, Goffinet G & Gaill F (2002) Does sulphide detoxication occur in the gills of the hydrothermal vent shrimp, Rimicaris exoculata? *Comptes Rendus Biologies* 325: 591-596.

Condon RH, Steinberg DK & Bronk DA (2010) Production of dissolved organic matter and inorganic nutrients by gelatinous zooplankton in the York River estuary, Chesapeake Bay. *Journal of Plankton Research* 32: 153-170.

Condon RH, Steinberg DK, del Giorgio PA, Bouvier TC, Bronk DA, Graham WM & Ducklow HW (2011) Jellyfish blooms result in a major microbial respiratory sink of carbon in marine systems. *Proceedings of the National Academy of Sciences* 108: 10225-10230.

Cottrell MT & Kirchman DL (2000) Community composition of marine bacterioplankton determined by 16S rRNA gene clone libraries and fluorescence in situ hybridization. *Applied and Environmental Microbiology* 66: 5116-5122.

Cottrell MT & Kirchman DL (2000) Natural assemblages of marine Proteobacteria and members of the Cytophaga-Flavobacter cluster consuming low- and high-molecular-weight dissolved organic matter. *Applied and Environmental Microbiology* 66: 1692-1697.

Crump BC, Kling GW, Bahr M & Hobbie JE (2003) Bacterioplankton community shifts in an Arctic lake correlate with seasonal changes in organic matter source. *Applied and Environmental Microbiology* 69: 2253-2268.

Cushing DH (1989) A difference in structure between ecosystems in strongly stratified waters and in those that are only weakly stratified. *Journal of Plankton Research* 11: 1-13.

Dale C & Moran NA (2006) Molecular interactions between bacterial symbionts and their hosts. *Cell* 126: 453-465.

Daniels C & Breitbart M (2012) Bacterial communities associated with the ctenophores *Mnemiopsis leidyi* and *Beroe ovata*. *FEMS Microbiology Ecology* 82: 90-101.

Davey ME & O'toole GA (2000) Microbial biofilms: from ecology to molecular genetics. *Microbiology and Molecular Biology Reviews* 64: 847-867.

Decho AW (1990) Microbial exopolymer secretions in ocean environments: their role (s) in food webs and marine processes. *Oceanography. Marine Biology Annual Review* 28: 73-153.

Decker M, Brown C, Hood R, Purcell J, Gross T, Matanoski J, Bannon R & Setzler-Hamilton E (2007) Predicting the distribution of the scyphomedusa *Chrysaora quinquecirrha* in Chesapeake Bay. *Marine Ecology Progress Series* 329: 99-113.

Delannoy CMJ, Houghton JDR, Fleming NEC & Ferguson HW (2011) Mauve Stingers (*Pelagia noctiluca*) as carriers of the bacterial fish pathogen *Tenacibaculum maritimum*. *Aquaculture* 311: 255-257.

Denner EBM, Mark B, Busse H-J, Turkiewicz M & Lubitz W (2001) *Psychrobacter proteolyticus* sp. nov., a psychrotrophic, halotolerant bacterium isolated from the Antarctic krill *Euphausia superba* Dana, excreting a cold-adapted metalloprotease. *Systematic and Applied Microbiology* 24: 44-53.

Dinasquet J, Granhag L & Riemann L (2012) Stimulated bacterioplankton growth and selection for certain bacterial taxa in the vicinity of the ctenophore *Mnemiopsis leidyi*. *Frontiers in Microbiology* 3.

Dinasquet J, Kragh T, Schrøter M-L, Søndergaard M & Riemann L (2013) Functional and compositional succession of bacterioplankton in response to a gradient in bioavailable dissolved organic carbon. *Environmental Microbiology* 15: 2616-2628.

Doyle TK, De Haas H, Cotton D, Dorschel B, Cummins V, Houghton JDR, Davenport J & Hays GC (2008) Widespread occurrence of the jellyfish *Pelagia noctiluca* in Irish coastal and shelf waters. *Journal of Plankton Research* 30: 963-968.

Dubilier N, Bergin C & Lott C (2008) Symbiotic diversity in marine animals: the art of harnessing chemosynthesis. *Nature Reviews Microbiology* 6: 725-740.

Ducklow HW & Mitchel R (1979) Bacterial populations and adaptations in the mucus layers on living corals. *Limnol. Oceanogr* 24: 715-725.

Eiler A, Langenheder S, Bertilsson S & Tranvik LJ (2003) Heterotrophic bacterial growth efficiency and community structure at different natural organic carbon concentrations. *Applied and Environmental Microbiology* 69: 3701-3709.

Eilers H, Pernthaler J & Amann R (2000) Succession of pelagic marine bacteria during enrichment: a close look at cultivation-induced shifts. *Applied and Environmental Microbiology* 66: 4634-4640.

Eilers H, Pernthaler J, Glöckner FO & Amann R (2000) Culturability and in situ abundance of pelagic bacteria from the North Sea. *Applied and Environmental Microbiology* 66: 3044-3051.

Eilers H, Pernthaler J, Peplies J, Glöckner FO, Gerdts G & Amann R (2001) Isolation of novel pelagic bacteria from the German Bight and their seasonal contributions to surface picoplankton. *Applied and Environmental Microbiology* 67: 5134-5142.

Elofsson R & Carlberg M (1989) Gland cells in the tentacles of the jellyfish *Cyanea lamarcki* reactive with an antibody against 5-hydroxytryptamine. *Cell and Tissue Research* 255: 419-422.

Faasse MA & Bayha KM (2006) The ctenophore *Mnemiopsis leidyi* A. Agassiz 1865 in coastal waters of the Netherlands: an unrecognized invasion. *Aquatic invasions* 1: 270-277.

Fenchel T (2008) The microbial loop – 25 years later. *Journal of Experimental Marine Biology and Ecology* 366: 99-103.

Ferguson HW, Christian MJD, Hay S, Nicolson J, Sutherland D & Crumlish M (2010) Jellyfish as vectors of bacterial disease for farmed salmon (*Salmo salar*). *Journal of Veterinary Diagnostic Investigation* 22: 376-382.

Fine M & Loya Y (2002) Endolithic algae: an alternative source of photoassimilates during coral bleaching. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 269: 1205-1210.

Finenko GA (2001) Chemical composition, respiration and feeding rates of the new alien ctenophore, *Beroe ovata*, in the Black Sea. *Hydrobiologia* 451: 177-186.

Fletcher M (1994) Bacterial biofilms and biofouling. *Current Opinion in Biotechnology* 5: 302-306.

Franzenburg S, Walter J, Künzel S, Wang J, Baines JF, Bosch TCG & Fraune S (2013) Distinct antimicrobial peptide expression determines host species-specific bacterial associations. *Proceedings of the National Academy of Sciences* 110: 3730-3738.

Fraune S & Bosch TCG (2007) Long-term maintenance of species-specific bacterial microbiota in the basal metazoan *Hydra*. *Proceedings of the National Academy of Sciences* 104: 13146-13151.

Fraune S & Bosch TCG (2010) Why bacteria matter in animal development and evolution. *BioEssays* 32: 571-580.

Fraune S, Abe Y & Bosch TCG (2009) Disturbing epithelial homeostasis in the metazoan *Hydra* leads to drastic changes in associated microbiota. *Environmental Microbiology* 11: 2361-2369.

Fraune S, Augustin R, Anton-Erxleben F, Wittlieb J, Gelhaus C, Klimovich VB, Samoilovich MP & Bosch TCG (2010) In an early branching metazoan, bacterial colonization of the embryo is controlled by maternal antimicrobial peptides. *Proceedings of the National Academy of Sciences* 107: 18067-18072.

Frias-Lopez J, Zerkle AL, Bonheyo GT & Fouke BW (2002) Partitioning of bacterial communities between seawater and healthy, black band diseased, and dead coral surfaces. *Applied and Environmental Microbiology* 68: 2214-2228.

Frias-Lopez J, Klaus JS, Bonheyo GT & Fouke BW (2004) Bacterial community associated with black band disease in corals. *Applied and Environmental Microbiology* 70: 5955-5962.

Friedrich AB, Fischer I, Proksch P, Hacker J & Hentschel U (2001) Temporal variations of the microbial community associated with the Mediterranean sponge *Aplysina aerophoba*. *FEMS Microbiology Ecology* 38: 105-113.

Frost J, Jacoby C, Frazer T & Zimmerman A (2012) Pulse perturbations from bacterial decomposition of *Chrysaora quinquecirrha* (Scyphozoa: Pelagiidae). *Hydrobiologia* 690: 247-256.

Fuhrman JA (1992) Bacterioplankton roles in cycling of organic matter: the microbial food web. *Primary productivity and biogeochemical cycles in the sea* 361-383.

Fuhrman JA (2006) Annually reoccurring bacterial communities are predictable from ocean conditions. *Proceedings of the National Academy of Sciences* 103: 13104-13109.

Gawel NJ & Jarret RL (1991) A modified CTAB DNA extraction procedure for *Musa* and *Ipomoea*. *Plant Molecular Biology Reporter* 9: 262-266.

Gerdts G, Wichels A, Döpke H, Klings K-W, Gunkel W & Schütt C (2004) 40-year long-term study of microbial parameters near Helgoland (German Bight, North Sea): historical view and future perspectives. *Helgoland Marine Research* 58: 230-242.

Gerdts G, Brandt P, Kreisel K, Boersma M, Schoo KL & Wichels A (2013) The microbiome of North Sea copepods. *Helgoland Marine Research* 67: 757-773.

GESAMP (1997) Opportunistic settlers and the problem of the ctenophore *Mnemiopsis leidyi* invasion in the Black Sea.

Ghabooli S, Shiganova T, Zhan A, Cristescu M, Eghtesadi-Araghi P & MacIsaac H (2011) Multiple introductions and invasion pathways for the invasive ctenophore *Mnemiopsis leidyi* in Eurasia. *Biology Invasions* 13: 679-690.

Gibbons M & Richardson A (2009) Patterns of jellyfish abundance in the North Atlantic. *Hydrobiologia* 616: 51-65.

Gilbert JA, Steele JA, Caporaso JG, Steinbruck L, Reeder J, Temperton B, Huse S, McHardy AC, Knight R, Joint I, Somerfield P, Fuhrman JA & Field D (2012) Defining seasonal marine microbial community dynamics. *ISME Journal* 6: 298-308.

Glöckner FO, Fuchs BM & Amann R (1999) Bacterioplankton compositions of lakes and oceans: A first comparison based on fluorescence in situ hybridization. *Applied Environmental Microbiology* 65: 3721-3726.

Gómez-Consarnau L, Lindh MV, Gasol JM & Pinhassi J (2012) Structuring of bacterioplankton communities by specific dissolved organic carbon compounds. *Environmental Microbiology* 14: 2361-2378.

Gómez-Pereira PR, Schüler M, Fuchs BM, Bennke C, Teeling H, Waldmann J, Richter M, Barbe V, Bataille E, Glöckner FO and Amann RI (2012) Genomic content of uncultured Bacteroidetes from contrasting oceanic provinces in the North Atlantic Ocean. *Environmental Microbiology* 14: 52-66.

González JM, Simó R, Massana R, Covert JS, Casamayor EO, Pedrós-Alió C & Moran MA (2000) Bacterial community structure associated with a dimethylsulfoniopropionate-producing North Atlantic algal bloom. *Applied and Environmental Microbiology* 66: 4237-4246.

Graf J & Ruby EG (1998) Host-derived amino acids support the proliferation of symbiotic bacteria. *Proceedings of the National Academy of Sciences* 95: 1818-1822.

Graham W & Bayha K (2007) Biological invasions by marine jellyfish. *Biological Invasions,* Vol. 193 (Nentwig W, ed.eds.), pp. 239-255. Springer Berlin Heidelberg.

Graham W, Pagès F & Hamner W (2001) A physical context for gelatinous zooplankton aggregations: a review. *Hydrobiologia* 451: 199-212.

Greve W (1970) Cultivation experiments on North Sea ctenophores. *Helgolander wiss. Meeresunters* 20: 304-317.

Greve W (1975) Phylum Ctenophora. Fiches identification zooplankton. 146: 1-6.

Grzymski JJ, Murray AE, Campbell BJ, Kaplarevic M, Gao GR, Lee C, Daniel R, Ghadiri A, Feldman RA and Cary SC (2008) Metagenome analysis of an extreme microbial symbiosis reveals eurythermal adaptation and metabolic flexibility. *Proceedings of the National Academy of Sciences* 105: 17516-17521.

Hahnke RL & Harder J (2013) Phylogenetic diversity of Flavobacteria isolated from the North Sea on solid media. *Systematic and Applied Microbiology* 36: 497-504.

Hamer HH, Malzahn AM & Boersma M (2011) The invasive ctenophore *Mnemiopsis leidyi*: a threat to fish recruitment in the North Sea? *Journal of Plankton Research* 33: 137-144.

Hamner W & Dawson M (2009) A review and synthesis on the systematics and evolution of jellyfish blooms: advantageous aggregations and adaptive assemblages. *Hydrobiologia* 616: 161-191.

Hansen GH & Olafsen JA (1999) Bacterial interactions in early life stages of marine cold water fish. *Microbial Ecology* 38: 1-26.

Hansson LJ & Norrman B (1995) Release of dissolved organic carbon (DOC) by the scyphozoan jellyfish *Aurelia aurita* and its potential influence on the production of planktic bacteria. *Marine Biology* 121: 527-532.

Hansson LJ, Moeslund O, Kiørboe T & Riisgård HU (2005) Clearance rates of jellyfish and their potential predation impact on zooplankton and fish larvae in a neritic ecosystem (Limfjorden, Denmark). *Marine Ecology Progress Series* 304: 117-131.

Harashima A, Kimoto T, Wakabayashi T & Toshiyasu T (2006) Verification of the silica deficiency hypothesis based on biogeochemical trends in the aquatic continuum of Lake Biwa–Yodo River–Seto Inland Sea, Japan. *AMBIO: A Journal of the Human Environment* 35: 36-42.

Hay S (2006) Marine Ecology: Gelatinous bells may ring change in marine ecosystems. *Current Biology* 16: 679-682.

Hay SJ, Hislop JRG & Shanks AM (1990) North Sea Scyphomedusae; summer distribution, estimated biomass and significance particularly for 0-group Gadoid fish. *Netherlands Journal of Sea Research* 25: 113-130.

Hedges JI (1992) Global biogeochemical cycles: progress and problems. *Marine Chemistry* 39: 67-93.

Heeger T & Möller H (1987) Ultrastructural observations on prey capture and digestion in the scyphomedusa *Aurelia aurita*. *Marine Biology* 96: 391-400.

Heindl H, Wiese J, Thiel V & Imhoff JF (2010) Phylogenetic diversity and antimicrobial activities of bryozoan-associated bacteria isolated from Mediterranean and Baltic Sea habitats. *Systematic and Applied Microbiology* 33: 94-104.

Helmholz H, Johnston B, Ruhnau C & Prange A (2010) Gill cell toxicity of northern boreal scyphomedusae *Cyanea capillata* and *Aurelia aurita* measured by an in vitro cell assay. *Hydrobiologia* 645: 223-234.

Hentschel U, Usher KM & Taylor MW (2006) Marine sponges as microbial fermenters. *FEMS Microbiology Ecology* 55: 167-177.

Hentschel U, Schmid M, Wagner M, Fieseler L, Gernert C & Hacker J (2001) Isolation and phylogenetic analysis of bacteria with antimicrobial activities from the Mediterranean sponges *Aplysina aerophoba* and *Aplysina cavernicola*. *FEMS Microbiology Ecology* 35: 305-312.

Heuchert A, Glöckner FO, Amann R & Fischer U (2004) *Psychrobacter nivimaris* sp. nov., a heterotrophic bacterium attached to organic particles isolated from the South Atlantic (Antarctica). *Systematic and Applied Microbiology* 27: 399-406.

Hiemenz PC & Rajagopalan R (1997) Principles of colloid and surface chemistry. *Marcel Dekker, New-York*.

Hoeger U (1983) Biochemical composition of ctenophores. *Journal of Experimental Marine Biology and Ecology* 72: 251-261.

Hofmann DK & Brand U (1987) Induction of metamorphosis in the symbiotic scyphozoan *Cassiopea andromeda*: role of marine bacteria and of biochemicals. *Symbiosis* 4: 99-116.

Hofmann DK, Neumann R & Henne K (1978) Strobilation, budding and initiation of scyphistoma morphogenesis in the rhizostome *Cassiopea andromeda* (Cnidaria: Scyphozoa). *Marine Biology* 47: 161-176.

Holmström C & Kjelleberg S (1999) Marine *Pseudoalteromonas* species are associated with higher organisms and produce biologically active extracellular agents. *FEMS Microbiology Ecology* 30: 285-293.

Holmström C, Egan S, Franks A, McCloy S & Kjelleberg S (2002) Antifouling activities expressed by marine surface associated *Pseudoalteromonas* species. *FEMS Microbiology Ecology* 41: 47-58.

Holst S (2012 a) Effects of climate warming on strobilation and ephyra production of North Sea scyphozoan jellyfish. *Hydrobiologia* 690: 127-140.

Holst S (2012 b) Morphology and development of benthic and pelagic life stages of North Sea jellyfish (Scyphozoa, Cnidaria) with special emphasis on the identification of ephyra stages. *Marine Biology* 159: 2707-2722.

Holst S & Jarms G (2006) Responses of solitary and colonial coronate polyps (Cnidaria, Scyphozoa, Coronatae) to sedimentation and burial. *Journal of Experimental Marine Biology and Ecology* 329: 230-238.

Holst S & Jarms G (2007) Substrate choice and settlement preferences of planula larvae of five Scyphozoa (Cnidaria) from German Bight, North Sea. *Marine Biology* 151: 863-871.

Holst S & Jarms G (2010) Effects of low salinity on settlement and strobilation of scyphozoa (Cnidaria): Is the lion's mane *Cyanea capillata* (L.) able to reproduce in the brackish Baltic Sea? *Hydrobiologia* 645: 53-68.

Holst S, Sötje I, Tiemann H & Jarms G (2007) Life cycle of the rhizostome jellyfish *Rhizostoma octopus* (L.) (Scyphozoa, Rhizostomeae), with studies on cnidocysts and statoliths. *Marine Biology* 151: 1695-1710.

Hopkinson BM & Barbeau KA (2012) Iron transporters in marine prokaryotic genomes and metagenomes. *Environmental Microbiology* 14: 114-128.

Hoppe H-G (1991) Microbial extracellular enzyme activity: A new key parameter in aquatic ecology. *Microbial Enzymes in Aquatic Environments,*(Chróst R, ed.eds.), pp. 60-83. Springer New York.

Hurtado LA, Mateos M, Lutz RA & Vrijenhoek RC (2003) Coupling of bacterial endosymbiont and host mitochondrial genomes in the hydrothermal vent clam *Calyptogena magnifica*. *Applied and Environmental Microbiology* 69: 2058-2064.

Hütz A, Schubert K & Overmann J (2011) *Thalassospira* sp. isolated from the oligotrophic eastern mediterranean sea exhibits chemotaxis toward inorganic phosphate during starvation. *Applied and Environmental Microbiology* 77: 4412-4421.

Ionescu D, Siebert C, Polerecky L, Munwes YY, Lott C, Häusler S, Bižić-Ionescu M, Quast C, Ramette A, Glöckner FO, Ramette A, Rödiger T, Dittmar T, Oren A, Geyer S, Stärk H-J, Sauter M, Licha T & Laronne JB (2012) Microbial and chemical characterization of underwater fresh water springs in the Dead Sea. *PLoS ONE* 7: e38319.

Ivanov V, Kamakin A, Ushivtzev V, Shiganova T, Zhukova O, Aladin N, Wilson S, Harbison GR & Dumont H (2000) Invasion of the Caspian Sea by the comb jellyfish *Mnemiopsis Leidyi* (Ctenophora). *Biological Invasions* 2: 255-258.

Ivanova EP, Kiprianova EA, Mikhailov VV, Levanova GF, Garagulya AD, Gorshkova NM, Vysotskii MV, Nicolau DV, Yumoto N, Taguchi T & Yoshikawa S (1998) Phenotypic diversity of *Pseudoalteromonas citrea* from different marine habitats and emendation of the description. *International Journal of Systematic Bacteriology* 48: 247-256.

Jensen PR & Fenical W (1994) Strategies for the discovery of secondary metabolites from marine bacteria: ecological perspectives. *Annual Reviews in Microbiology* 48: 559-584.

Jiao N, Herndl GJ, Hansell DA, Benner R, Kattner G, Wilhelm SW, Kirchman DL, Weinbauer MG, Luo T, Chen F & Azam F (2010) Microbial production of recalcitrant dissolved organic matter: long-term carbon storage in the global ocean. *Nature Reviews Microbiology* 8: 593-599.

Kan J, Suzuki MT, Wang K, Evans SE & Chen F (2007) High temporal but low spatial heterogeneity of bacterioplankton in the Chesapeake Bay. *Applied and Environmental Microbiology* 73: 6776-6789.

Kassabgy M (2011) Diversity and abundance of Gammaproteobacteria during the winter-spring transition at station Kabeltonne (Helgoland). *PhD thesis* 37-41.

Kim Y, Lee S, Hwang I & Yoon K (2012) Effect of temperature on growth of *Vibrio paraphemolyticus* and *Vibrio vulnificus* in Flounder, Salmon Sashimi and Oyster Meat. *International Journal of Environmental Research and Public Health* 9: 4662-4675.

Kirchman DL (2002) The ecology of Cytophaga–Flavobacteria in aquatic environments. *FEMS Microbiology Ecology* 39: 91-100.

Kisand V, Dagmar R & Meinhard S (2008) Significant decomposition of riverine humic-rich DOC by marine but not estuarine bacteria assessed in sequential chemostat experiments. *Aquatic Microbial Ecology* 53: 151.

Kittelmann S & Harder T (2005) Species- and site-specific bacterial communities associated with four encrusting bryozoans from the North Sea, Germany. *Journal of Experimental Marine Biology and Ecology* 327: 201-209.

Kodama Y, Stiknowati LI, Ueki A, Ueki K & Watanabe K (2008) *Thalassospira tepidiphila* sp. nov., a polycyclic aromatic hydrocarbon-degrading bacterium isolated from seawater. *International Journal of Systematic and Evolutionary Microbiology* 58: 711-715.

Koren O & Rosenberg E (2006) Bacteria associated with mucus and tissues of the coral *Oculina patagonica* in summer and winter. *Applied and Environmental Microbiology* 72: 5254-5259.

Kovacs A (2010) A systematic assessment of automated ribosomal intergenic spacer analysis (ARISA) as a tool for estimating bacterial richness. *Research in Microbiology* 161: 192-197.

Krause E, Wichels A, Giménez L, Lunau M, Schilhabel MB & Gerdts G (2012) Small Changes in pH have direct effects on marine bacterial community composition: A microcosm approach. *PLoS ONE* 7.

Kremer P (1977) Respiration and excretion by the ctenophore *Mnepiopsis leidyi*. *Marine Biology* 44: 43-50.

Kujawinski EB (2011) The impact of microbial metabolism on marine dissolved organic matter. *Annual Review of Marine Science* 3: 567-599.

Kushmaro A, Loya Y, Fine M & Rosenberg E (1996) Bacterial infection and coral bleaching. *Nature* 380: 396-396.

Lampert Y, Kelman D, Dubinsky Z, Nitzan Y & Hill RT (2006) Diversity of culturable bacteria in the mucus of the Red Sea coral *Fungia scutaria*. *FEMS Microbiology Ecology* 58: 99-108.

Landa M, Cottrell M, Kirchman D, Blain S & Obernosterer I (2013) Changes in bacterial diversity in response to dissolved organic matter supply in a continuous culture experiment. *Aquatic Microbial Ecology* 69: 157-168.

Larson RJ (1976.) Marine flora and fauna of the Northeastern United States, Cnidaria: Scyphozoa. *US Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service*.

Lassen S, Helmholz H, Ruhnau C & Prange A (2010) Characterisation of neurotoxic polypeptides from *Cyanea capillata* medusae (Scyphozoa). *Hydrobiologia* 645: 213-221.

Leitz T (1997) Induction of settlement and metamorphosis of Cnidarian larvae: Signals and signal transduction. *Invertebrate Reproduction & Development* 31: 109-122.

Leitz T & Wagner T (1993) The marine bacterium *Alteromonas espejiana* induces metamorphosis of the hydroid *Hydractinia echinata*. *Marine Biology* 115: 173-178.

Lesh-Laurie GE & Suchy PE (1991) Cnidaria: Scyphozoa and Cubozoa. *Microscopic anatomy of invertebrates* 2: 185-266.

Li Z, Hu Y, Liu Y, Huang Y, He L & Miao X (2007) 16S rDNA clone library-based bacterial phylogenetic diversity associated with three South China Sea sponges. *World Journal of Microbiology and Biotechnology* 23: 1265-1272.

Littman R, Willis B & Bourne D (2009) Bacterial communities of juvenile corals infected with different Symbiodinium (dinoflagellate) clades. *Marine Ecology Progress Serious* 389: 45-59.

Linnaeus C (1758) Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Editio decima, reformata. Laurentius Salvius: Holmiae. ii, pp 824.

Linnaeus C (1767) Systema naturae sive regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. *Laurentii Salvii, Holmiae.* 12th edn, vol 1: 533-1327.

Linnaeus C (1788) Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus differentiis, synonymis, locis. *Editio decima tertia, aucta reformata. In: Gmelin CJF, Beer GE (eds) Lipsiae* vol 1(5-6): 2225-3910.

Llobet-Brossa E, Rosselló-Mora R & Amann R (1998) Microbial community composition of Wadden Sea sediments as revealed by fluorescence in situ hybridization. *Applied and Environmental Microbiology* 64: 2691-2696.

López-García P, Duperron S, Philippot P, Foriel J, Susini J & Moreira D (2003) Bacterial diversity in hydrothermal sediment and epsilonproteobacterial dominance in experimental microcolonizers at the Mid-Atlantic Ridge. *Environmental Microbiology* 5: 961-976.

Lucas-Elio P, Hernandez P, Sanchez-Amat A & Solano F (2005) Purification and partial characterization of marinocine, a new broad-spectrum antibacterial protein produced by *Marinomonas mediterranea*. *Biochimica et Biophysica Acta (BBA) - General Subjects* 1721: 193-203.

Lucas C (2001) Reproduction and life history strategies of the common jellyfish, *Aurelia aurita*, in relation to its ambient environment. *Hydrobiologia* 451: 229-246.

Lucas CH, Graham WM & Widmer C (2012) Chapter Three - Jellyfish Life Histories: Role of polyps in forming and maintaining scyphomedusa populations. *Advances in Marine Biology,* Vol. 63 (Michael L, ed.eds.), 133-196. Academic Press.

Mai-Prochnow A, Evans F, Dalisay-Saludes D, Stelzer S, Egan S, James S, Webb JS & Kjelleberg S (2004) Biofilm development and cell death in the marine bacterium *Pseudoalteromonas tunicata*. *Applied and Environmental Microbiology* 70: 3232-3238.

Malmstrom RR, Straza TR, Cottrell MT & Kirchman DL (2007) Diversity, abundance, and biomass production of bacterial groups in the western Arctic Ocean. *Aquatic microbial ecology* 47: 45-55.

Mann AJ, Hahnke RL, Huang S, Werner J, Xing P, Barbeyron T, Huettel B, Stüber K, Reinhardt R, Harder J, Glöckner FO, Amann RI & Teeling H (2013) The genome of the alga-associated marine Flavobacterium *Formosa agariphila* KMM 3901T reveals a broad potential for degradation of algal polysaccharides. *Applied and Environmental Microbiology* 79: 6813-6822.

Manz W, Amann R, Ludwig W, Wagner M & Schleifer K-H (1992) Phylogenetic oligodeoxynucleotide probes for the major subclasses of Proteobacteria: Problems and Solutions. *Systematic and Applied Microbiology* 15: 593-600.

Manz W, Amann R, Ludwig W, Vancanneyt M & Schleifer K-H (1996) Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum Cytophaga-Flavobacter-Bacteroides in the natural environment. *Microbiology* 142: 1097-1106.

Martinez J, Smith DC, Steward GF & Azam F (1996) Variability in ectohydrolytic enzyme activities of pelagic marine bacteria and its significance for substrate processing in the sea. *Aquatic Microbial Ecology* 10: 223-230.

Maruyama A, Honda D, Yamamoto H, Kitamura K & Higashihara T (2000) Phylogenetic analysis of psychrophilic bacteria isolated from the Japan Trench, including a description of the deep-sea species *Psychrobacter pacificensis* sp. nov. *International Journal of Systematic and Evolutionary Microbiology* 50: 835-846.

Mary I (2006) SAR11 dominance among metabolically active low nucleic acid bacterioplankton in surface waters along an Atlantic meridional transect. *Aquatic Microbial Ecology* 45: 107-113.

McBride MJ, Xie G, Martens EC, Lapidus A, Henrissat B, Rhodes RG, Goltsman E, Wang W, Xu J, Hunnicutt DW, Staroscik AM, Hoover TR, Cheng Y-Q & Stein JL (2009) Novel features of the polysaccharide-digesting gliding bacterium Flavobacterium johnsoniae as revealed by genome sequence analysis. *Applied and Environmental Microbiology* 75: 6864-6875.

McCarthy MD, Hedges JI & Benner R (1998) Major bacterial contribution to marine dissolved organic nitrogen. *Science* 281: 231-234.

McFall-Ngai M & Ruby EG (1991) Symbiont recognition and subsequent morphogenesis as early events in an animal-bacterial mutualism. *Science* 254: 1491-1494.

Mills CE (2001) Jellyfish blooms: are populations increasing globally in response to changing ocean conditions? *Hydrobiologia* 451: 55-68.

Mitchell SO & Rodger HD (2011) A review of infectious gill disease in marine salmonid fish. *Journal of Fish Diseases* 34: 411-432.

Mitchell SO, Baxter EJ & Rodger HD (2013) Gill pathology in farmed salmon associated with the jellyfish *Aurelia aurita*. *Veterinary Record Case Reports* 1.

Möller H (1980) Population dynamics of *Aurelia aurita* medusae in Kiel Bight, Germany (FRG). *Marine Biology* 60: 123-128.

Moyer CL, Dobbs FC & Karl DM (1995) Phylogenetic diversity of the bacterial community from a microbial mat at an active, hydrothermal vent system, Loihi Seamount, Hawaii. *Applied and Environmental Microbiology* 61: 1555-1562.

Müller WA & Leitz T (2002) Metamorphosis in the Cnidaria. *Canadian Journal of Zoology* 80: 1755-1771.

Nagata T (2000) Production mechanisms of dissolved organic matter. *Wiley Series in Ecological and Applied Microbiology*.

Nagata T (2008) Organic matter–bacteria interactions in seawater. *Microbial Ecology of the Oceans* 207-241. John Wiley & Sons, Inc.

Nair S (2004) Bacterial associations: Antagonism to Symbiosis. 83-89.

Nemazie DA, Purcell JE & Glibert PM (1993) Ammonium excretion by gelationous zooplankton and their contribution to the ammonium requirements of microplankton in Chesapeake Bay. *Marine Biology* 116: 451-458.

Neulinger SC, Järnegren J, Ludvigsen M, Lochte K & Dullo W-C (2008) Phenotype-specific bacterial communities in the cold-water coral *Lophelia pertusa* (Scleractinia) and their implications for the coral's nutrition, health, and distribution. *Applied and Environmental Microbiology* 74: 7272-7285.

Niggl W, Naumann MS, Struck U, Manasrah R & Wild C (2010) Organic matter release by the benthic upside-down jellyfish *Cassiopea* sp. fuels pelagic food webs in coral reefs. *Journal of Experimental Marine Biology and Ecology* 384: 99-106.

Nussbaumer AD, Bright M, Baranyi C, Beisser CJ & Ott JA (2004) Attachment mechanism in a highly specific association between ectosymbiotic bacteria and marine nematodes. *Aquatic microbial ecology* 34: 239-246.

Nyholm SV & McFall-Ngai M (2004) The winnowing: establishing the squid-vibrio symbiosis. *Nature Reviews Microbiology* 2: 632-642.

Oberbeckmann S, Wichels A, Wiltshire K & Gerdts G (2011) Occurrence of *Vibrio parahaemolyticus* and *Vibrio alginolyticus* in the German Bight over a seasonal cycle. *Antonie van Leeuwenhoek* 100: 291-307.

Oberbeckmann S, Fuchs B, Meiners M, Wichels A, Wiltshire K & Gerdts G (2012) Seasonal dynamics and modeling of a *Vibrio* community in coastal waters of the North Sea. *Microbial Ecology* 63: 543-551.

Ogawa H & Tanoue E (2003) Dissolved organic matter in oceanic waters. *Journal of Oceanography* 59: 129-147.

Olesen NJ, Purcell JE & Stoecker DK (1996) Feeding and growth by ephyrae of scyphomedusae *Chrysaora quinquecirrha*. *Marine Ecology Progress Series* 137: 149-159.

Otero-González AJ, Magalhães BS, Garcia-Villarino M, López-Abarrategui C, Sousa DA, Dias SC & Franco OL (2010) Antimicrobial peptides from marine invertebrates as a new frontier for microbial infection control. *The FASEB Journal* 24: 1320-1334.

Ovchinnikova TV, Balandin SV, Aleshina GM, Tagaev AA, Leonova YF, Krasnodembsky ED, Men'shenin AV & Kokryakov VN (2006) Aurelin, a novel antimicrobial peptide from jellyfish *Aurelia aurita* with structural features of defensins and channel-blocking toxins. *Biochemical and Biophysical Research Communications* 348: 514-523.

Parsons TR & Lalli CM (2002) Jellyfish population explosions: revisiting a hypothesis of possible causes. *La mer* 40: 111-121.

Pernthaler A, Pernthaler J, Amann R, Kowalchuk G, Bruijn F, Head I & Elsas J (2004) Sensitive multi-color fluorescence in situ hybridization for the identification of environmental microorganisms *Molecular microbial ecology manual* 1 (Ed. 2): 711-725.

Péron F & Lesueur C (1809) Tableau des caractéres génériques et spécifiques de toutes les espéces de méduses connues jusqu'ace jour. *Annales Muséum Histroy National, Paris* vol 14: 325-366.

Pinhassi J & Hagstrom A (2000) Seasonal succession in marine bacterioplankton. *Aquatic Microbial Ecology* 21: 245-256.

Pinhassi J & Berman T (2003) Differential growth response of colony-forming alpha- and gamma-Proteobacteria in dilution culture and nutrient addition experiments from Lake

Kinneret (Israel), the eastern Mediterranean Sea, and the Gulf of Eilat. *Applied Environment Microbiology* 69: 199-211.

Pinhassi J, Sala MM, Havskum H, Peters F, Guadayol Ò, Malits A & Marrasé C (2004) Changes in bacterioplankton composition under different phytoplankton regimens. *Applied and Environmental Microbiology* 70: 6753-6766.

Pisut DP & Pawlik JR (2002) Anti-predatory chemical defenses of ascidians: secondary metabolites or inorganic acids? *Journal of Experimental Marine Biology and Ecology* 270: 203-214.

Pitt KA, Welsh DT & Condon RH (2009) Influence of jellyfish blooms on carbon, nitrogen and phosphorus cycling and plankton production. *Hydrobiologia* 616: 133-149.

Polz MF, Distel DL, Zarda B, Amann R, Felbeck H, Ott JA & Cavanaugh CM (1994) Phylogenetic analysis of a highly specific association between ectosymbiotic, sulfur-oxidizing bacteria and a marine nematode. *Applied and Environmental Microbiology* 60: 4461-4467.

Pruesse E (2007) SILVA: A comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research* 35: 71-88.

Pukall R, Kramer I, Rohde M & Stackebrandt E (2001) Microbial diversity of cultivatable bacteria associated with the North Sea bryozoan *Flustra foliacea*. *Systematic and Applied Microbiology* 24: 623-633.

Purcell J, Shiganova T, Decker M & Houde E (2001) The ctenophore *Mnemiopsis* in native and exotic habitats: U.S. estuaries versus the Black Sea basin. *Jellyfish Blooms: Ecological and Societal Importance,* Vol. 155 145-176. Springer Netherlands.

Purcell JE (2003) Predation on zooplankton by large jellyfish, *Aurelia labiata*, *Cyanea capillata* and *Aequorea aequorea*, in Prince William Sound, Alaska. *Marine Ecology Progress Series* 246.

Purcell JE & Arai MN (2001) Interactions of pelagic cnidarians and ctenophores with fish: a review. *Hydrobiologia* 451: 27-44.

Purcell JE & Decker MB (2005) Effects of climate on relative predation by scyphomedusae and ctenophores on copepods in Chesapeake Bay during 1987-2000. *Limnology and Oceanography* 50: 376-387.

Purcell JE, Uye SI & Lo WT (2007) Anthropogenic causes of jellyfish blooms and their direct consequences for humans: A review. *Marine Ecology Progress Series* 350: 153-174.

Purcell JE, Breitburg DL, Decker MB, Graham WM, Youngbluth MJ & Raskoff KA (2001) Pelagic cnidarians and ctenophores in low dissolved oxygen environments: A review. *Coastal Hypoxia: Consequences for Living Resources and Ecosystems,*77-100. American Geophysical Union.

Rahat M & Dimentman C (1982) Cultivation of bacteria-free *Hydra viridis*: missing budding factor in nonsymbiotic *Hydra*. *Science* 216: 67-68.

Raina J-B, Tapiolas D, Willis BL & Bourne DG (2009) Coral-associated bacteria and their role in the biogeochemical cycling of sulfur. *Applied and Environmental Microbiology* 75: 3492-3501.

Ranjard L, Brothier E & Nazaret S (2000) Sequencing bands of ribosomal intergenic spacer analysis fingerprints for characterization and microscale distribution of soil bacterium populations responding to mercury spiking. *Applied and Environmental Microbiology* 66: 5334-5339.

Rapoza R, Novak D & Costello JH (2005) Life-stage dependent, in situ dietary patterns of the lobate ctenophore *Mnemiopsis leidyi* Agassiz 1865. *Journal of Plankton Research* 27: 951-956.

Rappé MS, Vergin K & Giovannoni SJ (2000) Phylogenetic comparisons of a coastal bacterioplankton community with its counterparts in open ocean and freshwater systems. *FEMS Microbiology Ecology* 33: 219-232.

Reinheimer G (1986) Microbe-microbe interactions and interactions with other aquatic organisms. *Aquatic Microbiology* 4th Edition. John Wiley and Sons. New York: 149-169.

Richardson AJ, Bakun A, Hays GC & Gibbons MJ (2009) The jellyfish joyride: causes, consequences and management responses to a more gelatinous future. *Trends in Ecology and Evolution* 24: 312-322.

Riemann, L, Titelman, J, Bamstedt U (2006) Links between jellyfish and microbes in a jellyfish dominated fjord. *Marine ecology Progress series*, 325: 29-42.

Riemann L & Azam F (2002) Widespread N-Acetyl-d-Glucosamine uptake among pelagic marine bacteria and its ecological implications. *Applied and Environmental Microbiology* 68: 5554-5562.

Riemann L, Steward GF & Azam F (2000) Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. *Applied and Environmental Microbiology* 66: 578-587.

Rinehart KL (2008) Secondary metabolites from marine organisms. *Secondary Metabolites: Their Function and Evolution* 752: 236.

Ritchie KB (2006) Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. *Marine Ecology Progress Series* 322: 1-14.

Robidart JC, Bench SR, Feldman RA, Novoradovsky A, Podell SB, Gaasterland T, Allen EE & Felbeck H (2008) Metabolic versatility of the *Riftia pachyptila* endosymbiont revealed through metagenomics. *Environmental Microbiology* 10: 727-737.

Rohwer F, Seguritan V, Azam F & Knowlton N (2002) Diversity and distribution of coral-associated bacteria. *Marine Ecology Progress Series* 243: 1-10.

Rohwer F, Breitbart M, Jara J, Azam F & Knowlton N (2001) Diversity of bacteria associated with the Caribbean coral *Montastraea franksi*. *Coral Reefs* 20: 85-91.

Romanenko LA, Lysenko AM, Rohde M, Mikhailov VV & Stackebrandt E (2004) *Psychrobacter maritimus* sp. nov. and *Psychrobacter arenosus* sp. nov., isolated from coastal sea ice and sediments of the Sea of Japan. *International Journal of Systematic and Evolutionary Microbiology* 54: 1741-1745.

Romero J & Navarrete P (2006) 16S rDNA-based analysis of dominant bacterial populations associated with early life stages of coho salmon (*Oncorhynchus kisutch*). *Microbial Ecology* 51: 422-430.

Rosenberg E, Koren O, Reshef L, Efrony R & Zilber-Rosenberg I (2007) The role of microorganisms in coral health, disease and evolution. *Nature Reviews Microbiology* 5: 355-362.

Russell FS (1970) The medusae of the British Isles: 2. Pelagic scyphozoa with a supplement to the first volume on hydromedusae. *Cambridge University Press* London. XI: pp.283

Sanchez-Amat A, Lucas-Elío P, Fernández E, García-Borrónb JC & Solanob F (2001) Molecular cloning and functional characterization of a unique multipotent polyphenol oxidase from *Marinomonas mediterranea*. *Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology* 1547: 104-116.

Sapp M, Wichels A & Gerdts G (2007 a) Impacts of cultivation of marine diatoms on the associated bacterial community. *Applied and Environmental Microbiology* 73: 3117-3120.

Sapp M, Gerdts G, Wiltshire KH & Wichels A (2007 b) Bacterial community dynamics during the winter-spring transition in the North Sea. *FEMS Microbiol. Ecol.* 59: 622-637.

Sapp M, Schwaderer A, Wiltshire K, Hoppe H-G, Gerdts G & Wichels A (2007 c) Species-specific bacterial communities in the phycosphere of microalgae? *Microbial Ecology* 53: 683-699.

Schmahl G (1985) Bacterially induced stolon settlement in the scyphopolyp of *Aurelia aurita* (Cnidaria, Scyphozoa). *Helgoländer Meeresuntersuchungen* 39: 33-42.

Schneider G (1989) The common jellyfish *Aurelia aurita*: standing stock, excretion and nutrient regeneration in the Kiel Bight, Western Baltic. *Marine Biology* 100: 507-514.

Schneider G & Behrends G (1998) Top-down control in a neritic plankton system by *Aurelia aurita* medusae — a summary. *Ophelia* 48: 71-82.

Schuett C & Doepke H (2010) Endobiotic bacteria and their pathogenic potential in cnidarian tentacles. *Helgoland Marine Research* 64: 205-212.

Schuett C, Doepke H, Groepler W & Wichels A (2005) Diversity of intratunical bacteria in the tunic matrix of the colonial ascidian *Diplosoma migrans*. *Helgoland Marine Research* 59: 136-140.

Schuett C, Doepke H, Grathoff A & Gedde M (2007) Bacterial aggregates in the tentacles of the sea anemone *Metridium senile*. *Helgoland Marine Research* 61: 211-216.

Scolardi KM, Daly KL, Pakhomov EA & Torres JJ (2006) Feeding ecology and metabolism of the Antarctic cydippid ctenophore *Callianira antarctica*. *Marine Ecology Progress Series* 317: 111.

Sharp KH, Eam B, Faulkner DJ & Haygood MG (2007) Vertical transmission of diverse microbes in the tropical sponge *Corticium* sp. *Applied and Environmental Microbiology* 73: 622-629.

Shashar N, Cohen Y, Loya Y & Sar N (1994) Nitrogen fixation (acetylene reduction) in stony corals: Evidence for coral-bacteria interactions. *Marine ecology progress series* 111: 259-264.

Shcherbakova VA, Chuvil'skaya NA, Rivkina EM, Pecheritsyna SA, Suetin SV, Laurinavichius KS, Lysenko AM & Gilichinsky DA (2009) Novel halotolerant bacterium from cryopeg in permafrost: Description of *Psychrobacter muriicola* sp. nov. *Microbiology* 78: 84-91.

Shivaji S, Reddy GS, Suresh K, Gupta P, Chintalapati S, Schumann P, Stackebrandt E & Matsumoto GI (2005) *Psychrobacter vallis* sp. nov. and *Psychrobacter aquaticus* sp. nov., from Antarctica. *International Journal of Systematic and Evolutionary Microbiology* 55: 757-762.

Simon M, Glöckner FO & Amann R (1999) Different community structure and temperature optima of heterotrophic picoplankton in various regions of the southern Ocean. *Aquatic Microbial Ecology* 18: 275-284.

Solano F & Sanchez-Amat A (1999) Note: Studies on the phylogenetic relationships of melanogenic marine bacteria: Proposal of *Marinomonas mediterranea* sp. nov. *International Journal of Systematic Bacteriology* 49: 1241-1246.

Sommer, Stibor H, Katechakis A, Sommer F & Hansen T (2002) Pelagic food web configurations at different levels of nutrient richness and their implications for the ratio fish production: primary production. *Hydrobiologia* 484.

Stoecker DK, Michaels AE & Davis LH (1987) Grazing by the jellyfish, *Aurelia aurita*, on microzooplankton. *Journal of Plankton Research* 9: 901-915.

Sullivan B, Keuren D & Clancy M (2001) Timing and size of blooms of the ctenophore *Mnemiopsis leidyi* in relation to temperature in Narragansett Bay, RI. *Hydrobiologia* 451: 113-120.

Sunagawa S, DeSantis TZ, Piceno YM, Brodie EL, DeSalvo MK, Voolstra CR, Weil E, Andersen GL & Medina M (2009) Bacterial diversity and white plague disease-associated community changes in the Caribbean coral *Montastraea faveolata*. *ISME Journal* 3: 512-521.

Svane I & Dolmer P (1995) Perception of light at settlement: a comparative study of two invertebrate larvae, a scyphozoan planula and a simple ascidian tadpole. *Journal of Experimental Marine Biology and Ecology* 187: 51-61.

Taylor MW, Radax R, Steger D & Wagner M (2007) Sponge-associated microorganisms: Evolution, ecology, and biotechnological potential. *Microbiology and Molecular Biology Reviews* 71: 295-347.

Teeling H, Fuchs BM, Becher D, Klockow C, Gardebrecht A, Bennke CM, Kassabgy M, Huang S, Mann AJ, Waldmann J, Weber M, Klindworth A, Otto A, Lange J, Bernhardt J, Reinsch C, Hecker M, Peplies J, Bockelmann FD, Callies U, Gerdts G, Wichels A, Wiltshire KH, Glöckner FO, Schweder T & Amann RI (2012) Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. *Science* 336: 608-611.

Tendal OS, Jensen KR & Riisgård HU (2007) Invasive ctenophore *Mnemiopsis leidyi* widely distributed in Danish waters. *Aquatic Invasions* 2: 445–460.

Thakur NL, Anil AC & Muelle WEG (2004) Culturable epibacteria of the marine sponge *Ircinia fusca*: temporal variations and their possible role in the epibacterial defense of the host. *Aquatic microbial ecology* 37: 295-304.

Thakur NL, Hentschel U, Krasko A, Pabel CT, Anil AC & Muelle WE (2003) Antibacterial activity of the sponge *Suberites domuncula* and its primmorphs: potential basis for epibacterial chemical defense. *Aquatic Microbial Ecology* 31: 77-83.

Thuesen EV, Rutherford LD, Brommer PL, Garrison K, Gutowska MA & Towanda T (2005) Intragel oxygen promotes hypoxia tolerance of scyphomedusae. *Journal of Experimental Biology* 208: 2475-2482.

Tinta T, Malej A, Kos M & Turk V (2010) Degradation of the Adriatic medusa *Aurelia* sp. by ambient bacteria. *Hydrobiologia* 645: 179-191.

Tinta T, Kogovšek T, Malej A & Turk V (2012) Jellyfish modulate bacterial dynamic and community structure. *PLoS ONE* 7.

Titelman J, Riemann L, Sørnes TA, Nilsen T, Griekspoor P & Båmstedt U (2006) Turnover of dead jellyfish: stimulation and retardation of microbial activity. *Marine Ecology Progress Series* 325: 43-58.

Torkildsen L, Lambert C, Nylund A, Magnesen T & Bergh Ø (2005) Bacteria associated with early life stages of the great scallop, *Pecten maximus*: impact on larval survival. *Aquaculture International* 13: 575-592.

Uysal Z & Mutlu E (1993) Preliminary note on the occurrence and biometry of ctenophoran *Mnemiopsis leidyi* finally invaded Mersin Bay. *Doga-Tr J Zool* 17: 229-236.

Vinogradov ME, Shushkina EA, Musaeva EI & Sorokin PY (1989) Ctenophore *Mnemiopsis leidyi* (A-AGASSIZ) (Ctenophora, lobata)- new settlers in the Black Sea. *Okeanologiya* 29: 293-299.

Vrijenhoek R (2010) Genetics and evolution of deep-sea chemosynthetic bacteria and their invertebrate hosts. *The Vent and Seep Biota,* Vol. 33 (Kiel S, ed.eds.), pp. 15-49. Springer Netherlands.

Waddell B & Pawlik JR (2000) Defenses of Caribbean sponges against invertebrate predators. I. Assays with hermit crabs. *Marine Ecology Progress Series* 195: 125-132.

Wahl M, Jensen PR & Fenical W (1994) Chemical control of bacterial epibiosis on ascidians. *Marine Ecology Progress Series* 110: 45-57.

Wahl M, Jormalainen V, Eriksson B & Coyer J (2011) Stress ecology in Fucus: abiotic, biotic and genetic interactions. *Advances in marine biology* 59: 37-105.

Wang W, Wen B, Gasparich GE, Zhu N, Rong L, Chen J & Xu Z (2004) A spiroplasma associated with tremor disease in the Chinese mitten crab (*Eriocheir sinensis*). *Microbiology* 150: 3035-3040.

Webb KL & Johannes RE (1967) Studies of the release of dissolved free amino acids by marine zooplankton. *Limnology and Oceanography* 376-382.

Webster NS & Taylor MW (2012) Marine sponges and their microbial symbionts: love and other relationships. *Environmental Microbiology* 14: 335-346.

Webster NS, Taylor MW, Behnam F, Lücker S, Rattei T, Whalan S, Horn M & Wagner M (2010) Deep sequencing reveals exceptional diversity and modes of transmission for bacterial sponge symbionts. *Environmental Microbiology* 12: 2070-2082.

Wegley L, Edwards R, Rodriguez-Brito B, Liu H & Rohwer F (2007) Metagenomic analysis of the microbial community associated with the coral *Porites astreoides*. *Environmental Microbiology* 9: 2707-2719.

Wells ML (2002) Marine colloids and trace metals. *PhD thesis*.

West E, Welsh D & Pitt K (2009) Influence of decomposing jellyfish on the sediment oxygen demand and nutrient dynamics. *Hydrobiologia* 616: 151-160.

Wichels A, Würtz S, Döpke H, Schütt C & Gerdts G (2006) Bacterial diversity in the breadcrumb sponge *Halichondria panicea* (Pallas). *FEMS Microbiology Ecology* 56: 102-118.

Widersten B (1965) Genital organs and fertilization in some Scyphozoa. *Zool Bidr Upps* 37: 45-61.

Wieczorek SK & Todd CD (1998) Inhibition and facilitation of settlement of epifaunal marine invertebrate larvae by microbial biofilm cues. *Biofouling* 12: 81-118.

Williams WM, Viner AB & Broughton WJ (1987) Nitrogen fixation (acetylene reduction) associated with the living coral *Acropora variabilis*. *Marine Biology* 94: 531-535.

Woollacott RM & Hadfield MG (1996) Induction of metamorphosis in larvae of a sponge. *Invertebrate Biology* 115: 257-262.

Zeder M, Ellrott A & Amann R (2011) Automated sample area definition for high-throughput microscopy. *Cytometry Part A* 79: 306-310.

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Hiermit versichere ich, dass ich die vorliegende Dissertation mit dem Titel "Bacterial community associated with jellyfish"

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